

Comparison of the Influence of External Treatments on Oviposition by *Trichogramma pretiosum* and *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) into Stretched Plastic Artificial Eggs

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ABSTRACT

Treatment of the exterior of stretched plastic artificial eggs (SPAEs) with a hexane extract of *Helicoverpa zea* (Boddie) moth scales resulted in significant increases in the percentage of SPAEs into which females *Trichogramma pretiosum* Riley and *Trichogramma minutum* Riley oviposited and the number of eggs oviposited, compared to treatments with Elmer's School Glue®, gelatin, polyvinyl alcohol, an untreated control, water control, or hexanes control. Treatment with a mixture of Elmer's School Glue and moth scale extract was not significantly different from treatment with moth scale extract only. This information is useful in the development of a system for collecting *Trichogramma* spp. eggs for use in an automated *in vitro* mass rearing system.

RESUMEN

El tratamiento del exterior de huevecillos artificiales de plástico estirado (SPAEs) con el extracto producido con hexano de escamas de la palomilla *Helicoverpa zea* (Boddie) produjo un incremento significativo en el número de huevecillos ovipositados y en el porcentaje de SPAEs donde las hembras de *Trichogramma pretiosum* Riley y *Trichogramma minutum* Riley ovipositaron en comparación con los tratamientos en donde se usó la goma Elmer's School Glue, gelatina, alcohol polivinilo, control sin tratamiento, control usando agua o hexano solamente. El tratamiento con la mezcla de la goma Elmer's School Glue y extracto de escama de la palomilla no fue significativamente diferente del tratamiento con extracto de escama de palomilla únicamente. Esta información es útil para el desarrollo de un sistema de colección de huevecillos de *Trichogramma* spp para su uso en un sistema automatizado de producción masiva *in vitro*.

Additional Index Words: oviposition, egg collection, *in vitro* rearing, mass rearing

Trichogramma spp. attack over 400 pest species, mostly lepidopterans. On a world wide basis, some 32 million ha of agricultural and forest land are treated with *Trichogramma* spp. annually (Li, 1994). *Trichogramma* spp. have been used on corn, sugarcane, cotton, vegetables, and fruit trees in more than 30 countries (Li, 1994).

Limited production capacity and relatively high production costs are primary impediments to wider use of *Trichogramma* spp. in biological control (King, 1993). The vast majority of *Trichogramma* used in biological control are reared on factitious hosts. When factitious hosts are used for *Trichogramma* rearing, over 70% of the space used and costs are related to the rearing of the factitious hosts (Newton, 1993). *In vivo* rearing of *Trichogramma* spp. and rearing of factitious hosts were recently reviewed by Greenberg et al. (1996, 1998). The production costs for *Trichogramma* spp. could be reduced and capacity increased by the development of an automated *in vitro* mass rearing system.

in vitro mass rearing system.

There have been significant advances in the development of artificial diets for rearing *Trichogramma* spp. in China (Li, 1992), France (Grenier, 1994) and in United States (Xie et al., 1997a; Nordlund et al., 1997). However, the efficient use of artificial diets in mass rearing has been limited to date.

Various semiochemicals from hosts, including kairomones found in the scales of *Helicoverpa zea* (Boddie) moths, or plants that they feed on have been shown to intensify the searching behavior of *Trichogramma* spp. females, which subsequently results in increased rates of parasitism (Altieri et al., 1981; Jones et al., 1973; Lewis et al., 1972, 1975a, 1975b; Lewis and Martin, 1990; Nordlund et al., 1976, 1978; Shen et al., 1991). Also, hemolymph from oak silkworm, *Antheraea pernyi* (Guérin-Méneville) (Dai et al., 1988) or a mixture of saturated hydrocarbons identified from extracts of eggs of *Ostrinia nubilalis* Hübner stimulates the searching behavior of

