Increasing Production of *Trichogramma* by Substituting Artificial Diets for Factitious Host Eggs

S. M. Greenberg[1] and N. C. Leppla[2]

[1]USDA, ARS Beneficial Insect Research Unit, Weslaco, Texas 78596
and [2]University of Florida, Entomology and Nematology Department
Gainesville, Florida 32611

Presentation will be focused on propagation, quality control parasitoid *Trichogramma* spp. and predator *Chrysoperla* spp.

**Trichogramma* is the main biological control agent
- Attack more than 400 harmful insect species;
- Currently 11 million hectares of agricultural and forest land are treated with *Trichogramma* annually, in more than 40 countries;
- Experience in *Trichogramma* research accelerated the development of a new branch – industrial entomology, similar to research experience of *Drosophilla* , which promoted the development of genetics.

**Chrysoperla* spp.
key predator in many agricultural systems and ideal candidates for use in periodic release program.

Alternative Factitious Hosts

Host Species	Common name	Primary Countries
Sitotroga cerealella	Angoumois grain moth	Former USSR, E.Europe, North and South America
Ephestia kuehniella	Mediterranean flour fly	Western Europe
Corcyra cephalonica	Rice moth	China, Southeast Asia
Galleria mellonella	Greater wax moth	Research
Helicoverpa zea	Bollworm	Research
Manduca sexta	Tobacco Hornworm	Research
Samia cynthia ricini	Eri silkworm	China
Antheraea pernyi	Oak silkworm	China

For compare efficacy of different factitious hosts, we used the body length of *Trichogramma* spp. which is dependent of host egg sizes, on which parasitoid developed. The size of host eggs could be regarded as a criterion for host preference or acceptance.
We divided *Trichogramma* female body length (mm) from the frons to the tip of the abdomen of 4 categories of quality:
1 class >0.421;
2 class - 0.321 – 0.420;
3 class - 0.188 - 0.320;
Non-standard <0.187
Body length positive related with *Trichogramma* fecundity, emergence, sex ratio

Effect of Rearing Host on Quantity and Quality *Trichogramma*

Rearing Host	Size of Host Eggs, eggs/g	*Trichog-ramma*/ host egg	*Trichog-ramma* body length, mm	Prefer *Tri-chogramma* spp.
S. cerealella	50,086	1.0	0.189-0.275 Aver. 0.236	*T. evanescens, pintoi, maidis, pretiosum, minutum*
E. kuehniella	36,000	1.0-2.0	0.210-0.317 Aver. 0.279	*T. brassicae, evanescens*
G. mellonella	29,214	2.0	0.239-0.357 Aver. 0.319	*T. evanescens, pretiosum, minutum*
C. cephlonica	20,000	2.0-3.0	0.290-0.405 Aver.0.347	*T. ostriniae, chilonis, japonicum, evanescens*

Continuation

H. zea	11,212	2.0-4.0	0.310-0.425 Aver. 0.393	*T.evanescens, pretiosum, minutum, brassicae*
M. Sexta	759	4.5-8.9	0.400-0.561 Aver. 0.481	*T. evanescens, pretiosum, minutum*
Antheraea pernyi	1 cocoon=~10 gr, 1 gr contained 20 oak silkworm eggs	50.0-260.0, optimal 70.0	Optimal P:H ratio-4:1, > 80% progeny-females	*T. dendrolimi, chilonis, closteria*
Samia cynthia Ricini	1 cocoon= ~3 gr, 1 gr=50 eggs	27.0-60.0, Optimal 25.0	Optimal P:H- 2:1	*T.ostriniae, evamescens, preteosum, cacoeciae*

Influence of rearing host on the parasitization rate of *T. pretiosum* females in WAEs

Rearing host	% of WAEs parasitized	No. of parasitoid eggs per Female per WAE
M. sexta	83.3 ± 5.0a	19.6 ± 2.1a
H.zea	80.0 ± 4.7a	16.5 ± 2.9a
E. kuehniella	78.0 ± 5.4a	13.3 ± 1.6b
G. mellonella	76.7 ± 10.6a	9.4 ± 1.4b
S. Cerealella	49.5 ± 11.5b	6.5 ± 1.6b

