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## *In vitro* rearing of *Eucelatoria bryani* Sab. (Diptera Tachinidae) on tissue culture-based diets. (\*)<sup>(1)</sup>

### INTRODUCTION

*Eucelatoria bryani* Sab. is one of the most promising parasitoids for mass-rearing on artificial diet (Nettles, 1986). The rearing system results in adult yields around 50%, with 2315 flies for a one-liter diet (Bratti and Nettles, 1992), although the flies developed on artificial diet are smaller and less viable than those produced *in vivo*. Several attempts have been made to improve adult yield and quality. While many nutrient supplements have been tested with the Nettles diet (1986), the only significant results have been found with the addition of fresh egg yolk (1.6%) to a meridic diet, which increased the average adult yield (Nettles unpublished data).

Diets with insect material have been used successfully for rearing tachinid larval stages (Bratti and Campadelli, 1993) and tissue culture media has proved an effective ingredient for many parasitoid diets (Nettles, 1990). *E. bryani* grows well on diets without insect components, although when reared on a *Helicoverpa zea* Boddie pupal homogenate it registered adult yields similar to those achieved on a meridic diet and a 29% higher puparia weight (Bratti and Nettles, 1994).

*Galleria mellonella* L. pupal extract has proved very effective when mixed with other ingredients for *Exorista larvarum* (L.) (Bratti and Coulibaly, 1994; Bratti and Campadelli, 1993; Mellini *et al.*, 1993).

The present investigation tested sample tissue culture-based diets with and without *G. mellonella* pupal extract in order to improve the fly size of *in vitro*-reared *E. bryani*.

### MATERIALS AND METHODS

**Insect colonies.** *G. mellonella* was used as the factitious host for *E. bryani*. The Greater Wax Moth was reared according to the method of Campadelli (1973) and the adult parasitoids were reared after Coulibaly and Fanti (1991). Last-instar larvae of *G. mellonella* were superparasitized by exposing 10-15 insects

(\*) Accepted for publication January 15, 1994.

(<sup>1</sup>) Research supported by C.N.R, Bilateral Project Italy-USA N° 93.00174.CT06.

for 5-6 h to about 50 parasitoids and, after developing inside the host larva for about 24 h at 27-28°C, the *E. bryani* maggots were removed by dissection and placed on the diets using a method developed by Bratti and Nettles (1992).

### *In vitro* experiments

**Diets without insect component.** The three diets were based on the following tissue culture media (TCM): TNM-FH (Sigma Chemical Co., St. Louis, MO, USA); TC-100 (Sigma Chemical Co., St. Louis, MO, USA) modified with 5% Stock 'P'<sup>1</sup>, 0.2% vitamins<sup>1</sup>, 0.1% trace minerals<sup>1</sup>, 0.067% iron solution<sup>1</sup>, 0.32% glycerol (50% vol/vol in water, autoclaved) and 1% NuSerum (N° 50.000, Collaborative Research, Inc., Bedford, MA); IPL-52B (Hazelton Scientific Prod., Inc. Lenexa, KS) modified with 5% Stock 'P.' The techniques for preparing the modified TC-100 and IPL-52B are reported by Lynn (1989). Each TCM was supplemented with 10% fetal bovine serum (FBS) (Sigma Chemical Co, St. Louis, MO, USA), 2.6% soy flour, 1.6% egg yolk, 5% lipids (composition as Nettles *et al.*, 1980, diet) 1.5% agar, and a 0.01% antibiotic solution of gentamicin sulphate.

To a 50-ml flask containing 0.8 g egg yolk, 1.3 g soy flour and 0.75 g agar were added 38.65 ml TCM and 3.5 ml FBS, supplemented with 5 ml lipids. After adjusting the pH to about 6.75 (with KOH 6N and HCl 1N), the diets were boiled and autoclaved for 10 min at 120°C and transferred using a 5-ml syringe to Nunclon 96 Multiwell microplates. Three diets were compared and three replications performed: each cell contained one maggot for a total of 25-30 individuals per replication.