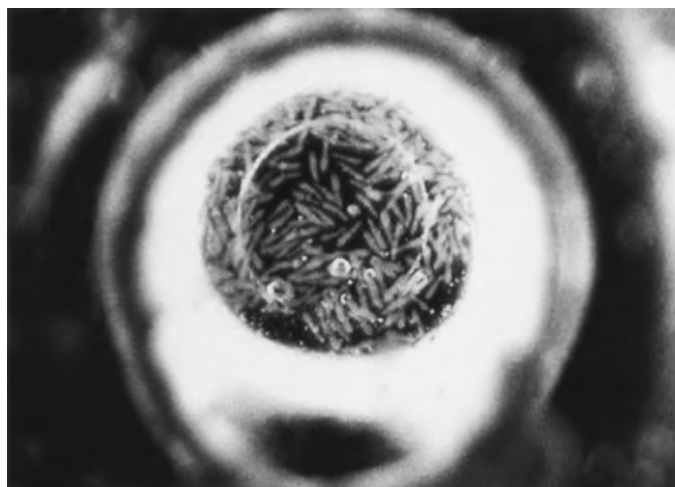


Fig. 1. *Trichogramma* spp. eggs in a stretched plastic artificial egg (from Nordlund et al. 1998).

Trichogramma brassicae Bezdenko females in a linear olfactometer (Renou et al., 1992).

Stretched plastic artificial eggs (SPAЕ) have been used for *in vitro* rearing of *Trichogramma* spp. in China, where several experimental machines for preparation of “host egg-cards” have been developed (Liu et al., 1995). In the Chinese system, *Trichogramma* females oviposit directly into the artificial diet, which is contained in the SPAEs. This can lead to inefficiencies in the system because some SPAЕ receive too few eggs while others receive too many.

Nordlund et al. (1998) proposed an automated mass rearing system in which the *Trichogramma* eggs are collected, quantitated, and mixed with an appropriate amount of diet, and then placed in rearing cells. This process could improve efficiency and quality, as well as facilitate development of an automated system. SPAEs can be used for collection of large numbers of *Trichogramma* spp. eggs (Fig. 1). However, it has been difficult to obtain a high rate of oviposition in SPAEs. Also, some oviposition stimulants (e.g. amino acids), when placed on the interior of the SPAEs can interfere with nutrition. Others, such as salts, have a deleterious effect on egg and larval development (Nettles et al, 1983; Qin and Wu, 1988).

There are reports that the presence of materials on the surface of SPAEs can result in increased numbers of eggs being oviposited by *Trichogramma* females. Grenier et al. (1993) reported that *O. nubilalis* moth scale extract sprayed on the surface of artificial eggs significantly increased the number of eggs laid by *T. brassicae* (15.0 or 2.5 times, respectively in comparison with control or solvent). Solutions or suspensions of polyvinyl alcohol, gelatin, white latex, agar, cassava (*Nanihot utilissimi*) powder, rice flour, and starch were used as synergists to improve oviposition in *in vitro* rearing of *T. dendrolimi* Matsumura, *T. contusum* Viggiani (= *T. chilonis* Ishii), and *T. evanescens* Westwood (Han et al., 1994). The authors observed that the ovipositional responses of the *Trichogramma* spp. to these materials was different. The best materials were polyvinyl alcohol and gelatin, gelatin, and polyvinyl alcohol and agar for *T.*

dendrolimi, *T. confusum*, and *T. evanescens*, respectively. Xie et al. (1997b) reported that Elmer’s School Glue® and Elmer’s Glue All® (Borden, Inc., Columbus, OH, USA) arrested movement and stimulated probing/oviposition by *T. pretiosum*, *T. minutum*, and *T. brassicae*.