Indices of Female *S. cerealella* Quality

Class	Size (mm)	Weight (mg)
1	≥ 7.1	≥ 8.4
2	6.5 – 7.1	6.8 – 8.4
3	5.9 - 6.4	5.2 – 6.7
Non-std	≤ 5.9	≤ 5.2

Correlates of Quality Indices

Class	Potential Fecundity	Mean Egg Volume (mm^3)
1	≥ 179	≥ 0.0279
2	142 - 179	0.0263
3	108 - 141	0.0231
Non std.	≤ 108	≤ 0.0217

Production *Trichogramma* on Different Factitious Hosts

Host	Production	Expenses
S. cerealella (in former USSR)	Increased from 4.5 to 9.4 g eggs S. c.\kg of barley kernels; 4-5 mln *Trichogramma* \day	Labor to produce 100,000 *S. cerealella* eggs ranged 0.14-0.27 man-h
S. cerealella (in the USA)	Increased from 6.0 to 12.0 g eggs S. c.\kg of wheat kernels; 5 mln *Trichogramma* \day	Labor required per production 100,000 *S. cerealella* eggs 0.239 man-h.

Continuation

Ephestia kuehniella on a mixture of wheat (40%) and corn (60%) flour	Increased from 3.1 to 7.6 g eggs E. k./kg of diet; about 3.5 mln *Trichogramma*/day	Reduced labor from 0.44 to 0.1 man-h / 100,000 E. k. eggs.
C. cephalonica on 90% wheat bran, 50% soybean, and 5% corn flour	Increased to 10 g eggs C.c..\kg of diet; 3.0 mln *Trichogramma* \day	
A. pernyi	A newly developed extractor can squeeze about 20,000 female/day and yield 120-130 kg of A.p eggs/ 280 mln *Trichogramma*/day	This device replaces more than 20 workers

Artificial Diet and Automated *in vitro* Rearing *Trichogramma*

*The Chinese first developed *in vitro* rearing of *Trichogramma* in commercial production.

*They used oligidic diets containing 27-50% of silkworm pupal haemolymph with chicken egg yolk, malt or milk, and Neisenheimer's salt mixture.

*They reared *T. dendrolimi*, *T. chilonis*, *T. cacoeciae*, *T. evanescens*, *T. ostriniae*, and *T. japonicum* by using wax-vaseline capsules and handling drops, later a plastic "chorion" from different thickness of polyethylene or polypropylene films.

*In China a computer controlled machine automatically completes all five egg production processes.
*The production capacity of the machine is 1,200 egg-cards/ hour, which can be used to produce 6-7 x 10^6 *Trichogramma*.
*About 5x10^6 *Trichogramma* can be reared on one liter of the oligidic diet (at $2.84/liter, diet cost is $0.06/100,000 adults).

*Biological Control units of USDA, ARS at Mississippi and Texas (Nordlund D. A., WuZ. X., Cohen A. C., and Greenberg S. M.) developed *in vitro* rearing of *T. minutum* and *T. pretiosum* for ten continuous generations with quality assessment comparisons of *in vitro* and *in vivo* reared adults.
**In vitro*-reared larvae required one day longer to reach the adult stage than did insects reared on *Helicoverpa zea* eggs.
*Adult longevity, number of eggs parasitized, and *Trichogramma* adult female body length was greater for insects reared *in vitro*.

Trichogramma spp.	Host	Host Egg/mm^3	*Trichogramma* /in host egg	*Trichogramma* length, mm
evanescens, minutum, pretiosum	WAE	5.4	6.5-19.6	0.430-0.588
evanescens, minutum, pretiosum	SPAE	3.8	3.8-7.4	0.421-0.496

*There was a higher percentage of deformed adults *in vitro* culture, generally insects with distended abdomens

