Boll. Ist. Ent. "G. Grandi" Univ. Bologna, 53: 109-119, 1999.

MARIA LUISA DINDO, LUCA SIGHINOLFI, GUIDO CAMPADELLI, PIERO BARONIO
Istituto di Entomologia "G. Grandi", Università di Bologna

Laboratory Evaluation of Parasitism of Wax Moth and Gypsy Moth Larvae by *Exorista larvarum* (L.) cultured *In Vivo* and *In Vitro* (*)

Introduction

In the last twenty years, significant advances have been made in the development of *in vitro* rearing techniques for entomophages (Thompson, 1999; Thompson and Hagen, 1999). Success has been achieved in the mass culturing on artificial diets of a number of predators and idiobiotic parasitoids, including *Geocoris punctipes* (Cohen, 1985), *Podisus maculiventris* and *Podisus sagitta* (DeClercq and Degheele, 1992), *Catolaccus grandis* (Burks) (Rojas *et al.*, 1996), and *Trichogramma* spp. (Li *et al.*, 1988; Grenier, 1994; Liu *et al.*, 1995).

Attainment of high adult yields is not, however, the only important issue involved in rearing entomophages on artificial diets. Just as important is the evaluation and maintenance of their quality (Grenier *et al.*, 1994; Thompson and Hagen, 1999). Recent studies have addressed this topic (Morales-Ramos *et al.*, 1996; Nordlund *et al.*, 1997; Cohen *et al.*, 1999).

Exorista larvarum (L.) is a polyphagous gregarious larval parasitoid of Lepidoptera. In cork-oak forests in Sardinia it is very important as a natural antagonist of *Lymantria dispar* (L.) (Luciano and Prota, 1984), *Malacosoma neustria* (L.) (Delrio *et al.*, 1983) and *Tortrix viridana* L. (Delrio *et al.*, 1988). This tachinid is one of the most promising parasitoids for *in vitro* mass rearing. Its complete development was obtained on various artificial diets devoid of insect components, with adult yields of 43-44% (Mellini and Campadelli, 1995), of 55% (Bratti *et al.*, 1995) and of 33% (Dindo *et al.*, 1999). The adults obtained in all media mated and the females oviposited on *G. mellonella* larvae, producing a second generation *in vivo*.

Some information is available on the quality of the *in vitro*-reared *E. larvarum* adults. The puparia produced on the diet developed by Mellini and Campadelli (1995), comprising skimmed milk, chicken egg yolk, yeast extract and saccharose, were similar in size to those usually obtained from *G. mellonella* (Mellini and Campadelli, 1996a). Moreover, Dindo *et al.* (1999) showed that, in the laboratory,

(*) Accepted for pubblication: November 15, 1999.

the longevity and the fecundity of females obtained on a meat homogenate-based diet were comparable to those of females emerged from puparia formed in *G. mellonella* larvae. As yet, however, the capability to parasitize natural and unnatural hosts of *E. larvarum* reared on artificial media had not been compared with that of *in vivo*-cultured parasitoids.

The present paper reports laboratory results of the evaluation of the parasitism of the factitious host *G. mellonella* and of the natural host *L. dispar* by *E. larvarum* females obtained *in vivo* and *in vitro*.

Materials and Methods

Insects. The specimens of *E. larvarum* and *G. mellonella* utilised in the present study were collected from laboratory-reared colonies held at the Institute of Entomology "G. Grandi".

The *E. larvarum* colony was established in 1992 from adults which had emerged from *L. dispar* and *H. cunea* Drury larvae field-collected in the province of Bologna. From the start *G. mellonella* was utilised as a factitious host for this parasitoid. The flies were kept in Plexiglas cages (40×30×30 cm) in a rearing chamber at 26±1°C, 75±5% RH with a 16:8 L:D photoperiod, and fed on lump sugar and cotton balls soaked in a honey and water solution. The *G. mellonella* colony was maintained at 30±1°C, 65±5% RH, in complete darkness. The larvae were fed on the diet developed by Sehnal (1966) and modified by Campadelli (1973). Upon attaining maturity they were exposed to parasitoid females (Mellini *et al.*, 1993a).