In this study, we compared the response of *T. pretiosum* and *T. minutum* females to SPAEs that had been treated with a hexane extract of *H. zea* moth scales (Jones et al., 1973), Elmer’s School Glue, a mixture of School Glue and moth scale extract, gelatin, polyvinyl alcohol, untreated control, water control, or hexane control. As mentioned above, all of these materials have been shown to influence the host selection or oviposition behavior of *Trichogramma* spp. However, we were interested in identifying the most promising material(s) for use in future system development efforts. The measurements recorded were the percentage of SPAEs into which *T. pretiosum* and *T. minutum* females oviposited and the number of eggs deposited.

Table 1. Influence of different treatments on the percentage of stretched plastic artificial eggs (SPAЕs) into which *Trichogramma pretiosum* Riley and *Trichogramma minutum* Riley females oviposited.

Treatment	Percentage of SPAEs with <i>Trichogramma</i> eggs ^z	
	<i>T. pretiosum</i>	<i>T. minutum</i>
Untreated Control	16.2 ± 2.9 c	14.8 ± 1.3 d
School Glue	40.0 ± 1.6 b	34.8 ± 1.3 bc
Moth Scale Extract (MSE)	58.1 ± 4.2 a	62.8 ± 3.0 a
School Glue-f-MSE	59.5 ± 2.6 a	64.8 ± 2.5 a
Gelatin	45.2 ± 1.3 b	51.4 ± 1.6 b
Polyvinyl Alcohol	42.9 ± 2.2 b	41.9 ± 1.7 bc
Water	15.7 ± 3.0 c	12.4 ± 2.1 d
Hexane	33.3 ± 2.1 b	28.1 ± 2.5 c

F=39.8; df=7, 16; P<0.001 F=93.2; df=7, 16; P<0.001

^zMeans (±SEM) in each column, followed by different letters are significantly different at the 5% level, as determined by Tukey studentized range test.

Table 2. Influence of different treatments on the total number of eggs deposited by *T. pretiosum* or *T. minutum* females in an oviposition arena (=70 SPAEs).

Treatment	Mean of Eggs/Oviposition Arena ^z	
	<i>T. pretiosum</i>	<i>T. minutum</i>
Untreated Control	80 ± 12 c	200 ± 10 d
SchoolGlue	252 ± 12 b	439 ± 31 c
Moth Scale Extract (MSE)	518 ± 14 a	878 ± 40 a
School Glue + MSE	513 ± 24 a	906 ± 40 a
Gelatin	330 ± 18 b	666 ± 18 b
Polyvinyl Alcohol	268 ± 25 b	523 ± 31 bc
Water	93 ± 24 c	178 ± 23 d
Hexane	202 ± 5 b	389 ± 36 c

F=84.4; df=7, 16; P<0.001 F=83.3; df=7, 16; P<0.001

^zMeans (± SEM) in each column, followed by different letters are significantly different at the 5% level, as determined by the Tukey studentized range test.

MATERIALS AND METHODS

The *T. pretiosum* and *T. minutum* used in this study were maintained at the Biological Control of Pests Research Unit, Weslaco, Texas, USA on irradiated (25 kr, Cs 137 source, 641 sec) *H. zea* eggs. Rearing was conducted in 2 x 15 cm glass tubes at $26 \pm 1^\circ\text{C}$, 75% RH, and a 14L:10D photoperiodic regime. The *H. zea* eggs were attached to paper strips (2.5 x 12.5 cm) with chicken egg albumen. Two cardboard strips with up to 0.66 g (ca. 7260 eggs) of unparasitized *H. zea* eggs were inserted into a vial, which was then taped, open end to open end to another vial containing *Trichogramma* that had begun to emerge as adults. The latter was covered with black paper. A ratio of ca. 1 parasitoid female : 10 host eggs was maintained and the exposure period was ca. 24 h. The oviposition arena used in these experiments was a modified form of the arena developed by the Chinese scientists (Li et al., 1988) and consisted of two plastic rings to hold the film with SPAEs in place (Xie et al., 1997b). One of the rings was then taped to a 6 cm diameter Petri dish (with masking tape), which contained the *Trichogramma*. SPAEs were held with the convex side toward the *Trichogramma*.