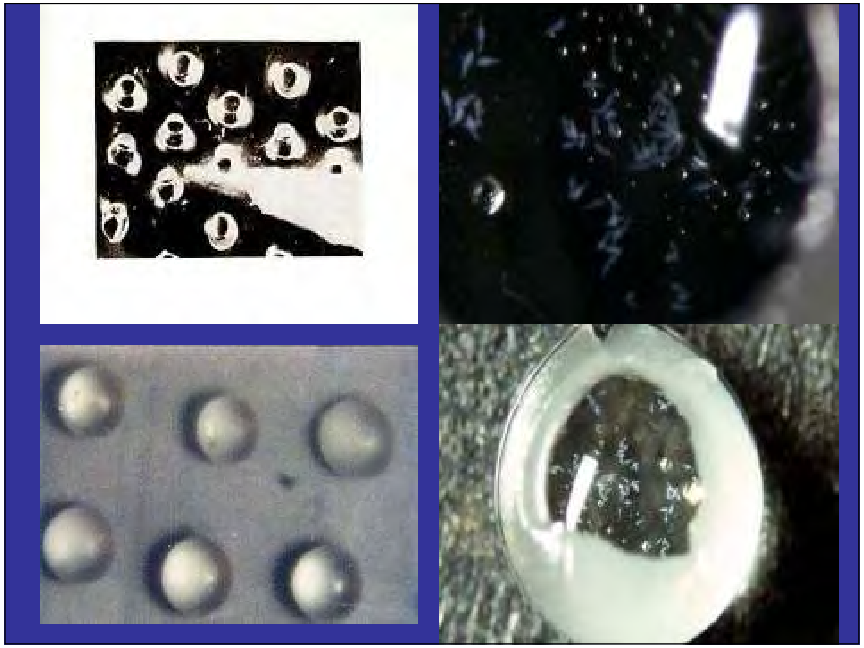
The diet used for *in vitro* rearing of *Trichogramma*

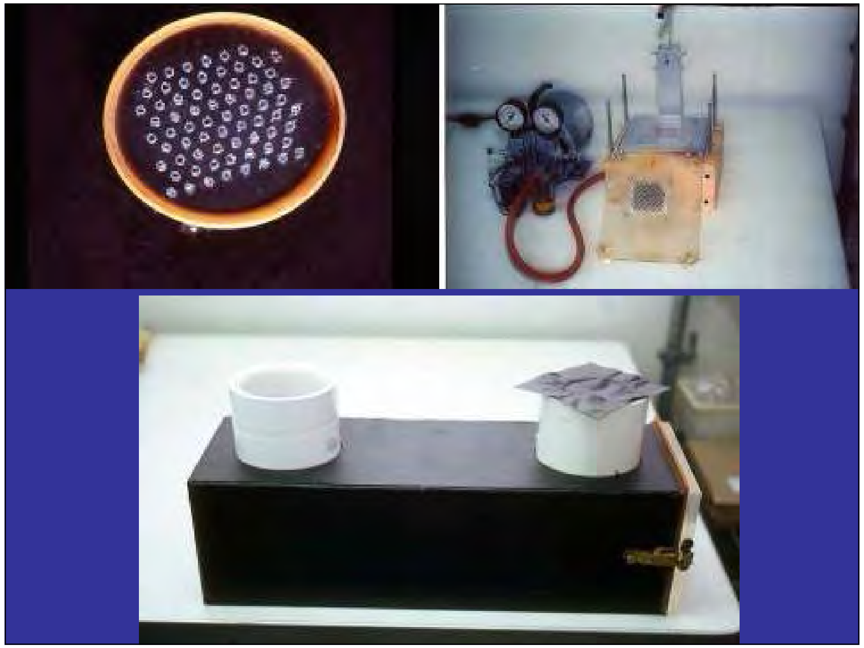
Component	Percentage
7% Yeast Extract solution	10.0
Free Amine III	5.0
10% suspension of nonfat dry milk	15.0
Chicken egg yolk	25.0
Chicken embryo extract	15.0
Manduca sexta egg liquid	30.0

We recognized that the diet used was not practical enough because the *Manduca sexta* egg juice component, and also concerned about the high percentage of deformed individuals in the *in vitro* culture. Improvements of our understanding of the feeding behavior of *Trichogramma* larvae showed that parasitoid larvae should be fed a semi-solid food, more concentrated nutritionally.

It has been difficult to obtain high rates of uniform oviposition in artificial diet filled artificial eggs.
Because *in vitro* rearing of *Trichogramma* spp. will require the ability to collect large quantities or parasitoid eggs. Wax-vaseline (WAEs) and Stretched plastic artificial eggs (SPAEs) have been used .
Eggs are removed from the SPAEs by filtering the oviposition solution and then mixed with the diet.