L. dispar larvae were obtained from egg masses collected between the end of February and the beginning of March1999 in forests of *Quercus suber* L. at Padula, near Tempio Pausania, by prof. Pietro Luciano, of the Institute of Agricultural Entomology of the University of Sassari. The egg masses were individually placed inside 16.5-cm-diameter Petri dishes, and stored in the fridge at 4-6°C (Ridet, 1972) until the beginning of the experiments, which were performed between May and September 1999. The first and the last egg masses were taken out of the fridge at the end of May and at the beginning of September respectively, i.e. 90 and 180 days after having been collected in the forest. The egg masses were transferred into plastic boxes (25.5×12.5×8.5 cm), and placed in a rearing chamber at 26±1°C, 75±5% RH with a 16:8 L:D photoperiod. Before placing the eggs in the boxes, the latter were disinfected with a 4% sodium hypochlorite solution. Eggs hatched in 2-3 days. The larvae were fed on fresh leaves of *Quercus robur* L. which were changed daily. The leaves were rinsed in fresh water and dried with blotting-paper before use.

In vitro rearing of E. larvarum. The artificial rearing of *E. larvarum* was performed on the diet developed by Mellini and Campadelli (1995). The diet contained 77% skimmed milk, 14% chicken egg yolk, 7% yeast extract and 2% saccharose, and was prepared according to the method exhaustively described by Farneti *et al.* (1998). About 60 ml diet per replicate were prepared and

distributed into 5-cm-diameter glass Petri dishes (10-12 ml per Petri dish). The resulting five Petri dishes were in turn put into a 16.5-cm-diameter glass Petri dish which was then wrapped in tinfoil. The dish was stored in the fridge at 4-6°C for the parasitoid eggs which were to be placed onto the diet within the following 48 hours, or in the freezer at –18°C in case of longer times. The frozen diet was left to defrost at room temperature for 1 hour before use.

E. larvarum eggs were collected from superparasitized *G. mellonella* larvae, disinfected with 60% ethanol, rinsed twice with sterile water and finally transferred onto the diet by the method described by Mellini and Campadelli (1994). About 60 eggs were placed in each Petri dish so that each replicate consisted of about 300 eggs. The Petri dishes were kept in darkness at 26±1°C and 70% RH until puparium formation. After forming the puparia were rinsed and used for the experiment as described in the section below.

In the present study, adult yields and puparium weight, which had already been studied by Mellini and Campadelli (1995; 1996a), were not recorded. Throughout the experiment, puparium production was however more than sufficient for the trials to be performed as described below.

Evaluation of parasitism of G. mellonella and L. dispar larvae by E. larvarum cultured in vivo and in vitro. Each replicate was performed employing an equal number (100-125) of newly-formed *E. larvarum* puparia obtained *in vitro* as above described (A), and *in vivo* from *G. mellonella* (B). The A- and the B-puparia were separately placed in a Plexiglas cage (40×30×30 cm). After 7-8 days the adults started emerging. Emergence was complete within 24-72 hours. The adults were fed as in the standard *in vivo* rearing procedure.

According to Hafez (1953) *E. larvarum* females mate as soon as they emerge. At 26°C the pre-oviposition period lasts 2-3 days. Dindo *et al.* (1999) showed that most eggs are laid by both the *in vivo* and *in vitro*-reared females between the 5^{th} and the 8^{th}-9^{th} day following emergence. In view of the foregoing, in the present study both the A- and the B-females were separated into two groups 7-8 days after the beginning of the emergence and then transferred into Plexiglas cages (20x20x20 cm). The resulting four cages were intended for the trials with *L. dispar* (A1 and B1) and *G. mellonella* (A2 and B2) respectively. The following 4 treatments were thus compared.

A1: larvae of *L. dispar* exposed to *in vitro*-reared females;
A2: larvae of *G. mellonella* exposed to *in vitro*-reared females;
B1: larvae of *L. dispar* exposed to *in vivo*-reared females;
B2: larvae of *G. mellonella* exposed to *in vivo*-reared females.

The larval stages of *L. dispar* were identified according to Fraval *et al.* (1984). As suggested by prof. Luciano, four-instar larvae were selected for parasitisation. The larvae were selected during the larval-larval apolysis between the 3^{rd} and the 4^{th} instar. They were easily recognisable as the detached cuticle was clearly visible, especially around the head capsule. The larvae were exposed to parasitoid females within 24 hours after the ecdysys. In *L. dispar*, the average duration of the 4^{th} larval stage actually varies depending on temperature and other environmental

factors, but is generally longer than 3 days (Fraval *et al.*, 1984). As the *E. larvarum* eggs hatch in 3 days (Hafez, 1953), parasitoid egg losses due to host moulting were avoided.

The mean weight (± s.d.) of the gypsy moth larvae utilised throughout the experiment was mg 364.6±152.7 (n=114) and mg 406±100.6 (n=126) in treatment A1 and B1 respectively.

