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In Vitro Rearing of Parasitoids of Insect Pests in China

Li-ying Li

Guangdong Entomological Institute, Guangzhou, 510260, China

ABSTRACT Since 1975, the studies on *in vitro* rearing *Trichogramma* spp., *Anastatus japonicus* Ashmead, *Telenomus dendrolimusi* Chu, *Dibrachys cavus* Walker, *Habrobracon hebetor* (Say), *Bracon greeni* Ashmead have been conducted successfully in China. The simulated "host-eggs" are made of polyethylene or polypropylene semispherical capsules, containing artificial diets, in which insectan pupal hemolymph is the main component. Manufacture of simulated "host egg-cards" has been experimentally mechanized. The simulated "host larvae" are made of small rectangular parafilm or cotton-paper bags, containing artificial diets with insectan pupal hemolymph as the main component. Mass production of *in vitro* reared *Trichogramma* spp. and *Anastatus japonicus* and its utilization in the fields showed good effectiveness in controlling cotton bollworm, pine caterpillars, sugarcane borers and litchi stink bug.

KEY WORDS Insect rearing, *Trichogromma, Amastatus japonicus, Telenomus dendrolimusi, Dibrachys cavus, Habrobracon hebetor, Bracon greeni*

INTRODUCTION

As the main procedure in biological control of agricultural and forestrial insect pests, mass production of several species of parasitoids has been conducted in China. Their factitious hosts for mass production are still the eggs or larvae of insects. For instance, the eggs of *Antheraea pernyi* Guerin-Meneville, *Philosamia cynthia ricini* Donovan, *Corcyra cephalonica* (Stainton) are used for mass rearing *Trichogramma* spp., the first 2 hosts are also used for mass production of *Anastatus japonicus*. The larvae of *Pectinophora gossypiella* Saunders is the factitious host for mass producing *Dibrachys cavus* and *Bracon greeni*. The larvae of *Galleria mellonella* can be used also in mass producing *Habrobracon hebetor* or *Bracon greeni*. However, searching for more convenient and cheaper methods of parasitoids' mass rearing is still an important challenge in biological control. In China, great advances have been achieved in research on artificial diets of above mentioned parasitoids during the last 15years. Manufacture of the simulated "host eggs" and "larvae" for *in vitro* rearing these parasitoids is developed rapidly too. In this paper, artificial diets and simulated hosts of these parasitoids are described. The results of augmentative releases of *in vitro* reared *Trichogramma dendrolimi, T. confusum*, and *Anastatus japonicus* in the fields for controlling insect pests are showed.

ARTIFICIAL DIETS

1) For *Trichogramma* spp.

16 species of *Trichogramma* were tested : *T. dendrolimi, T. closterae, T. confusum, T. evanescens, T. ostriniae, T. japonicum, T. cordubensis, T. embryophagum, T. cacoeciae, T. nagarkattii, T. trjapitzini, T. maidis, T. nubilale, T. pretiosum, T. chilotraea,* and *T. neustadt.* The first 6 species are indigenous in China, the others were introduced from USSR, France, USA, Thailand and Germany. There is no specific requirement of nutrition for different species of *Trichogramma.*

a) Crude diet No.A, designed by the Guangdong Entomological Institute(GEI)

Pupal hemolymph of *Antheraea pernyi* or *Philosamia cynthia ricini*(heated in a water bath at 60℃ for 4 min.)……40%, Chicken yolk……20%, 10% malt solution……30%, Neisenheimer's salt solution……10%, Penicillin……400 units/ml. Streptomycin……400 units/ml.

b) Crude diet No.B, designed by the Wuhan Unversity

Pupal holotissues of *Antheraea pernyi*……30%, Hen yolk……14%, 10% skim milk solution……26 %, Distilled water……30%, Penicillin……500 units/ml. Canamycin 50 μg/ml.

In either the diet No. A or No. B, *Trichogramma* spp. developed normally, with more than 60% pupation and adult emergence. The development of *T. dendrolimi,* reared *in vitro* successively for many generation, is showed in Table 1.

Table 1. Development of *Trichogramma dendrolimi,* reared *in vitro* successively for 61 generations (Da Kai-jia etc. 1985)

No. generation	1-10	11-20	21-30	31-40	41-50	51-60
% parasitism	100	100	100	100	100	100
% adult emergence	89 ± 3	87± 8	86±10	82±14	82±18	46±23
no. adult/host-egg	60 ±27	55±12	55±11	59±20	60±17	33±13
Coefficient reproduction	9.6± 7	10± 3	10± 2	7± 3	10± 6	
% ♀ progeny	76 ± 7	81± 7	83± 6	77± 6	80± 7	83± 6
% adult with expanded wings	77 ±12	87± 7	90± 4	85± 4	86± 6	81± 6
% normal adult	81 ±15	92± 7	95± 4	95± 4	92± 5	90± 8
no. normal adult per host-egg	42 ±7.6	38± 9	41±13	40±19	40±20	12± 9

c) Diet No.C, designed by the Zoological Institute, Academy Sinica in cooperation with the GEI

A mixture of yeast hydrolysate, calf serum and Grace's solution(1:1:1)……60%, Chicken embryo extract……10%, Chicken yolk……15-20%, 10% skim milk solution……10-15%.

