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Comparison of Insect-material in a Meridic Diet for *Exorista larvarum* L. (Dipt. Tachinidae) *In Vitro* Rearing (*)([1])

Introduction

The presence of host materials in artificial diets often strikingly improves the yield and quality of many parasitoids reared *in vitro*. Poor rearing results recorded in diets without insect components is strong evidence, but not proof, that at least some species of parasitoids are dependent on host chemicals (Nettles, 1990). Diet with host-component have been tested on several tachinids, the varying extent of their success depending on species and amount of insect-material in the diet (Tab. 1). *Exorista larvarum* L., a parasitoid of *Lymantria dispar* L., *Hyphantria cunea* Drury and other Lepidoptera (Herting, 1960), grows well on diets containing a *Galleria mellonella* L. pupal extract (Bratti and Coulibaly,1993; Mellini *et al.*, 1993b) and homogenate (Mellini *et al.*, 1993 c). The *G.mellonella* pupal homogenate has shown a marked effectiveness at low concentrations (5 and 2.5%) and, at least for the tested diets, proved indispensable for development *in vitro* of *E. larvarum* (Bratti and Coulibaly, 1993; Mellini *et al.*, 1993 c).

The results obtained using low amount of an insect largely available as the Greater Wax Moth, may allow to prepare economic diets for mass rearing this tachinid. But, as reported by Nettles (1990), the identification and synthesis of certain low-molecular-weight host chemicals present in hemolymph or in other host parts to add to the diet is expected to result in a more economical means of *in vitro* rearing of certain species of parasitoids. The first step is thus to discover the insect component that, when added to the other diet ingredients, promotes the best growth and development of *E. larvarum* and then to identify and incorporate into the diet the active chemicals contained in this material. The present study reports and discusses a comparison four sources of insect material in a meridic diet to determine the most effective one for the development *in vitro* of *E. larvarum*.

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Tab 1. Tachinids reared on diets with insect components

Species	Stage Reared	Diet Ingredients	Results	References
Pseudogonia rufifrons Wied.	First Instar	*G. mellonella* Pupal Homogenate, Dextran, Grace's medium	Slight Growth	Baronio and Sehnal,1980
	First Instar	*G. mellonella* Pupal Homogenate, Agar and Gentamicin	2.5% Adult Yields	Bratti and Monti,1988
	First Instar	*G. mellonella* Pupal Extract and Gentamicin	90.3% Second Instar	Bratti, 1990
	First Instar	*G. mellonella* Pupal Extract, Nettles Diet and Agar	23.3% Puparia Yields	Bratti and Benini,1991
	First Instar	*G. mellonella* Pupal Extract, Threlaose, Chicken egg yolk, Bovine Serum, Gentamicin and Agar	15% Puparia Yields	Mellini *et al.*,1993b
	Second Instar	*G. mellonella* Pupal Homogenate, Agar and Gentamicin	1.5% Adult Yields	Bratti and Monti,1988
Archytas marmoratus (Tns.)	First Instar	*H. zea* Pupal and Larval Hemolymph, Cholesterol	Slight Growth	Bratti,1993
	Second Instar	*H. zea* Pupal Homogenate, Nettles Diet	40% Puparia Yields	Bratti,1993
Lydella * *thompsoni* Hert.	First Instar	*Ostrinia nubilalis* Pupal Extract and Cotton	No Growth	Bratti, 1993
Palexorista * *laxa* (Curran)	First Instar	*M. sexta* Larval Hemolymph	10% Puparia Yields	Bratti and Nettles,1988
	First Instar	*M. sexta* Larval Hemolymph, Soy residue	13.1% Adult Yields	Bratti and Nettles, 1988
	First Instar	*H. zea* Pupal Homogenate, Agar and Gentamicin	80% Adult Yields	Bratti and Nettles (un.)
Eucelatoria * *bryani* Sab.	First Instar	*H. zea* Pupal Homogenate Agar and Gentamicin	51.1% Adult Yields	Bratti and Nettles (un.)
	First Instar	TNM-FH, Fetal Bovine Serum, *H. zea* Pupal Extract Chicken Egg Yolk, Agar and Gentamicin	64.4% Adult Yields	Bratti and Nettles (un.)
	First Instar	TNM-FH, Fetal Bovine Serum, *G. mellonella* Pupal Extract, Chicken Egg Yolk, Agar and Gentamicin	66.7% Adult Yields	Bratti and Nettles (un.)
Exorista * *larvarum* L.	Egg	Sf-900, Fetal Bovine Serum, *G. mellonella* Pupal Extract, Chicken Egg yolk, Agar and Gentamicin	55.6% Adult Yields	Bratti and Coulibaly,1993
	Egg	SCHNEIDER'S, Fetal Bovine Serum, *G.mellonella* Pupal Extract,Chicken Egg yolk, Agar and Gentamicin	51.8% Adult Yields	Bratti and Coulibaly,1993
	Egg	Bovine Serum, *G. mellonella* Pupal Extract or Homogenate Agar and Gentamicin	62% Adult Yields	Mellini *et al.*, 1993c