Five (treatments A1 and A2) and six (treatments B1 and B2) replicates were performed.

In all treatments, three host larvae per female were exposed. The number of females utilised for each treatment and replicate was adjusted according to the number of the four-instar larvae of *L. dispar* available for the experiment. The same number of *G. mellonella* mature larvae was used in treatments A2 and B2 as that of *L. dispar* larvae employed in treatments A1 and B1, respectively. Hence, for each replicate, the number of larvae (and parasitoid females) was the same in treatments A1 and A2 as in treatments B1 and B2, as shown in Table 1. For *L. dispar*, some larvae not exposed to parasitoids and maintained as controls were also employed for each replicate (Table 1), so as to be able to determine the mortality rate not ascribable to successful parasitization.

Tab.1 - Number of larvae of *L.dispar* (= number of larvae of *G. mellonella*) used in each replicate for treatments A1 and A2 (*in vitro* trials), B1 and B2 (*in vivo* trials) and employed as controls.

Replicate	Number of larvae used in treatments A1 and A2	Number of larvae used in treatments B1 and B2	Number of *L. dispar* larvae used as controls
1	30	30	11
2	30	30	10
3	15	6	6
4	12	30	4
5	27	18	10
6	—	12	9
Total	114	126	50

The mean weight (± s.d.) of the *G. mellonella* larvae utilised throughout the experiment was mg 312.8±36.7 and mg 333.5±24 in treatments A2 and B2 respectively.

In all treatments, the larvae were removed from the parasitoid cage after 5-6 eggs had been laid on their body. Each was then transferred separately inside 5-cm-diameter plastic Petri dishes which were in turn placed in the rearing chamber at 26±1°C, 75±5% RH, with a 16:8 L:D photoperiod. The *L. dispar* larvae were daily supplied with small pieces of leaves of *Quercus robur* until death. The *G. mellonella* larvae were not fed.

Upon formation, puparia were rinsed, weighed and placed singly into glass tubes. Parasitoid sex was determined upon adult emergence.

The data were processed according to the following parameters:

1. percentage of successfully parasitized larvae (=larvae from which puparia were obtained/larvae contaminated with parasitoid eggs x 100);
2. female puparial weight in mg;
3. male puparial weight in mg;
4. parasitoid larval development time in days (from egg to puparium);
5. female parasitoid pupal development time in days (from puparium to adult);
6. male parasitoid pupal development time in days;
7. percentage of larval mortality (=larvae from which puparia were obtained + dead larvae which did not produce puparia/ larvae contaminated with parasitoid eggs x 100) in the case of *L. dispar*.

Statistical analysis

A factorial analysis of variance was made of the data (Zar, 1984) for the first six parameters (2x2 factors tested for the rearing technique and host effect) (STATISTICA for WINDOWS, 1995). *L. dispar* larval mortality data were analysed by the one-way variance of analysis. Means were compared using the Tukey's HSD multiple range test where significant differences were recorded (a= 0.05) The percentage values were transformed for the analysis using an arcsine transformation (Mosteller and Youtz, 1961).

Results

Successfully parasitized larvae percentages were higher in *G. mellonella* than in *L. dispar*. The host effect was significant. Neither the rearing technique effect nor interaction were significant. The values for the *in vitro*-reared females were however lower than those for the females obtained *in vivo*. (Table 2).

Neither rearing technique nor host effect were found to significantly affect male puparial weight. Nor was interaction significant. On the other hand, the weights of the progeny of the *in vitro*-reared females were higher than those of the progeny of the *in vivo*-reared ones. For this parameter, the rearing technique effect was significant. (Table 2).

Parasitoid development time from egg to pupa and from pupa to adult did not reveal any marked difference among the treatments. The rearing technique and host effects, as well as interaction, were not significant.

In *L. dispar* larvae contaminated by *E. larvarum* females (either produced *in vitro* or *in vivo*) mortality percentages were very high, notwithstanding the fact that successfully parasitized larvae percentages were rather low. Larval mortality percentages were 77.2±8.1 and 82.1±7.2 for the larvae contaminated by *in vitro*- and *in vivo*-reared females, respectively. Both values were significantly higher (p=0.003*) than that for the controls (27.1±12.1). No significant difference was found between the larvae parasitized by *in vitro*- and *in vivo*-reared females.

In *G. mellonella* almost all the larvae which were not successfully parasitized developed up to the adult stage.

Tab.2. - Evaluation of parasitism of the natural host *Lymantria dispar* and of the factitious host *Galleria mellonella* by *Exorista larvarum* cultured *in vitro* and *in vivo*. Means ± s.e.

