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In Vitro Rearing of *Exorista larvarum* (L.) on Diet without Insect Components.*(1)

Introduction

Exorista larvarum (L.), a parasitoid of *Lymantria dispar* L., *Hyphantria cunea* Drury and other Lepidoptera (Herting, 1960), can be mass-reared on diets with insect components (Mellini *et al.*, 1993a,b; Bratti and Coulibaly, 1995; Bratti and Campadelli, 1993). The procedure followed for its rearing *in vitro* is shown in figure 1. The analysis of the proteins and aminoacids contained in the pupal-extracted hemolymph of the factitious host *Galleria mellonella* L. (Bratti and Benini, 1992) has proved a valuable tool in elucidating both the importance of these chemicals and their eventual concentrations in diet use. Laboratory investigations of *in-vitro* rearing approaches for *Pseudogonia rufifrons* Wied. (Bratti and Monti, 1988; Bratti, 1989; Mellini *et al.*, 1993a) have played a fundamental role in perfecting these techniques, and the studies of Nettles *et al.* (1986) on *E. bryani* and of Bratti and Nettles (1988) on *Palexorista laxa* (Curran) have led to improvements in diet preparation and efficiency. Also to be recalled is the basic work done by Mellini (1975) on the *in-vitro* rearing of parasitoids, which has provided excellent guidelines in the choice of nutrients and rearing techniques.

Yet, despite the advances reported to date, two key problems remain to be resolved in achieving cost-effective mass rearing. The first is to eliminate completely from diets host components without adversely affecting insect yields and quality traits. The second is to eliminate host parasitization for egg collection. This would result in the rearing of the parasitoid in a continuous cycle with the eggs being directly laid on the diet. The indirect system involves the use in any case of a factitious host and, hence, an increase of the work load that necessarily raises production costs.

The present study focuses on the first of these two issues and reports the testing of host-component-free diets based on TNM-FH and SCHNEIDER'S, media which have been successfully employed in the rearing of other tachinids (Bratti and Coulibaly, 1995; Bratti and D'Amelio, 1994; Bratti and Nettles, unpublished data).

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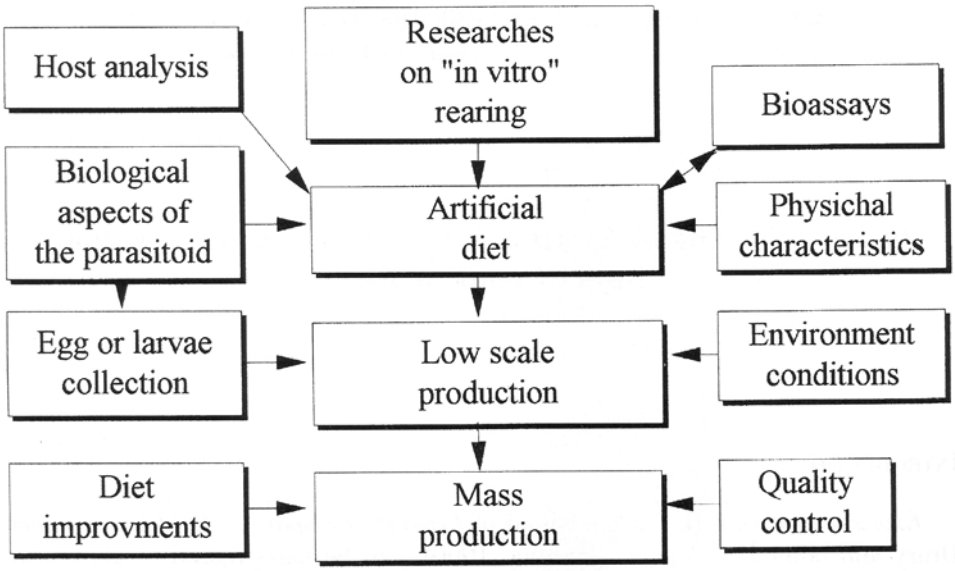

Figure 1 - System followed for *Exorista larvarum in vitro.*

Material and Methods

The experiment was run in three parts. In the first we compared four diets: two containing the tissue culture media SCHNEIDER'S and TNM-FH (Sigma Chemical Co., St. Louis, MO, USA) and two concentrations (5 and 10%) of fresh chicken egg yolk (CEY). Given the good response of TNM-FH in the first part, the second tested five diets based on this medium at the varying CEY concentrations of 0, 5, 10, 15 and 20%. The third part involved rearing consecutive generations of the parasitoid on artificial diet.