* Species reared to the adult stage also on diets without insect components.

Material and methods

Insect colonies. *G. mellonella* was used as the factitious host for *E. larvarum*. *G. mellonella* larvae were reared on a semi-artificial diet (Campadelli, 1973) and stored at 30°C, r.h. 70% in complete darkness. The adult parasitoids were reared by a method developed by Coulibaly (unp.) at 27°C, r.h. 75% and a 16:8 photoperiod. The fly colony was initiated from collections of *L. dispar* and *H. cunea* larvae and pupae.

Diet composition. All four diets had a volume composition of 35.25% Schneider's tissue culture media (TCM) (Sigma Chemical Co., St. Louis, MO, USA), 35.25% bovine serum (BS) (Sigma Chemical Co.,St. Louis, MO, USA), 2% chicken egg yolk (CEY), 2.5 % insect material (IM), 25% agar-water suspension (6% agar, for a 1.5% final agar concentration) and 0.01% of gentamicin sulphate (Sigma Chemical Co., St. Louis, MO, USA). All of these materials except CEY were sterilized: the agar suspension by autoclaving for 10 min at 120°C and 1 bar and the other ingredients by filtering. The IMs were *G. mellonella* and *Hyphantria cunea* pupal extract (GMPE and HCPE), prepared after Bratti (1990), and *Manduca sexta* L. larval (MSLH) and *Antheraea perny* Suer. pupal hemolymph (APPH), prepared after Xie *et al.*, (1986). The introduction of BS depend on the good results achieved using this ingredient in the diets for *Pseudogonia rufifrons* Wied. and *E. larvarum* (Mellini *et al.*, 1993a, b)

To 0.3 ml fresh CEY that were dissolved in a 25-ml sterile beaker with 0.375 ml IM were added and mixed, using a sterile pipette, 5.28 ml BS and 5.28 ml Schneider's TCM. The beaker was then covered with a piece of aluminum foil and put in a water-bath at 50°C for about 15 min. The agar suspension was prepared and mixed with the other diet ingredients after Bratti and Coulibaly (1993). All the diets were placed in a Multiwell 24 Falcon dish, from 12 to 15 cells being filled for a total diet amount of 15 ml.

Experimental design. The tested diets differed only as to the kind of insect material employed, i.e, GMPE, HCPE, MSLH or APPH. Each diet was poured in a Multiwell Falcon dish and two eggs were put in each cell for a total of 24-30 eggs per diet. The eggs were collected, dispensed and surface-disinfected after Bratti and Coulibaly (1993). The number of hatched eggs was determined after 3 days by visually counting the empty shells. Each test was replicated four times. All the instruments and glassware were sterilized by autoclaving for 12 min at 120°C, and all operations, including visual examination, were performed under a laminar flow hood. The Multiwell dishes were put singly in glass Petri dishes (170 mm x 25 mm) and kept in a growth room at 27°C and 75% r.h. during the experiment. Each newly formed puparium was transferred to a new Multiwell dish until adult emergence.