Parameter	Rearing technique	Host				
		L. dispar	*G. mellonella*	Rearing technique effect (df =1,18)	Host effect (df = 1,18)	Interaction (df = 1,18)
% successfully parasitized larvae	*IN VITRO*	19.8±6.5	76.4±6.6	p = 0.23	*p = 0.0001	p = 0.51
	IN VIVO	22.7±5.8	87.3±2.9			
Female puparial weight (mg)	*IN VITRO*	49.6±10.5	45.8±8.9	*p = 0.009	p=0.69	p = 0.20
	IN VIVO	31.8±9.7	38.9±7.3			
Male puparial weight (mg)	*IN VITRO*	35.5±23.9	48.2±7.7	p = 0.70	p = 0.24	p = 0.46
	IN VIVO	37.9±16.6	40.9±7.5			
Parasitoid larval development time (days)	*IN VITRO*	10±1.4	10±1.7	p = 0.88	p = 0.36	p = 0.36
	IN VIVO	10.5±1.5	9.3±1.03			
Parasitoid female development time from pupa to adult (days)	*IN VITRO*	9.3±0.9	8±1.4	p = 0.64	p = 0.14	p = 0.56
	IN VIVO	8.6±1.5	8.1±1.2			
Parasitoid male development time from pupa to adult (days)	*IN VITRO*	7.9±1.9	7.7±0.9	p = 0.78	p = 0.58	p = 0.82
	IN VIVO	7.9±1.4	7.5±0.8			

Discussion

The diet developed by Mellini and Campadelli (1995) is the cheapest and the easiest to prepare among those so far developed for *E. larvarum*. According to Mellini and Campadelli (1996a), on this diet the adult yields are lower than those reported by Bratti *et al.* (1995) on a diet based on tissue culture media, but were higher than those (35-40%) obtained *in vivo* from the factitious host *G. mellonella*. Moreover, when parasitoids were initially provided with 0.2 ml diet each (as in the present study), Mellini and Campadelli (1996a) obtained puparia weighing 39-40 mg on average, a mean weight similar to that of the puparia usually formed in *G. mellonella* when no more than 3-4 eggs are oviposited on each host (Mellini and Campadelli, 1996b). Since weight may be correlated with individual fitness in parasitoids produced both *in vivo* (Doutt *et al.*, 1976) and *in vitro* (Rojas *et al.*, 1996), it can be presumed that the adults emerged from puparia formed on the diet developed by Mellini and Campadelli (1995) are as fit as those obtained from *G. mellonella* larvae which have not been heavily superparasitized

On a commercial beef homogenate-based diet, Dindo *et al.* (1999) obtained

puparia weighing more than those usually formed in monoparasitized *G. mellonella* larvae. Notwithstanding, fly longevity and fecundity of the females reared on this medium were not significantly higher than those of the females emerged *in vivo* from the above mentioned puparia. Moreover, on this diet, which is rather complicated to handle due to the homogenate consistency, adult yields were lower than those obtained on Mellini and Campadelli's medium (1995). In view of the above, the latter was selected for the *in vitro* production of the flies utilised in the present study.

In relation to the parameters considered in the present study no substantial difference was found to exist between the capability of *E. larvarum* females produced *in vitro* and that of the flies reared *in vivo* to parasitize the natural and the unnatural host in the laboratory. The only parameter significantly affected by the rearing technique effect was female puparial weight which was found to be higher for the host larvae parasitized by the *in vitro*-reared females. A similar trend was also observed for male puparial weight in *Galleria*, but this parameter was not seen to be significantly affected by the rearing technique adopted.

Parasitoid weight may depend on a number of factors including host size (Baronio *et al.*, 1982, Mendel, 1986), species (Moratorio, 1987) sex (Kraaijeveld *et al.*, 1999) and age (Baronio *et al.*, 1982; Mellini, 1984, 1986). To the best of our knowledge, the hypothesis that the size of parasitic insects may be influenced by the size of their parents has not been supported by any research. Notwithstanding, a possibility that should not be ruled out is that the progeny (especially female) of the *in vitro*-reared flies was found to weigh more than the progeny of the *in vivo*-reared ones due to higher parental size. In fact, the puparia produced in the diet developed by Mellini and Campadelli (1995) were heavier on average than those altogether formed *in vivo* since puparia weighing less than 30 or even 20 mg frequently form in *G. mellonella* due to high superparasitization level (Mellini and Campadelli, 1996b).