The solution of 5% FreAmine III, 30% chicken egg yolk, 20% TNM-FH Insect Medium, and 45% Rinaldini salt inside the SPAEs would stimulate oviposition and support about 70% of eggs hatch within 2 days of oviposition. *Trichogramma* eggs do not survive exposure to a simple FreAmine III solution for than a few hours.





Influence of different materials applied to exterior of SPAEs on arrestants and probing/oviposition by *Trichogramma*

Treatment	Percent of SPAEs with eggs		Mean numbers of eggs/ oviposition arena	
	T. pretiosum	*T. minutum*	*T. pretiosum*	*T. minutum*
Control	16.2c	14.8d	80.0c	200.0d
School glue	40.0b	34.8c	252.0b	439.0c
Moth scale ext.	58.1a	62.8a	518.0a	878.0a
Gelatin	45.2b	51.4b	330.0b	665.0b
Polyvinyl Alcohol	42.9b	41.9c	268.0b	523.0c
Hexane	33.3b	-	202.0b	-
Water	15.7 c	15.3d	93.0c	163.0d

Oviposition in SPAEs by *T. minutum* Females Reared on Different Hosts

Hosts	SPAEs containing eggs (%)	*Trichogramma* eggs/ oviposition arena (70 SPAEs per 16 cm^2 area)
Sitotroga cerealella	30.9c	380.3c
Helicoverpa zea	76.2 b	1,383.7b
WAEs	89.3a	2,051.0a

96-well tissue culture plates (Falcon 3070 Microtest III) was used for *in vitro* rearing parasitoids, that are similar to the 32 cell larval rearing trays used for rearing lepidopterous insects. This indicates a potential for producing cells that can serve as both the rearing container and release capsule.

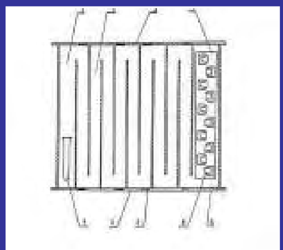
Assuming that we can use similar sized rearing cell for *Trichogramma* as for Lepidopterous insects with existed machine, which is reasonable, 6 h production run per day, a 7 day per week operation, and 4,000 *Trichogramma* per cell, the weekly production capacity for this machine will be 4.2 billion per week.

The main indirect ways what increase numbers of production *Trichogramma*:
* Inducing diapause in *Trichogramma* spp. that was instrumental for developing short- and long-term cold storage strategies.
* Establishing a culture of *Trichogramma* for mass rearing by selecting species, adapted to regional conditions and pests.
* The genetic consequences of mass rearing on the production culture must also be considered.
* Optimizing the packaging and shipping of *Trichogramma* related with strategies and equipments for it releases.
* Improving the quality of mass-reared *Trichogramma* and methods it assessment.

Quality Control Characteristics for Stock and Marketable *Trichogramma*

Characteristic	Stock culture	Marketable product
Appearance and color	Black, tinged with blue	
Parasitized eggs/g	>60,000	>80,000
Parasitism	60%	80%
Emergence	80%	90%
Females	65%	50%
Fecundity, eggs/female	35.0	20.0
Deformed individuals	<3%	<5%
Searching ability, % parasitized eggs/female/24 h	≥ 45	≥ 30

Device for evaluation Integral Index Quality of Trichogramma



1. Section for release parasitoid
2. Cover
3. Twisting canal
4. Vial with Trichogramma
5. Ventilation window
6. Section for parasitism
7. Tray
8. Cards with host eggs

Integral Index of Quality (IIQ)
IIQ is ≥ 50.1% is estimated as a High
IIQ is 30.1-50.0 % - Medium
IIQ is ≤30% - Low

Chrysoperla rufilabris all instars was reared on *Sitotroga cerealella* eggs and 3 artificial diet (Hassan and Hagen, 1978; Vanderzant, 1973; and Nepomnyashchaya et a., 1979). The predator all biological characteristics were significantly higher when it reared on *S. cerealella* eggs. While the cost of eggs necessary to feed 1,000 *C. rufilabris* larvae was over 17-times the cost of the diet ($10.31 vs $0.00655). For presentation artificial diet to *Chrysoperla* were tested: capillary tubes, 6-8 mm long, with 250-280 µm inside diameter; small cubes (ca 0.75 cm3) of cellulose sponge saturated with diet; Agarose based jelly, and WAX artificial eggs. More technologically were capillary tubes and Agarose based jelly.