A polypropylene film, 22.9 μm thick (Catalogue # ACE 11166, ACE Hardware Corporation, Oak Brook, IL) was used for preparation of the SPAEs. The SPAEs were prepared using a device consisting of male and female molds. The female mold was a piece of perforated metal (#9255T64, McMaster-Carr, Atlanta, GA), with 3.175 mm diameter holes, which was attached to a vacuum source. The male mold consisted of a metal pin, 2 mm in diameter x 1.5 mm long. The polyvinyl film was placed on top of the female mold, and a vacuum (ca. 60 mm Hg) was pulled. The male mold was then pressed into the female mold, by hand, and removed. After the appropriate number of SPAEs were formed (70 in a 16cm² area), the vacuum was released and the polypropylene film was removed and placed in the two rings of the oviposition arena.

The SPAEs were then treated on the exterior surface with

one of the treatment solutions, at a rate of 0.04 ml/16 cm², or left untreated as a control. Treatment solutions were: 1) 0.001 gm/ml hexane extract of *H. zea* moth scales (Jones et al., 1973); 2) 10% aqueous solution of polyvinyl alcohol (Aldrich Chemical Co. Inc., Milwaukee, WI); 3) 10% aqueous solution of gelatin (Difco Laboratories, Detroit, MI); 4) 70% aqueous suspension of Elmer's School Glue; 5) 70% aqueous suspension of Elmer's School Glue + 0.001 gm/ml hexane extract of *H. zea* moth scales (V:V); 6) deionized water; 7) hexane, technical grade (Fisher Scientific).

The surface treatments were brushed onto the exterior of the SPAEs ca. 3 h prior to exposure to parasitoids. After surface treatment, the SPAEs were filled with an excess (10 ml/oviposition arena) of a 0.01% sodium bisulfite solution (Baker Chemical Co., Phillipsburg, NJ) (Wu et al. unpublished data) and any air bubbles that formed in the individual SPAEs were removed by using a syringe. The filled SPAEs were then exposed to *Trichogramma* (ca. 10 females/SPAЕ) for 24 h. The number of females was purposely low, to facilitate the counting of eggs. After exposure the number of SPAEs with eggs and the number of eggs in each SPAЕ were counted. Five classes, based on the number of eggs (0-10, 11-20, 21-30, 31-40, or >40 eggs) in each SPAЕ, were used to analyze the distribution of *Trichogramma* eggs in SPAEs. This experiment was replicated 3 times (1 replicate = 70 SPAEs).

Statistical analyses were conducted using analysis of variance (ANOVA) and Tukey's studentized range test using SYSTAT (Wilkinson et al. 1992).

RESULTS AND DISCUSSION

All treatments, except the water control, significantly increased the mean percentage of SPAEs with eggs, for both *T. pretiosum* (33.3 - 59.5%) and *T. minutum* (28.1 - 64.8%), compared with the untreated controls (16.2 and 14.8%, respectively (Table 1). Moth scale extract and School Glue + moth scale extract resulted in the highest percentage of SPAEs with eggs, but were not significantly different from each other.

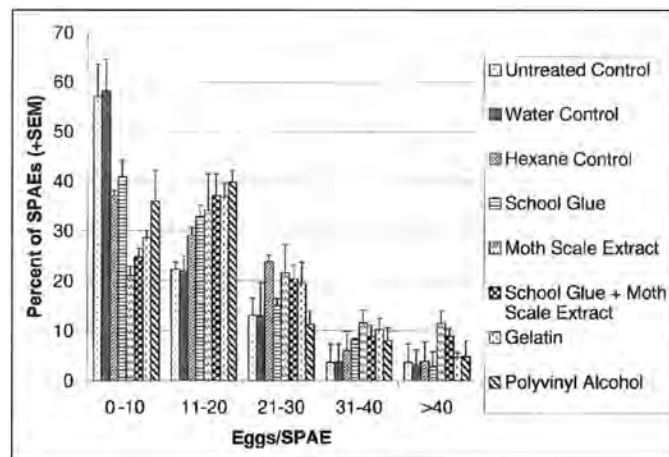


Fig. 3. Distribution of *Trichogramma minutum* eggs in stretched plastic artificial eggs, which were treated on the exterior surface with one of several materials with the potential to increase oviposition.