Comparison of SCHNEIDER'S and TNM - FH at 5 and 10% CEY. The ingredients of the media included yeast extract (Sigma Chemical Co., St. Louis, MO, USA), wheat germ (Sigma Chemical Co., St. Louis, MO, USA), bovine serum (Sigma Chemical Co., St. Louis, MO, USA), gentamicine sulphate(solution, 10 mg/ml) (Sigma Chemical Co., St. Louis, MO, USA), fresh CEY and agar. Table 1 lists the exact composition of the four tested diets.

Table 1 - Composition of the four diets employed in the first part of the research.

TNM-FH ml	SCHNEI. ml	Yeast extract mg	Wheat germ mg	CEY ml	Bovine serum ml	Agar mg	Gentam. sulphate ml
14.4	---	200	200	2 (10%)	3.2	300	0.75
---	14.4	200	200	2 (10%)	3.2	300	0.75
15.2	---	210	210	1 (5%)	3.4	300	0.75
---	15.2	210	210	1 (5%)	3.4	300	0.75

Preparation. The same procedure was employed for all the diets, the ingredients being prepared in two steps before being mixed together.

Step 1: the yeast extract, agar and wheat germ, the latter being finely ground in a Girmi blender for homogenous mixing, were weighed and placed in a 50-ml beaker. SCHNEIDER'S or TNM-FH was then poured from sterile pipette into the beaker, the mixture stirred to dissolve all the solid parts and the beaker placed in autoclave about 10' to sterilize the solution at 120°C and 1 atm. Once cooled to 90°C, the beaker was removed from the autoclave and its solution poured into a 25-ml beaker.

Step 2: to a 25-ml beaker sterilized in autoclave for 15' at 120°C and 1 atm were added bovine serum, fresh CEY and a gentamicine sulphate solution; the three liquid ingredients were introduced respectively with a 5-ml pipette, a 5-ml syringe and a 1-ml syringe, all single-use and sterile. The beaker was then sealed with aluminium foil and placed in bain marie in a glass vessel at 50-55°C.[2] The ingredients were not sterilized in autoclave in that, except for the CEY, the other two are supplied filtered and sterilized by the manufacturer; preliminary tests also showed that both CEY and bovine serum undergo chemical and physical changes at high temperature that make the diet unappetizing.

The ingredients in steps 1 and 2 were then mixed together and the resulting diet, before solidifying, was distributed by 5- and 10-ml syringes to 24-well Nunclon dishes, each well containing 0.8-1 ml of the diet. Prior to the distribution of the biological material, the diet-containing dishes were kept 24 h at room temperature and relative humidity (rh) to let the diet's surface dry and prevent the eggs being covered by a liquid film.

Biological material. The eggs to be placed on the diet were collected and distributed after Bratti and Coulibaly (1994). The eggs were removed by special spatu-

(2) Keeping the nutrient suspension inside the beaker around 50°C is dictated by the need for a minimum temperature difference between the autoclaved suspension and that in the beaker so as to prevent the agar solidifying too quickly and forming lumps that would impede proper homogenization of the diet.

las and dipped in a drop of either TNM-FH or SCHNEIDER'S (depending on type of diet) on a glass slide, washed several times and placed two-three per cell by Pasteur pipette on the medium.

Comparison of TNM - FH diets at varying CEY amounts. The diets containing TNM-FH of the first part were the base ones: three other CEY amounts were tested in addition to the 5 and 10% starting concentrations; their exact composition is listed in table 2.
Preparation.The diets were prepared as in Part 1 above.
Biological material. The eggs were removed as in Part 1 above and they were dipped in liquid TNM-FH.

Rearing of consecutive generations on artifici al diet. The *E. larvarum* adults that emerged from the tested diets were kept in a standard rearing cage for nine days, after which last-instar larvae of *G. mellonella* were introduced to allow egg laying. The eggs were collected as above and distributed on the diet (table 1 ,TNM-FH at 10% CEY) inside Petri dishes after Mellini *et al.* (1993). The adults that subsequently formed on this diet were collected in another rearing cage and, after about a week, used for egg laying on the host. The eggs were then removed and placed on the same diet.This procedure was replicated over five consecutive generations to test the mating and parasitizing ability of the adults reared *in vitro*.

Experimental design and statistical analysis. The comparison of the 2 tissue culture media per 2 CEY concentrations was replicated 5 times; each replication employed 45-50 eggs. The five CEY concentrations were tested in four replications, about 40 eggs per replication being employed.