Calculation of L3, puparia and adult yields was based on the number of eggs hatched on the diets; the percentage of hatched eggs was also calculated. Puparia eclosion percentages were calculated on the number of puparia. Puparia weight 24 h after forming and the development time from egg laying to puparia eclosion were also recorded. The data were analyzed using CSS: Statistica (1991) by Anova. Tukey's HSD test was used to determine significantly different means.

Results and discussion

No significant differences were found among diets as to the number of hatched eggs: the roughly 80% value is similar to that registered with diets containing a greater amount of GMPE (Bratti and Coulibaly, 1993) or in standard rearing (Coulibaly, unpublished data). A higher mortality *in vitro* had been expected as the eggs sometimes remained soaked for a couple of days in a liquid film that, had it not been subsequently absorbed by the agar diet, could have caused the death of the embryo by asphyxia. This phenomenon did not occur indicates that diet had no influence on this biological parameter.

The L3 and puparia yields were not affected by the kind of IM (Tab. 2). The puparia weights formed on HCPE diet were significantly higher (over 20%) than those on MSLH diet but do not differ from the other two diets (Tab. 2). Despite the average weight values, puparia eclosion was low in all the tests (30-44% did not develop into adults). A diet containing 2.5% GMPE (Bratti and Coulibaly, 1993) showed eclosion values over 80% from puparia with an average weight around 30 mg. This diet differed from those tested in the present study only by the addition of fetal bovine serum (FBS) instead of BS and a higher concentration of TCM (60% Schneider's against the roughly 32% in the others). Although BS has proven to be an effective ingredient when combined with 20% GMPE for both *E. larvarum* and *P. rufifrons* (Mellini *et al.*, 1993 a,b), it generally exerts a less stimulating growth effect *in vitro* on the insects' cells than FBS (Ignoffo *et al.*, 1973). Thus, the combination in the diet of BS with a lower concentration of TCM, which is richer in nutrient substances than vertebrate serum, may explain the failure of adult formation in the puparia and the ensuing poor rate of eclosion.

Tab. 2 - Development parameters of *Exorista larvarum* maggots reared *in vitro*.

	Percentages					
Diet	Hatched Eggs	L3 Yield	Puparia Yield	Adult Yield	Puparia Eclosion	Puparia weight** mg
MSLH BD*	78.3 ± 4.5	54.5 ± 7.1	48.3 ± 6.1	33.8 ± 6.8	69.0 ± 9.2	31.2 ± 1.4 a
APPE BD	76.8 ± 4.1	68.8 ± 7.5	59.1 ± 5.0	32.5 ± 5.2	55.9 ± 8.7	33.6 ± 1.3 ab
GMPE BD	79.3 ± 1.4	74.1 ± 5.7	61.6 ± 3.0	39.1 ± 3.1	64.0 ± 6.9	36.1 ± 1.4 ab
HCPE BD	79.6 ± 5.9	66.5 ± 6.5	56.7 ± 4.7	40.7 ± 5.2	70.0 ± 11.3	38.4 ± 1.4 b
df= 3,16	3,16	3,16	3,16	3,16	3,252	
F = 0.15	1.34	1.35	0.41	0.57	4.74	
P = 0.9264	0.2952	0.2935	0.7464	0.6416	0.0031	

* BD= Based Diet

** Means followed by the same letter in the column do not differ significantly.

The adult yields were about 17-18% higher in the HCPE and GMPE than in the other two diets. These values are similar to those found by Bratti and Coulibaly (1993) with diets containing the same GMPE concentration. Development

time from oviposition to emergence on the other hand varied from 18 to 20 days a range similar to that found for *in vivo* rearing (Coulibaly, unpublished).