In diet No.C, that is a medium devoid of insectan additives, *T. dendrolimi, T. confusum* and *T. pretiosum* developed completely with 17-36% pupation and 1-2% adult emergence, but, more than 40 % of the adults had abnormal wings and developed weakly. Up to now, there is no significant advance in research on chemically defined medium of *Trichogramma.*

Table 2. Development of *Anastatus japonicus*, reared *in vitro* for 3 generations successively (Xin Jia-qi, Li Li-ying 1990)

No. generation	1	2	3
% parasitism	42 ± 6	40 ± 9	44 ± 2
% hatched larva	96 ± 5	94 ± 5	94 ± 4
% pupation	78 ± 5	83 ± 6	72 ± 3
% adult emergence	96 ± 3	91 ± 5	95 ± 2
% adult with expanded wings	99 ± 1	97 ± 3	99 ± 1
% ♀ progeny	82 ± 8	89 ± 3	90 ± 1

2) For *Anastatus japonicus*(Designed by GEI)

Pupal hemolymph of *Antheraea pernyi*……44.4%, 10% malt solution……22.2%, Chicken yolk……22.2%, Neisenheimer's salt solution……11.2%, Penicillin……400 units/ml. Streptomycin……400 units/ml.

A. japonicus developed in this diet quite well, sometimes better than in the eggs of *Antheraea pernyi*, with 40-44% parasitism, 72-83% pupation, 97-99% adult emergence and high percentage of females (more than 82%). The development of *A. japonicus*, reared *in vitro* for 3 generation successively, was showed in Table 2.

3) For *Telenomus dendrolimusi*(Cao Ai-hua, Gao Yi-guang etc. 1991)

Telenomus dendrolimusi completed its development from egg to adult in the diet containing pupal hemolymph of *Antheraea pernyi*, chicken yolk and 10% skim milk solution. The development of *T. dendrolimusi*, reared in this diet, was similar to that in the eggs of *Dendrolimus punctata*.

4) For *Dibrachys cavus*(Lu Wen-qing and Lang Suo, 1981)

Eggs of *Dibrachys cavus*, laid on the larvae of *Pectinophora gossypiella* and transferred on the simulated "larvae" containing 100% pupal hemolymph of *Antheraea pernyi*, developed to adults with 50% pupation and 60% adult emergence.

5)For *Habrobracon hebetor* and *Bracon greeni*(designed by GEI)

Eggs of both *Habrobracon hebetor* and *Bracon greeni*, laid on larvae of great wax moth and transferred to the simulated "larvae" containing 80% of pupal hemolymph of *Antheraea pernyi*, 10% of chicken yolk and 10% of skim milk solution, developed completely to adults. Their larval survival, percentage of pupation and adult emergence were 72% & 90%, 48% & 71%, 24% & 69%, respectively.

MANUFACTURE OF SIMULATED "HOST EGG-CARDS" AND "LARVAE"

The simulated "host eggs" are made of artificial chorion, capsuled with the artificial diets mentioned above. Based on the research on correlation of the variant ovipositor size and ovipositional behaviour of *Trichogramma* spp. and *Anastatus japonicus* with the *in vitro* rearing efficiency, Guangdong

Entomological Institute has selected some kinds of plastic film as material for making simulated chorion :

For *Trichogramma evanescens*, *T. ostriniae*, *T. maidis*, *T. nubilale*, *T. neustadt*, *T. pretiosum*, *T. chilotraea*, *T. embryophagum*, *T. cacoeciae*, *T. nagarkattii*, *T. japonicum* and *T. trjapitzini*……polyethylene film of 10-18㎛ thickness.

For *T. dendrolimi*, *T. confusum*, *T. cordubensis* and *T. closterae*……polyethylene film of 34-65㎛ thickness or polypropylene film of 32-36㎛ thickness. For *Anastatus japonicus*……polypropylene film of 32-36㎛ thickness.

There are 2 methods of making simulated "host egg-cards", designed by GEI, either for *Trichogramma* spp. or *Anastatus japonicus*:

a) Bag-form "host egg-cards"

33 or more semispherical convex capsules (2.5-3 mm diameter, distance between 2 capsules-5 mm) are made on half of a piece of plastic film(the size of film depends on the number of capsules), the medium is poured into these capsules, then the film with capsules is folded with the other half of the film without capsules. Between the two halves there is space to provide aeration for parasitoids development. Three sides of the "bag" are sealed by plastic sealer. An another "bag-form host egg-card", designed by Wuhan University, is similar in principle, but on the bottom film there are empty convex semispherical capsules opposite the plane between the dieted capsules on upper film in order to separate the upper and bottom films.

b) Tri-ring-form "host egg-cards"

2 pieces of plastic film are stretched tight separately by three hard plastic rings (the upper and bottom diameter of each ring are 5.5 cm & 5.8 cm, 5.4 cm & 5.6 cm, 5.2 cm & 5.4 cm, respectively). On the upper plastic film there are 21-29 semispherical convex capsules (2.2-3 mm diameter) containing 3-5 μ l. medium. Between the 2 pieces of film there is space for aeration.