The Nunclon dishes containing the eggs were put singly in glass Petri dishes (170 x 25 mm), covered with aluminium foil and kept at room temperature (25-28°C) and relative humidity. Each new puparium was weighed within 24-48 h of formation and transferred to a well in new Nunclon dishes that were stored in a growth chamber at 25°C and 75% rh until adult emergence. The yield percentages of puparia and adults were calculated on the initial egg number; the adult emergence percentages are given by the ratio of emerged individuals to puparia number. The weights of the puparia formed over 24 h were also recorded. All the instruments and glassware were sterilized by autoclaving for 20' at 120°C, and all steps were performed under laminar-flow hood.

A factorial analysis (2x2) was performed on the data for the comparison of TNM-FH and SCHNEIDER'S with the two CEY concentrations (5 and 10%), and analysis of variance on the data comparing the 5 CEY concentrations. LSD was employed to determine the significant difference of the means; the percentages were analysed by transforming the data to the arcsin of the square root. The data analysis program was CSS: STATISTICA (1993).

Table 2 - Composition of the diets with different percentages of CEY.

Ingredients → CEY ml ↓	TNM-FH ml	Yeast extract mg	Wheat germ mg	Bovine serum ml	Agar mg	Gentam. sulphate ml
0 (0%)	16	220	220	3.6	300	0.75
1 (5%)	15.2	210	210	3.4	300	0.75
2 (10%)	14.4	200	200	3.2	300	0.75
3 (15%)	13.6	190	190	3.0	300	0.75
4 (20%)	12.8	180	180	2.8	300	0.75

RESULTS

Comparison of SCHNEIDER'S and TNM - FH at 5 and 10% CEY. The interaction of the two media and CEY concentrations was not significant for insect development rates (Tab. 4). This led to the media and the concentrations being compared separately, although once again the differences were not significant (Tab. 4). The values recorded for the comparison were generally high: from 51 to 66% for puparia, from 43 to 58.6% for adults and even 75-87.4% for adult emergence respectively for 5 and 10% of CEY(Tab. 3).

Yet this picture slightly changes if the average puparia weights are analysed. For, though interaction is not significant, the differences between media (TNM-FH and SCHNEIDER'S) and between CEY amounts (5 and 10%) are significant (Tab. 4). TNM-FH proved best, as did 10% CEY, the concentration that coincided with the highest weight values (Tab. 3).

Table 3 - Development parameters of *Exorista larvarum* maggots reared on TNM-FH and SCHNEIDER'S based-diets containing two different percentages of CEY.

	Puparia weight mg (s.e.)		Puparia Yield		Adult Yield		Adult emergence	
CEY percentages →	5 %	10 %	5 %	10 %	5 %	10 %	5 %	10 %
TNM-FH	49.6 ± 1 .1	60.1 ± 1.3	51.6 ± 8.5	66.5 ± 7.4	43.1 ± 9.3	58.6 ± 7.7	80.8 ± 4.6	87.4 ± 3.0
SCHNEIDER'S	47.4 ± 1.2	55.8 ± 1.4	52.7 ± 8.6	58.9 ± 7.2	43.3 ± 6.0	45.8 ± 8.4	83.3 ± 3.0	75.2 ± 6.3

Table 4 - Results of statistical analyses of data in table 3.

	Interaction (TNM-FH, SCHNEIDER'S) X (CEY percentages)			TNM-FH vs SCHNEIDER'S			5% vs 10% CEY		
	F	df	P	F	df	P	F	df	P
Puparia weight	0.66	1,540	0.41	6.33	1,540	0.012	53.8	1,540	< 0.01
Puparia yield	0.37	1,16	0.54	0.19	1,16	0.66	1.7	1,16	0.21
Adult yield	0.72	1,16	0.4	0.65	1,16	0.43	1.24	1,16	0.28
Adult emergence	2.13	1,16	0.16	1.14	1,16	0.29	< 0.01	1,16	0.94

Comparison of TNM - FH diets at varying CEY amounts. While the puparia, adult and emergence rates did not evince significant differences, they did show a fairly typical pattern: they rose from 0 to 15% and then declined in the diet containing 20% CEY. As shown in table 5, the puparia percentage was about 45% for the diet without CEY, 69% for 10% CEY and 60% for the higher CEY. The differences in adult yield are very close to being significant (P=0.06): table 5 evinces that the highest values (about 56%) are recorded at 5, 10 and 15% CEY. The adult emergence data present a similar picture: the differences between diets are less marked yet the highest values, from 80 to 85%, are recorded for the same diets.

Significant differences are instead found in the mean puparia weights. The weight values without CEY are less than 30 mg, climb to 52 at 5% and progressively rise to 64.6 mg at 20% CEY (Tab. 5). The puparia had a somewhat lighter colour than *in vivo* ones and evinced no malformations. The average test development time of 13-14 days from egg to puparium does not differ from that recorded for rearing on the factitious host.