Apart from tachinids (Tab. 1), a number of Hymenoptera parasitoids have been reared on artificial diets containing host material in concentrations ranging from 20 to 50%, including *Catolaccus grandis* Burks and *Bracon mellitor* Say (Guerra, 1992), *Telenomus heliothidis* Ashm. (Strand *et al.*, 1985), *Trichogramma pretiosum* Riley (Xie *et al.*, 1986; Strand and Vinson, 1985), *T. pretiosum* and *T. confusum* (Coop.Res.Group Hubei, 1985) and *Brachymeria intermedia* Nees (Dindo and Campadelli, 1993). The growth and development of *E. larvarum* depend on IM factors (Bratti and Coulibaly, 1993) that are found not only in the host species *H. cunea* but also in non-permissive hosts. This lack of obligatory species specificity is evinced even for other parasitoids. For example, *Palexorista laxa* develops to the adult stage with a diet based on last-instar hemplymph of *M. sexta* (Bratti and Nettles, 1988), and the larvae of *Eucelatoria bryani* evince adult yields around 60% on diets containing 10% GMPE in combination with TCM and CEY (Bratti, unpublished data). Ferkovich *et al.* (1991) preconditioned the IPL-52 B TCM with host fat body and two insect cell lines. The *Lymantria dispar* cell line, which was derived from the fat body of a non-permissive host, promoted *Microplitis croceipes* (Cresson) egg development as did other hemolymphs from four non-permissive lepidopteran hosts (Greany, 1986; Greany *et al.*, 1989).

E. larvarum manifests a notable nutritional adaptability in being able to grow and develop on a number of diets, so long as IM is included in the diet (Bratti and Coulibaly, 1993). Especially noteworthy are the good results with diets containing GMPE (2.5%): they entirely comparable to those found with HCPE diets and show that mass-rearing of this parasitoid is practicable. GMPE can thus be employed to determine the factor or factors found in the insect material that influence the *in vitro* growth and development of this tachinid.

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SUMMARY

Pupae of *Hyphantria cunea* (Drury), *Galleria mellonella* L. and *Antheraea perny* Suer. and full-grown larvae of *Manduca sexta* L.were tested as sources of insect material for *Exorista larvarum* diet. The diet consisted of 35.25% Schneider's medium for *in vitro* insect tissue culture, 35.25% bovine serum, 2% egg yolk, 2.5% insect material, 25% agar suspension in water (1.5% final concentration) and 0.01% gentamicin sulphate. The hatch rate for the four diets was roughly 80%; the pupae and adult yields, which ranged respectively from 48 to 62% and from 32 to 40%, were not influenced by the kind of insect material employed. Pupal weights were higher on average (36-38 mg) for the individuals reared on the diets containing *H.cunea* and *G.mellonella* pupal extract.

Key Words: Artificial diet, parasitoid, insect material, biological control, *Exorista larvarum*, *Lymantria dispar*, *Hyphantria cunea*.

Comparazione di materiale proveniente da insetti diversi in una dieta meridica per l'allevamento di *Exorista larvarum* L. (Dipt.Tachinidae)

RIASSUNTO

Si sono sperimentate, per *Exorista larvarum* L., quattro diverse fonti di materiale di insetto nella dieta, tre provenienti da pupe di *Hyphantria cunea* (Drury), *Galleria mellonella* L. e *Antheraea perny* Suer. e una da larve mature di *Manduca sexta* L. La dieta era così costituita: 35.25% Schneider's, substrato per la coltura *in vitro* dei tessuti d'insetto, 35.25% di siero bovino, 2% di tuorlo d'uovo, 2.5% di materiale proveniente da insetti, 25% di una sospensione di agar in acqua (concentrazione finale dell'1.5%) e lo 0.01% di solfato di gentamicina.

La percentuale di schiusa delle uova si è aggirata, per tutte e 4 le diete, attorno all'80%. Le rese in pupari (dal 48 al 62% circa) ed in adulti (dal 32 al 40%) non sono state influenzate dal tipo di materiale d'insetto utilizzato. I pesi dei pupari sono mediamente superiori (attorno ai 36-38 mg) per gli individui sviluppatisi nelle diete contenenti estratto di pupe di *H.cunea* e *G.mellonella*.

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