**Insect-component diets.** Four TCM were employed in the diets: TNM-FH, TC 100 modified, IPL-52 B modified and SCHNEIDER'S (Sigma Chemical Co., St. Louis, MO, USA). All the diets had a volume composition of 53.5% TCM, 6% FBS, 15% *G. mellonella* pupal extract (GMPE) prepared according to the method of Bratti (1990), 1.5% egg yolk, 25% agar-water suspension (6% agar), for a 1.5% final diet concentration, and 0.01% gentamicin sulphate. A 15-ml batch of each diet was prepared and dispensed according to the methods of Bratti and Coulibaly (1994). Four diets were compared in three replications: each cell contained about 1 ml diet and two maggots, for a total of 30 individuals.

### *In vivo* experiments

The parasitizing ability of the diet-reared adults was tested by placing the newly emerged flies (males and females) in a plexiglass cylinder (160 mm diam, 170 mm ht) at 27°C and 70% rh. Two last-instar *G. mellonella* larvae per *E. bryani* female (emerged 9 days earlier) were exposed for parasitization. The host lar-

(<sup>1</sup>) Composition of:

a) Stock 'P': 2.5 g phytone peptone (N° 11906 BBL, Cockeysville, MD), 1.5 g liver digest (N° L. 27, Oxoid USA, Columbia, MD), 100 ml water.

b) Iron solution: 82.3 FeSO<sub>4</sub>·H<sub>2</sub>O, 53.2 mg aspartic acid, 100 ml water.

c) Vitamin Stock: 4.8 mg thiamine-HCl, 4.8 mg riboflavin, 4.8 mg calcium pantothenate, 12 mg pyridoxine HCl, 9.6 mg para-amino benzoic acid, 4.8 mg niacin, 4.8 mg biotin, 60 ml water.

d) Trace minerals: 4.0 mg ZnCl<sub>2</sub>, 2.0 mg MnCl<sub>2</sub>·4H<sub>2</sub>O, 19.5 mg CuCl<sub>2</sub>·2H<sub>2</sub>O, 100 ml water.

vae were removed after about 20 min and placed in Petri dishes supplied with abundant food until fly puparia formed. This operation was repeated every two days in a week.

In all *in vitro* experiments maggots were kept at 27°C and 90% rh. Calculations of percentage yields of third instars, puparia and adults were based on the number of maggots alive 24 h after transfer to artificial medium (base=100%). Puparia eclosion percentage (development of puparia to adults) was based on the number of puparia. Puparia weights were determined 24 h after formation. Newly formed puparia were placed in vials until adult emergence.

Materials such as instruments and glassware were sterilized by autoclaving for 15 min at 121°C. All *in vitro* rearing operations were performed under laminar flow hood. The data were analyzed by ANOVA (CSS: STATISTICA, 1991) and the percentage values transformed using arcsin transformation (Mosteller and Youtz Tables, 1961). When F test was significant, Tukey's HSD multiple comparison test was used, and when there was no homogeneity of variance, the data were analyzed after Kruskal-Wallis's non-parametric procedure (CSS: STATISTICA, 1991).

## RESULTS AND DISCUSSION

### *In vitro* experiments

**Diets without insect component.** Diet sterilization by autoclaving definitely altered their chemical and physical characteristics, and can explain the poor viability and yield of adults in all three tested diets. Note too that most of the reared maggots evinced a brownish colouring of the malpighian tubes, a symptom of nutritional imbalance. Nevertheless, the data in table 1 clearly indicate that the modified IPL-52B diet produced the highest adult yield (about 23 %), puparia eclosion (about 60%) and puparia weights. One of the most significant differences between the tested diets is the free-aa concentration, which was highest in IPL-52B for all except histidin and glycin. The importance of the free amino acids for (the deve-

Tab. 1 - *In vitro* rearing of *Eucelatoria bryani* on tissue culture based-diets without insect component.