The maggots hatched from the eggs after about two days, immediately penetrated the diet and formed, as in the host, a kind of respiratory funnel. It is important that during this stage there is no surface liquid, which would drown the maggots. The first instars moulted to the next stage three days after hatching, remaining in the same position as before. Once grown, they started digging fairly large interconnecting chambers in the nutrient mass, which changed from an initial gel-like texture to, in what remained, a pulpy mush. Noteworthy too is that upon maturity many larvae exhibited the same behaviour found *in vivo*, i.e. they left the medium and pupated in the immediate vicinity.

An average of about 20% of the eggs regularly failed to hatch either because they were sterile or had been damaged in removal and transfer to the diet. Bacterial and fungal contamination was very limited in the experiment: the few recorded cases involved only a few wells and the parasitoid was almost always able to complete its development.

Table 5 - Development parameters of *Exorista larvarum* maggots reared on diets with different percentages of CEY.

Percentages of CEY in the diet	Puparia weight mg*	Puparia Yield	Adult Yield	Adult emergence
0	27.8 ± 0.9 a	44.8 ± 11.7	31.9 ± 10.0	63.0 ± 16.7
5	52.2 ± 1.5 b	65.9 ± 4.7	56.1 ± 3.8	85.2 ± 2.7
10	60.9 ± 1.6 c	68.8 ± 3.3	56.1 ± 4.8	81.2 ± 4.5
15	62.7 ± 1.4 c	69.2 ± 4.7	55.6 ± 4.9	80.2 ± 3.3
20	64.6 ± 1.8 c	60.2 ± 6.8	45.7 ± 3.2	77.5 ± 5.8
df	4,519	4,15	4,15	4,15
F	82.12	2.10	2.79	0.88
P	< 0.01	0.13	0.06	0.49

* Means in the column followed by the same letter are not significantly different (L.S.D test, $P < 0.05$).

Rearing of consecutive generations on artificial diet. The adults produced *in vitro* were able to develop over at least five generations on artificial diet without any contact between parasitoid and host except for egg laying. The puparia weighed about 50 mg and the adults maintained their vital characteristics unaltered. Rearing was not extended beyond the fifth generation as this span was held to be sufficient proof that *E. larvarum* can develop on artificial diet without its quality characteristics being adversely affected, at least in the laboratory.

Conclusions

The biological characteristics of *E. larvarum* make this parasitoid particularly suitable to *in vitro* rearing. They include non-synchronized development with the host, high fertility, gregariousness and polyphagy (Bratti, 1994). These traits have been evident in all their potentiality since the first experiments. Bratti and Campadelli (1993), listing the tachinids tested on meridic and oligidic diets containing host material, point out that *E. larvarum* is the one that has registered the best adult yield and quality results. Mellini *et al.* (1993b) developed a diet based on bovine serum and a technique that, by placing the freshly laid eggs in culture, enabled *E. larvarum* to register about 60% development rate of adults weighing about 45 mg.

These diets, though including about 20% host material, are easy to prepare, have low-cost ingredients and, according to Mellini and Campadelli (1994), are

also suitable to mass rearing. Supporting this statement is the fact that the diets used for the *Trichogramma* spp., the only parasitoid reared *in vitro* and employed in biological pest control, contain a concentration of host material (often of hemolymph or egg juice) that varies from 10 to 40% (Strand and Vinson, 1985; Xie *et al.*, 1986a,b).

The marked aptitude of *E. larvarum* to develop on artificial diet has also been shown experimentally by Bratti and Coulibaly (1995) and Bratti (1994). The diets used in these tests were based on TNM-FH, CEY (1.5%) and a 10% maximum of host-pupal extract. The tachinids reared, all developing on host-larvae, were *P. laxa*, *E. bryani* and *E. larvarum*, the latter two exhibiting the best results. Here it was found that when the amounts of the other ingredients were kept constant and the concentration of host material was lowered from 5 to 0%, *E. larvarum* evinced a sharp decline in development and puparia weights.