Relation of *Ch. carnea* Pupal Weight To Biological Parameters*

Class	Pupal WT, mg	Emergence, (%)	Females, %
1	3.1-5.5	17.5	28.5
2	5.6-7.0	52.0	48.0
3	7.1-9.5	77.2	67.2
4	9.6-12.0	85.9	81.7
5	>12.1	100.0	97.2

*All-Russian Institute of Plant Protection, St. Petersburg
SARC-ARS-USDA, Weslaco Texas

Suggestions for discussion:

1. Status of Augmentation Biological Control- I don't want to repeat about usefulness of biocontrol against insects, diseases, and weeds. Everybody in this room knows it very well. By having an effective mass-rearing program the augmentative biological control must be recognized as an important alternative to chemical control. But today augmentative biological control is applied on only 16,000,000 ha worldwide, 0.046% land under culture, while 150 species of natural enemies (parasitoids, predators, and pathogens) are currently commercially available for augmentative form of biocontrol (van Lenteren et al., 2006). From 16.0 mln ha of augmentative biocontrol, 11.0 mln ha fit of *Trichogramma* and current area of *Trichogramma* use is 3-times lower than it was at the end of the 20-th century. But this valuable parasitoid has much more potential resources for use.

2. Barriers to Implementation-The main reasons why application of *Trichogramma* in practical goals were limited: many successful scientific developments were not introduced to farmers and producers, from one side, and some scientific studies with *Trichogramma* were closed and didn't finished to logical end, from other side. The farmers lost interest in *Trichogramma* in the last 10 years. They don't want to taking risk with *Trichogramma*, have problems with parasitoid variability in quality, with storage, transportation, and releases. Farmers will rather pay more expensive, and be sure that their production is secure.

3. Analysis of Opportunities to Increase Augmentation Biological Control-

a. Create a one year International team of scientists, practical workers different specialties which will analyze situation with *Trichogramma* and other augmentative form of biological control program.

b. Found if they can compete with synthetic chemical, biopesticides, their place and role in IPM systems; rational volume of production and application in the nearest future; what should be done for technology transfer; what studies need to be finished, continued, and original studies need be started; how long does it need to continue; who (independently from countries) can be involved in the studies; how much will cost for this project.

c. And next year these two papers should be on the table for discussion. Financing this project can be from International organization, for example FAO. We need to fight for biocontrol, *Trichogramma*, one of the main component of augmentative biocontrol, and it's mean we will fight for clean environment, safety humanity, social effect.

4. Coordination of Natural Enemy Production-

4a. Improve mass rearing beneficials, including the main biological control agent, *Trichogramma*. Now in North America, we have 130 suppliers, which sell 100 biocontrol agents, among them *Trichogramma* suppliers are in Canada – 8, Mexico – 22, and USA – 48. They all are working without coordinator of distribution their products and methodological help for propagation, which make augmentative form of biological control in practical use significantly lower than they potentially could be.

4b. For improving those it needs to be organized Scientifically-Production Association [SPA] (International, for example: SPA for North America [Canada, Mexico, and USA], or incite separate countries) which are doing studies (improving, completion current, or start new) needed for serve zone; assessment on their experimental field biological control technologies before they will be transferred to practice; produce experimental equipment for propagation beneficial and releases and non-standard equipment on their experimental plants; produce stock culture of beneficial on their experimental production laboratories; organize training and education farmers, methodological control of producers.

4c. Mass propagation of wide-use beneficial, for example, *Trichogramma* should be, in our opinion, on analogy with seed Companies that produce seed elite (superior stock) and provide with them farmers for mass growing crops. Big biological control Companies will select specific and intraspecific form of species, establish the initial culture by collecting insects from the field (F1 and F2 progeny), introduce it in laboratory on natural host eggs when qualitative indices are stabilized close to those with wild population.

4d. Stock cultures need be provided to small laboratories which will rear *Trichogramma* on factitious hosts and sell it to farmers. Each batch need to be followed by passport included quality indices and recommendation for application.