In China, there are several experimental machines for making "host egg-cards" automatically. On the machine, designed by GEI and the Experimental Factory of Guangdong Academy of Sciences in 1985-1986, the process of making semispherical capsules, pouring medium into capsules and covering bottom film are conducted synchronously, operated by 1 person. In a minute it produces 14 pieces of egg-cards with 608 simulated eggs. Every day 6 million of Trichogrammatid wasps or 300 thousand of *Anastatus japonicus* can be produced. The percentage of parasitism, pupation and adult emergence of *Trichogramma* spp. and *Anastatus japonicus*, reared on the egg-cards, manufactured by this machine, were 72.4-100%, 55.7-94.3%, 28.6-70.4%, respectively for different species of parasitoids. Another assembly line with a series of machines for making simulated "host-eggs", for making egg-cards", for storaging reared parasitoids and their shipping was designed by Wuhan University. It can produce 6400 "egg-cards" or 30 million of Trichogrammatid wasps every day. The percentage of parasitism, adult emergence and females of *T. dendrolimi*, *T. confusum*, reared with the simulated "egg-cards", produced on such mechanized line, was 85-95%, 85-90% and more than 85%, respectively.

2) Simulated "larvae" for *Dibrachys cavus*, designed by Lu Wen-qing, Lang Suo, 1981. The eggs of *D. cavus*, laid on the larvae of *Pectinophora gossypiella*, transferred to the surface of a round bag-form simulated "larvae", made of cotton-paper film covered with a piece of plastic film, 100% of pupal hemolymph of *Antheraea pernyi* was injected into the space between these pieces of films, separated by 2 steel or rubber rings.

3) Simulated "larvae" for *Habrobracon hebetor* and *Bracon greeni*, designed by GEI. A piece of parafilm (1×1 cm) was expanded into 2×4 cm, sterilized in 75% alcohol for 15 min., dried and folded as a small rectangular bag, in which 0.5 ml. of medium was injected, then the opened end was sealed. Since the direct oviposition of parasitoids on the simulated "larvae" has not been successful, we have to make the parasitoids to lay their eggs on the larvae (last instar) of great wax moth, and transfer 6 of these eggs to the surface of each simulated larva. When the braconid larvae hatched, they suck the medium through the parafilm and complete development on the surface of the simulated "larvae".

RESULTS OF AUGMENTATIVE RELEASES OF *IN VITRO* REARED PARASITOIDS

Some field trials on the effectiveness of augmentative releases of *Trichogramma* spp. and *Anastatus japonicus*, reared *in vitro*, to control insect pests in the fields, showed good results(Table 3). During 1981-1990, *in vitro* reared *T. confusum* was released in 1,000 ha. of sugacane fields to control *Chilo sacchariphagus* and *Chilo infuscatellus*. Egg parasitism of the sugarcane borers reached 61% & 90% respectively, while in the control area it was only 5-6%. The effectiveness of *T. dendrolimi* and *Anastatus japonicus* in controlling cotton bollworm, pine caterpillar and litchi stink bug was better than the parasitoids, reared with the eggs of *Antheraea pernyi*.

Table 3. Controlling effect of parasitoids, reared *in vitro* (Dai Kai-jia etc. 1988; Han Shi-chou etc. 1990)

Parasitoid	Target pest	Treatment	no. wasp/ha.	% egg parasitism
Trichogramma dendrolimi	*Heliothis armigera*	Reared *in vitro*	150,000	92.3 ± 7.58
		Reared *in vivo**	150,000	88.6 ± 5.9
		CK	0	29.93±17.13
	Dendrolimus punctata	Reared *in vitro*	300,000	83.36± 7.78
		Reared *in vivo**	300,000	42.43±10.78
		CK	0	0
Trichogramma confusum	*Chilo sacchariphagus*	Reared *in vitro*	150,000	90. 4
		Reard in *vivo**	150,000	92. 6
		CK	0	6. 1
	Chilo infuscatellus	Reared *in vitro*	150,000	60. 9
		Reared *in vivo**	150,000	67. 2
		CK	0	5. 0
Anastatus japonicus	*Tessaratoma papillosa*	Reared *in vitro*	125,000	85.77
		Reard *in vivo**	125,000	75.00
		CK	0	32.66

* Reared with the eggs of *Antheraea pernyi*.

PROBLEM FOR STUDY IN THE NEAR FUTURE

1) Insect hemolymph is a very important component in artificial diets for *in vitro* rearing all of the parasitoids mentioned in this paper. Some compounds of the insect hemolymph seem unalternative for pupation and adult emergence. What are these compounds and their functions?

2) Contamination by microorganisms is the main problem in mass production of all these parasitoids with *in vitro* methods. It must be solved.

3) Stability of parasitazation of simulated "host eggs" by egg parasities should be improved. The direct oviposition of 3 species of ectoparasitoids on simulated "larvae" is the important problem for practical use of these ectoparasitoids, reared *in vitro*. Some practically available kairomones & ovipositional stimulants should be studied and requisite.

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