It is worth recalling that although host components are necessary in these diets, this does not rule out the possibility of developing other diets which do not contain them. For example, *Trichogramma* spp., *Pteromalus puparum* L., *E. bryani*, *P. laxa*, *Bracon melitor* Say and *Catolaccus grandis* Burks are parasitoids that can develop successfully to varying extent on both types of medium (Bratti and Coulibaly, 1995). This is borne out by the results of the present study: the biological parameter values of *E. larvarum* are better for all the tested diets than those recorded for the same parasitoid on diets with pupal extracts and homogenates (Mellini *et al.*, 1993b; Bratti and Coulibaly, 1995). A similar situation is reported for the ectoparasitoid *C. grandis*, which registered far higher development rates (about 70%) on meridic than on hemolymph diet (Guerra *et al.*, 1993). This contrasts with the performance of *T. dendrolimi*, which on a diet free of host hemolymph yielded adults (about 15%) marked by poor vitality and deformities (Wu and Qin, 1982).

Neither the SCHNEIDER'S nor TNM-FH medium, used in the present study as the basic ingredient in the tested diets, significantly affected adult yields or adult emergence rates. A similar finding is reported by Bratti and Coulibaly (1995), who in a comparison of TNM-FH, SCHNEIDER'S, EX-CELL 400 and SF-900 found but minimum differences in the puparia, adult and emergence rates of *E. larvarum*. This response contrasts with that of another tachinid, *E. bryani*, reared on TNM-FH and SCHNEIDER'S based-diets: adult yields on the former were about 55% against the latter's 30% (Bratti and Nettles, in press). Bratti and D'Amelio (1994) confirmed this finding for *E. bryani*, noting that when the insect material only was removed from the diet the yield rates uniformly dropped far below those recorded on the Nettles (1986) diet.

TNM-FH proved clearly better for puparia weights of *E. larvarum* in the present study. These data confirm the suitability of this insect cell culture medium,which is used in varying amounts in diets for *Trichogramma* spp. (Bratti, 1990).

The data on CEY concentration show that when it rose from 15 to 20%, yields fell, albeit not significantly, from 55.6 to 45.7% for adult yield and from 80.2 to 77.5% for adult emergence. This is a fairly common phenomenon in that the important thing in such diets is not so much large amounts of a given nutrient as the balance of the various ingredients (Singh, 1977). Given the considerable con-

tent of lipids in egg yolk, a toxic reaction of these substances at high concentrations cannot be ruled out. The puparia weights varied markedly at the tested CEY concentrations with respect to adult yields. When egg is completely absent from the diet, individuals weigh about a third less than they do on diet with 10-20% CEY. Egg yolk has a number of other substances that, in addition to lipids, are important to insect nutrition. It contains approximately 48.7% water, 16.4% proteins (including free aminoacids), 0.21% carbohydrates and 33% lipids (with a 1.6 mg/100 g cholesterol count).

In the oligidic diets for *T. preticsum*, Xie *et al.* (1986a,b) combined hemolymph and powdered milk in a 25% concentration, whereas the fairly successful artificial diet for *Trichogramma confusum* Vigg. and *T. dendrolimi* had these ingredients in a 14% concentration (Coop. Prov. Res. Hubei, 1985). Liu and Wu (1982) were the first to develop for *T. confusum*, *T. pretiosum* and *T. dendrolimi* an oligidic diet free of insect components containing from 10 to 20% CEY, although adult yields were very low. Ding *et al.* (1980) reported good rearing results for *Tetrastichus schoenobii* Ferr. on a diet containing 20% CEY, while on a similar pabulum but with 35% CEY. Strand *et al.* (1985) reared *Telenomus heliothidis* Ashm. to the adult stage. This material has proved beneficial not only to egg parasitoids but also to *Habrobracon hebetor* Say, which was reared on a diet similar to that for *Trichogramma* spp. (Xie *et al.*, 1989) and for *E. bryani*. Nettles (unpublished data) has increased by about 20% the average adult yield of the latter by adding 1.6% CEY.

The data of the present study indicate that the diet containing TNM-FH and 10-15% fresh CEY is the most suitable of those tested for the rearing of *E. larvarum*. This diet (with 10% CEY) was then used to rear five consecutive generations of the parasitoid without any drop in adult vitality. Only for a few *Trichogramma* species have more consecutive generations been recorded and in these cases the diets had a marked host-hemolymph content. The present findings are especially noteworthy because they were recorded for diets entirely free of host components yet capable of quantitative and qualitative adult yields even higher than those registered for rearing on the factitious host *G. mellonella*. They are in effect even more significant given that the tests started with freshly laid eggs and not, as for other tachinids, with eggs extracted from the uterus or with maggots taken from dissected hosts (Bratti, 1994). Mass-rearing is thus fully practicable in so far as the diet is concerned. There now remain to be resolved the issues of direct egg-laying on the pabulum and the effectiveness in the field of these parasitoids.