Diet (*)	Percentages				
	L3 Yield (**)	Puparia yield (**)	Adult yield (**)	Puparia eclosion (**)	Puparia weight mg +
TC-100 M	44.1 ± 13.6 a	17.0 ± 6.8 a	0.0 ± 0.0 a	0.0 ± 0.0 a	5.5 ± 0.2 a
IPL-52B M	57.3 ± 1.3 a	37.1 ± 4.1 a	22.6 ± 5.6 b	59.3 ± 9.3 b	8.8 ± 0.2 b
TNM-FH	62.7 ± 1.9 a	31.7 ± 7.0 a	1.3 ± 1.3 a	4.8 ± 4.8 a	6.5 ± 0.4 a
df =	2,8	2,8	2,8	2,8	N=76
F =	1.4	2.7	23.1	18.3	H=31.7
P =	0.31	0.14	0.01	0.03	<0.01

(\*) Diet composition: 77.3 % Tissue culture medium, 7% FBS, 10% lipids , 1.5% agar , 1.6% egg yolk, 2.6% soy flour, 0.01% gentamicin sulphate.

Means in a column followed by the same letters are not significantly different.

(\*\*) ANOVA, P <0.05, Tukey HSD multiple range test). (+ Kruskal-Wallis's non parametric procedure).

lopment of *E. bryani* was pointed out by Nettles (1986; 1987), who found that without them the larvae did not develop beyond third instar even with an abundant supply of proteins. This species, like *Phryxe caudata* Rond. (Grenier *et al.*, 1986), exhibits a low endopeptidasic activity and hence finds it difficult to digest proteins. A comparison of these results with a modified IPL-52 B and those with the Nettles (1986) diet, which had a far higher amount of free amino acids, shows the latter as more effective, especially in terms of adult yield. TC-100 M, while nutritionally much richer than TNM-FH, did not register better adult yield and puparia weight results than the latter. None of the tested diets showed significant differences as to third-instar and puparia yields.

**Diets with insect component.** The *E. bryani* development parameters averaged much higher scores for diets with as opposed to those without GMPE. Adults yields, which ranged from 21 to 27 %, showed no significant differences between diets (Tab. 2). The same pattern also held for the remaining biological parameters. TNM-FH diet showed the highest adult yield (about 80% puparia eclosion) and average puparia weight (16.5 mg). Because of the GMPE supplement, the notable difference in chemical composition of the three diets did not affect amount and viability of adult yield. GMPE has proved to be an important ingredient, even at low concentrations, for the development of *Exorista larvarum* (Bratti and Coulibaly, 1994; Mellini *et al.*, 1993) and of the first instar of *Pseudogonia rufifrons* Wied. (Bratti, 1990). Yet, while the adult yield is lower with the diets tested in the present study than with the Nettles (1986) and the Bratti and Nettles (1992) diets, average puparia weight proved to be higher with the present ones than with any of the diets tested to date for this tachinid.

Tab. 2 - *In vitro* rearing of *Eucelatoria bryani* on tissue culture based-diets with insect component.

Diet(*)	Percentages				
	L3 Yield	Puparia yield	Adult yield	Puparia eclosion	Puparia weight mg
TC-100 M	43.3 ± 3.1	33.2 ± 5.6	21.4 ± 5.4	62.6 ± 8.1	14.2 ± 0.7
IPL-52B M	48.6 ± 12.1	39.3 ± 7.3	23.8 ± 3.3	62.9 ± 8.9	15.8 ± 0.8
SCHNEIDER'S	63.0 ± 6.8	50.4 ± 4.9	25.0 ± 4.5	48.8 ± 5.2	16.5 ± 0.6
TNM-FH	61.9 ± 13.5	39.4 ± 5.3	27.3 ± 1.8	78.8 ± 11.3	16.5 ± 0.6
df =	3,11	3,11	3,11	3,11	3,141
F =	1.05	1.5	0.42	1.9	1.6
P =	0.42	0.29	0.74	0.21	0.19

(\*) Diet composition: 52.5% Tissue culture medium, 6% FBS, 25% agar-water, 15% GMPE, 1.5% egg yolk, 0.01% gentamicin sulphate

### *In vivo* experiments

This study focused exclusively on adults reared with GMPE-supplemented diets. These adults were capable of mating, parasitizing the host larvae and then producing viable adults. Yet, because the data set is too limited for reliable statistical analysis, the data reported in table 3 are merely indicative. The individuals raised on the TNM-FH and SCHNEIDER'S diets appear to be more effective in parasitizing the host as they produced higher adult yields from the host larvae (Tab. 3).