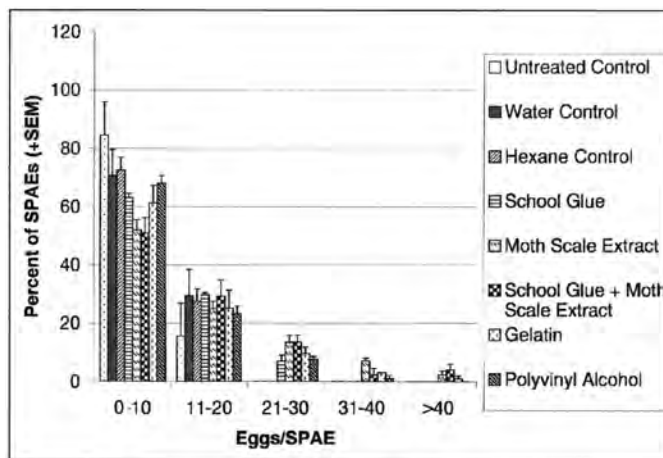


Fig. 2. Distribution of *Trichogramma pretiosum* eggs in stretched plastic artificial eggs, which were treated with one of several materials with the potential to increase oviposition.

With *T. pretiosum*, responses to School Glue, gelatin, polyvinyl alcohol, or hexane were not significantly different from each other, but were significantly higher than the response to either the water or untreated controls. However, with *T. minutum*, the percentage of SPAEs, which had been treated with gelatin, with eggs was significantly higher than that of the SPAEs treated with School Glue, polyvinyl alcohol, or hexane (Table 1). Interestingly, Guerra et al (1994) reported that n-hexane elicited oviposition behavior in *Catolaccus grandis* (Burks) females.

The treatments also resulted in a significant increase in the total number of *Trichogramma* eggs oviposited in an oviposition arena (Table 2). Moth scale extract resulted in a 6.5 fold increase in the number of eggs oviposited by female *T. pretiosum*, when compared to the untreated control and a 3.2, 6.4, 4.1, 3.4. and 2.5 fold increase when treated with School Glue, School Glue + moth scale extract, gelatin, or hexane, respectively. For *T. minutum*, the increases were 4.4, 2.2, 4.5, 3.3, 2.6, and 1.9 fold, respectively.

There were also apparent differences in the distribution of eggs in the SPAEs (Fig. 2 and 3). The untreated control had the highest percentage of SPAEs with 10 or fewer eggs than any of the other treatments (84.8 for *T. pretiosum* and 57.0 for *T. minutum*). Treatment with moth scale extract resulted in 23.0% and 44.7% SPAEs having 21 or more eggs for *T. pretiosum* or *T. minutum*, respectively. With *T. pretiosum*, only treatment with moth scale extract, School Glue + moth scale extract, or gelatin resulted in any SPAEs containing more than 40 eggs.

Identification of surface treatments for SPAEs that result in the maximum number of oviposition by *Trichogramma* spp. females is important to the development of a system for collecting eggs for use in an automated in vitro mass rearing system. Clearly, treatment of SPAEs with *H. zea* moth scale extract results in a greater increase in oviposition than any of the other treatments tested. Though the moth scale extract is not currently commercially available, *H. zea* moth scales (and a variety of other lepidopterous scales) can be obtained relatively easily from a variety of insect rearing facilities. The extract is relatively easy and inexpensive to prepare. Since moth scale extract is not viscous, it can easily be sprayed on a surface with a low pressure air brush, which would facilitate automated preparation of oviposition arenas in a mass rearing system.

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