Key words: *In vitro* rearing, tachinids, tissue culture media, biological control

SUMMARY

For mass-rearing *Exorista larvarum* (L.), a parasitoid of *Lymantria dispar* L., *Hyphantria cunea* Drury and other Lepidoptera, two key problems need to be resolved. The first is to eliminate completely from diets host components without adversely affecting insect yields and quality traits. The

second is to eliminate host parasitization for egg collection. In the present study are reported the results of *E.larvarum in vitro* rearing on diets without insect components and containing mainly TNM-FH or SCHNEIDER'S and egg yolk (CEY) at varying concentrations (from 0 to 20%). The results indicate that the diet containing TNM-FH and 10-15% fresh CEY is the most suitable of those tested for the rearing of *E.larvarum*. This diet (with 10% CEY) was then used to rear five consecutive generations without any drop in adult vitality. Mass-rearing is thus fully practicable in so far as the diet is concerned. There now remain to be resolved the issues of direct egg-laying on the pabulum and the effectiveness in the field of these parasitoids.

Allevamento *in vitro* di *Exorista larvarum* (L.) su diete meridiche prive di componenti di insetto.

RIASSUNTO

Per poter allevare massivamente su dieta artificiale *Exorista larvarum* (L.), parassitoide di numerosi Lepidotteri tra cui *Lymantria dispar* L. e *Hyphantria cunea* Drury, è necessario risolvere due problemi fondamentali. Il primo consiste nell'ottenere una dieta a basso costo, priva di componenti entomatiche, in grado di garantire la produzione di insetti di alta qualità. Il secondo riguarda la possibilità di eliminare il metodo della superparassitizzazione dell'ospite per raccogliere le uova da porre in coltura.

In questo lavoro si sono sperimentate varie diete, senza componenti di insetto, basate principalmente su due "media" per le colture cellulari:SCHNEIDER'S e TNM-FH, ai quali sono state addizionate varie concentrazioni di tuorlo fresco di uovo di gallina (da 0 al 20%).

I risultati indicano che, fra tutte le diete sperimentate, quelle che contengono TNM-FH più il 10 e 15% di tuorlo d'uovo hanno consentito di ottenere la maggior resa di adulti (55-56%) ed un soddisfacente peso medio dei pupari (attorno ai 62 mg). In seguito la dieta a base di TNM-FH con il 10% di tuorlo d'uovo è stata utilizzata per allevare il parassitoide per 5 generazioni consecutive senza che gli adulti manifestassero cali evidenti di vitalità.

Si può quindi affermare che l'allevamento massale, almeno per ciò che riguarda la dieta, è praticabile. Rimane ora da verificare la possibilità di ottenere direttamente la deposizione delle uova del parassitoide sulla dieta oltrechè l'efficacia di questi insetti, prodotti *in vitro*, in applicazioni in pieno campo.

REFERENCES CITED

BRATTI A., 1989. - Allevamento *in vitro* di *Pseudogonia rufifrons* Wied. (Diptera Tachinidae)su omogeneizzato di crisalidi di *Galleria mellonella* L. e su diete meridiche. - *Boll. Ist. Entom. "G.Grandi" Univ. Bologna,* 44:11-22.

BRATTI A., 1990. - Tecniche di allevamento *in vitro* per gli stadi larvali di Insetti entomofagi parassitoidi. - *Boll. Ist. Entom. "G.Grandi" Univ. Bologna*, 44: 169-220.

BRATTI A., 1994. - Principi generali per l'allestimento di diete artificiali per gli stadi larvali dei Ditteri Tachinidi e nuovi contributi nel campo della sperimentazione. - M.A.F. Convegno "Lotta Biologica" Acireale 1991, (Coord. G. Viggiani), pp. 41-54.

BRATTI A., 1994. - Una dieta di base per l'allevamento *in vitro* di tre specie di Ditteri Tachinidi: *Palexorista laxa* Curran, *Eucelatoria bryani* Sab. e *Exorista larvarum* (L). - Atti XVII Congresso Naz. Ent., Udine, pp.705-706.

BRATTI A., MONTI M., 1988. - Allevamento *in vitro* delle larve di *Pseudogonia rufifrons* Wied. (Diptera Tachinidae) su omogeneizzato di crisalidi di *Galleria mellonella* L. (Lep. Galleriidae). - *Boll. Ist. Entom. "G. Grandi" Univ. Bologna,* 43: 115-126.

BRATTI A., NETTLES W. C. JR., 1988. - *In vitro* rearing of *Palexorista laxa* Curran (Diptera Tachinidae) on hemolymph-based diets. - *Boll. Ist. Entom. "G. Grandi" Univ. Bologna*, 43: 25-30.