Tab. 3 - Capability of parasitization of *E. bryani* adults developed on diets with insect component.

Diet(*)	N° of host larvae used for parasitization	N° of parasitized host larvae	Percentage of parasitization	N° of <i>E. bryani</i> adults	<i>E. bryani</i> adults developed from host (mean)(**)
Adults from:					
TC-100 M	38	23	60.5%	40	1.05
IPL-52B M	40	27	67.5%	66	1.65
TNM-FH	44	31	70.5%	77	1.75
SCHNEIDER'S	28	21	75%	52	1.86

(\*) Diet composition: 52.5% Tissue culture medium, 6% FBS, 25% agar-water , 15% GMPE, 1.5% egg yolk, 0.01% gentamicin sulphate.

(\*\*) Ratio between the *E. bryani* adults formed and host larvae used in the experiment.

The tissue culture media, even when supplemented with vitamins, minerals and proteins, proved to be effective only with the addition of host component, their low concentration of free amino acids being a possible cause of this drawback. The amino-acid concentration increases with the addition of GMPE, even when added in low amounts (15%). The attaining of puparia weights averaging around 16-17 mg, 50% and 14% than those found respectively with the Bratti and Nettles (1992) diet and that of *H. zea* pupal homogenate (Bratti and Nettles, 1994), strongly suggests that the size of adults reared *in vitro* can be further enhanced.

Key words: *In vitro* rearing, tachinids, tissue culture media, parasitoids.

#### Acknowledgements

The authors wishes to thank Dr W.C.Nettles Jr of Biological Control of Pests Research Unit, Weslaco Tx, who supplied *Eucelatoria bryani* colonies and Dr Nina Barcenas of Centro de Genética, Chapingo Mexico for supplying part of the tissue culture media.

#### SUMMARY

*Eucelatoria bryani* Sab. was reared on tissue culture based-diets with and without *G. mellonella* pupal extract in order to improve the adult yield and fly size of *in vitro*-reared parasitoids.

The tissue culture media, even when supplemented with vitamins, minerals and proteins, proved to be effective only with the addition of host component, their low concentration of free amino acids being a possible cause of this drawback. The amino-acid concentration increases with the addition of *G. mellonella* extract, even when added in low amounts (15%). While the adult yields were lower compared to Nettles diet (1986) the puparia weights (averaging around 16-17 mg) were the highest never achieved on artificial diets. This finding strongly suggests that the size of adults reared *in vitro* can be further enhanced.

The adults reared on host component supplemented diet were capable of mating, parasitizing the host larvae and then producing viable adults.

Allevamento *in vitro* di *Eucelatoria bryani* Sab. (Dipt.Tachinidae) su diete a base di substrati per le colture cellulari.

#### RIASSUNTO

*Eucelatoria bryani* Sab. è stata allevata su diete a base di substrati per le colture cellulari di insetti con e senza estratto di pupe di *Galleria mellonella* L. Lo scopo della ricerca era di migliorare le rese in adulti e, soprattutto, le dimensioni degli individui prodotti artificialmente.

I substrati per le colture cellulari, anche se arricchiti con vitamine, minerali e proteine sono efficaci solo se contengono materiale proveniente dall'ospite. La bassa concentrazione in aminoacidi liberi di tali substrati potrebbe essere la ragione della loro scarsa resa. Aggiungendo, anche se in basse percentuali (15%) l'estratto di crisalidi di *G.mellonella*, la concentrazione di aminoacidi liberi aumenta. Dalle diete contenenti materiale proveniente dall'ospite si sono ottenute rese in adulti inferiori a quelle derivate dalla dieta di Nettles (1986), ma pesi dei pupari più elevati (16-17 mg). Il significativo incremento ponderale dei pupari fa ben sperare circa la possibilità di migliorare ulteriormente le dimensioni dei parassitoidi allevati *in vitro*.

Gli adulti provenienti dalle diete, arricchite con materiale dell'ospite, sono in grado di parassitizzare l'ospite di sostituzione e di dare origine ad una nuova generazione.

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