BRATTI A., BENINI S., 1991. - Allevamento *in vitro* delle larve di *Pseudogonia rufifrons* Wied. (Diptera Tachinidae). Prove su diete subnaturali e meridiche. - *Boll. Ist. Entom. "G. Grandi" Univ. Bologna,* 46:71-75.

BRATTI A., NETTLES W. C. JR., 1992. - *In vitro* rearing of *Eucelatoria bryani*: improvements and evaluation affecting efficency. - *Entomol. Exp.Appl.* 63: 213-219.

BRATTI A., CAMPADELLI G., 1993. - Comparison of insect material in a meridic diet for *Exorista larvarum* (L.)(Diptera Tachinidae) *in vitro* rearing. - *Boll. Ist. Entom. "G. Grandi" Univ. Bologna*, 48: 59-65.

BRATTI A., COULIBALY A. K., 1995. - *In vitro* rearing of *Exorista larvarum* (L.) (Diptera Tachinidae) on tissue culture media based diets. - *Entomol. Exp.Appl.* 71:45-51.

BRATTI A., D'AMELIO L., 1994. - *In vitro* rearing of *Eucelatoria bryani* Sab. (Diptera Tachinidae) on tissue culture based diets. - *Boll. Ist. Entom. "G. Grandi" Univ. Bologna*, 48:109-114.

COOP. RES. GROUP OF ARTIFICIAL HOST EGG OF TRICOGRAMMA, 1985. - Study in artificial host egg-E II for *Trichogramma*. - *J. Wuhan Univ.*, 4: 1-10.

DING H. C., QUI H. G., HWANG C. B., 1980. - *In vitro* rearing of an egg parasitoid, *Tetrastichus schoenobii* Ferriere (Hymenoptera: Tetrastichidae). - *Contributions Shangai Inst. Entomol.*, 1: 55-59.

GAO Y. G., DAI K. J., SHONG L. C., 1982. - Studies on the artificial host egg for *Trichogramma*. - *Les Colloques de l'INRA* N°9. Les Trichogrammes Antibes (France), 20-23 Avril 1982.

GREANY P., 1980. - Growth and development of an insect parasitoid *in vitro*. - *Amer. Zoo*l., 20: 946.

GRENIER S., GREANY P. D., COHEN A. C., 1993. - Potential for mass release of insect parasitoids and predators through development of artificial culture techniques. Pest management in the Subtropics Biological Control. A Florida Perspective. - Editors: D.Rosen, F.D.Bennet and J.L.Capinera, 520 pp.

GRENIER S., BONNOT G., DELOBEL B., LAVIOLETTE P., 1978. - Développement en milieu artificiel du parasitoide *Lixophaga diatraeae* Towns. (Diptera Tachinidae). Obtention de l'imago a partir de l'oeuf. - *C. R. Acad. Sc. Paris ser. D*, 287: 535-538.

GUAN X. C., WU Z. X., WU T. N., FENG H., 1978. - Studies on rearing *Trichogramma dendrolimi* Mats. *in vitro*. - *Acta Entom. Sin.* , 21:122.

GUERRA A. A., MARTINEZ S., 1994. - An *in vitro* rearing system for the propagation of the ectoparasitoid *Catolaccus grandis*. - *Entomol. Exp. Appl.*, 72: 11-16.

GUERRA A. A., ROBORACHER K. M., MARTINEZ S., 1993. - *In vitro* rearing of *Bracon mellitor* and *Catolaccus grandis* with artificial diets devoid of insect components. - *Entomol. Exp. Appl.*, 68: 303-308.

HAFEZ M., 1953. - Studies on *Tachina larvarum* L. (Diptera Tachinidae) III.Biology and life history. - *Bull. Soc. Fouad Ier d'Entomologie*, 37: 305-335.

HERTING B., 1960. - Biologie der westpaläarktischen Raupenfliegen Dipt. Tach.- *Monogr. z. Angew. Entom.* 16: 188 pp.

IGNOFFO C. M., SHAPIRO M., DUNKEL D., 1973. - Effects of insects, mammalian, and avian sera on *in vitro* growth of the *Heliothis zea* Cells. - *Ann. Entomol. Soc. Ann.*, 66 (1): 170-172.

IRIE K., XIE Z. N., NETTLES W. C. JR., MORRISON R. K., CHEN A. C., HOLMAN G. M., VINSON S. B., 1987. - The partial purification of a *Trichogramma pretiosum* pupation factors from hemolymph of *Manduca sexta*. - *Insect Biochem.*, 17: 269-275.

LIU W. H., WU Z. X., 1982. - Recent results in rearing *Trichogramma in vitro* with artificial media devoid of insectan additives. - *Acta Entomol. Sin.*, 25: 160-163.

LUCIANO P., PROTA R., 1984. - La dinamica di popolazione di *Lymantria dispar* L. in Sardegna. III. Indicatori biologici della gradazione. - *Frustula Entomologica*, 7-8: 613-630.

MELLINI E., 1975. - Possibilità di allevamento di insetti entomofagi parassiti su diete artificiali. - *Boll. Ist. Entom. Univ. Bologna*,32: 257-290.

MELLINI E., 1992. - Ditteri entomofagi, occasionali determinatori di miasi nell'uomo. - *Natura e Montagna*, 39: (3-4): 41-48.

MELLINI E., CAMPADELLI G., 1994. - Positivi risultati nell'allevamento su diete artificiali del parassitoide *Exorista larvarum* (L.) (Dipt. Tachinidae).- *Informatore Fitopatologico*, 2/94: 44-49.

MELLINI E., CAMPADELLI G., DINDO M. L., 1993 a). - Possibile impiego di plasma bovino nell'allestimento di diete artificiali per le larve del parassitoide *Pseudogonia rufifrons* Wied. - M.A.F. - Convegno "Lotta Biologica" Acireale 1991, (G. Viggiani), pp. 145-150.

MELLINI E., CAMPADELLI G., DINDO M. L., 1993 b). - Artificial culture of the parasitoid *Exorista larvarum* (L.) (Dipt. Tachinidae) on bovine serum based diets. - *Boll. Ist. Entom. "G.Grandi" Univ. Bologna*, 47: 221-229.

MELLINI E., CAMPADELLI G., DINDO M. L., 1993 c). - Artificial culture of the parasitoid *Exorista larvarum* (L.) (Dipt. Tachinidae) on oligidic media: improvements of techniques. - *Boll. Ist. Entom. "G. Grandi" Univ. Bologna*, 48: 1-10.

NETTLES W. C. JR., 1981. - Effect of water deprivation and subsequent exposure to water of the response of *Eucelatoria* sp. to okra volatiles and to water vapor. - *Environ. Entomol.*, 10: 111-113.

NETTLES W. C. JR., 1986. - Effects of soy flour, bovine serum albumin and three amino acid mixtures and growth and development of *Eucelatoria bryani* (Dipt. Tachinidae) reared on artificial diets. - *Environ. Entomol.*, 15: 1111-1115.

NETTLES W. C. JR., 1990. - *In vitro* rearing of parasitoids: role of host factors in nutrition. - *Arch. Insect Biochem. Physiol.*, 13: 167-175.

REINECKE I. P., 1985. - Nutrition: Artificial diets. - In: Comprehensive insect Physiology, Biochemistry and Pharmacology (Eds. G.a. Kerkut and L.I. Gilbert)Vol. 4., pp. 391-419, CRC Press, Cleveland.

SINGH P., 1977. - Artificial diets for insects, mites and spiders. - IFI/Plenum , New York, Washington and London,pp. 594.

STRAND M. R., VINSON S. B., 1985. - *In vitro* culture of *Trichogramma pretiosum* on an artificial medium. - *Entomol. Exp. Appl.*, 39: 203-209.

STRAND M. R., VINSON S. B., NETTLES W. C. JR., XIE Z. N.,1988. - *In vitro* culture of the egg parasitoid *Telenomus heliothidis:* the role of teratocytes and medium comsumption in development. - *Entomol. exp. appl.*, 46: 71-78.

WU Z. X., QUIN J., LI T. X., CHANG Z. P., LIU T. M., 1982. - Culturing *Trichogramma dendrolimi in vitro* with artificial media devoid of insect materials. - *Acta Ent. Sin.*, 22: 122-126.

XIE Z. N., NETTLES W. C. JR, MORRISON R. K., IRIE K., VINSON S. B., 1986 a).- Effect of ovipositional stimulants and diets on the growth and development of *Trichogramma pretiosum in vitro.* - *Entomol. exp. appl.*, 42: 119-124.

XIE Z. N., NETTLES W. C. JR, MORRISON R. K., IRIE K., VINSON S B., 1986 b). - Three methods for the *in vitro* culture of *Trichogramma pretiosum* Riley. - *J. Entomol. Sci.*, 21: 133-138.

XIE Z. N., LI L., XIE Y., 1989. - *In vitro* culture of *Habrobracon hebetor* Say (Hym.: Braconidae).- *Chin.J.Biol.control,* 5:49-51.