Further study is required to test the efficacy in the forest of *E. larvarum* reared *in vitro*. It will also be necessary to investigate whether the capability of the females produced *in vitro* to parasitize natural and unnatural hosts decreases when parasitoids are maintained for many generations *in vitro*. It is worth pointing that in the present study, despite the lack of significance, the percentage of successfully parasitized larvae was lower for the *in vitro*- than for the *in vivo*-reared females. After several other generations of culturing on artificial diet this phenomenon may become even more evident. In principle, culturing of parasitoids for too many generations on an artificial diet may not be advisable if good genetic traits are to be retained (Grenier *et al.*, 1994). The fecundity of *Catolaccus grandis* (Burks) females has been shown by Morales-Ramos *et al.* (1996) to be significantly reduced after 10 generations of *in vitro* rearing. Moreover, field tests have revealed that the dispersal ability from the release point of *in vitro*-reared *C.grandis* is lower than that of parasitoids cultured on the natural hosts. Otherwise, a decrease in parasitoid quality might also result from continuous *in vivo* mass culture (Mackauer, 1980).

In the present study, the percentages of successfully parasitized larvae were found to be significantly lower in the natural than in the unnatural host, whether

exposed to *in vivo*- or *in vitro*-reared females. This phenomenon may be ascribable to a number of factors. In the first place, as the integument of *G. mellonella* larvae is smooth, contrary to that of *L. dispar* larvae, *E. larvarum* macrotype eggs adhere better to the host body of *G. mellonella* than to that of *L. dispar*. The result is that parasitoid losses due to egg detachment occur more frequently in the case of the natural host than in that of the unnatural one.

Moreover, a hypothesis for the lower parasitization percentages observed in the natural as compared to the unnatural host is that in old host-parasitoid associations as the one between *L. dispar* and *E. larvarum*, parasitoids and hosts may develop some degree of balance; in new host-parasitoid associations as the one between *G. mellonella* and *E. larvarum*, instead, no interspecific balance has been evolved by the parasitoid and the host. This hypothesis was suggested by Pimentel (1963) and Hokkanen and Pimentel (1984) after comparing a number of new versus old parasitoid-host associations. The potential evolution taking place over 200 years of interaction between *Nezara viridula* (L.) and its tachinid parasitoid *Trichopoda pennipes* (Fabr.) in tropical America has been investigated by Hokkanen (1983) leading him to conclude that this host had evolved a reproductive success in resisting parasitoid attack 10% better than that of hosts from another geographic region which had never been exposed to this tachinid.

It should also be noted that neither development times nor puparial weights were significantly affected by the host effect. All weights, except that of the female progeny of the *in vivo*-reared flies, were even higher in *Galleria* than in *Lymantria* despite the size of the latter being bigger than that of the former and the fact that no more than 2 puparia per larva were ever produced by the natural as compared to the factitious host. Puparial weights, therefore, further suggest *G. mellonella* to be more suitable for *E. larvarum* than *L. dispar*.

Finally, it is worth pointing that in *L. dispar* larvae contaminated by *E. larvarum*, either produced in the diet or in the factitious host, mortality was much higher than in controls. Apart from successful parasitization, mortality was thus due to other causes, probably including partial parasitoid activity and/or viruses or other pathogens. This situation may occur also in natural environment. Studies by Luciano and Prota (1984) have underscored the fact that high *L. dispar* larval mortality caused by pathogens restricts viable *E. larvarum* action in gypsy moth populations in cork-oak forests in Sardinia. Parasitization rates reached as high as 50%, but were generally lower. We can assume that, since in parasitized population larval mortality may be very high despite the low parasitization percentages, inundative release of *E. larvarum* in the forest may be effective against *L. dispar*, if made at the right time and intensity.

Acknowledgements

A special thank-you to Prof. P. Luciano for supplying *L. dispar* egg masses and for his useful suggestions.

This research was supported by Italian Research and University Ministry (MURST ex-40%).

SUMMARY

Parasitism of the factitious host *Galleria mellonella* and of the natural host *Lymantria dispar* by the tachinid *Exorista larvarum* cultured *in vitro* and *in vivo* was evaluated in the laboratory. The *in vivo* and *in vitro* cultures were performed on *G. mellonella* and on an oligidic diet composed of skimmed milk, egg yolk, yeast extract and saccaharose, respectively.

With reference to the parameters examined (namely the percentage of successfully parasitized larvae, parasitoid puparial weights and development times), the results suggest that there was no substantial difference between the *in vitro* and *in vivo* reared parasitoids. Female puparial weight was even significantly higher when the host larvae were parasitized by the *in vitro*-reared females.

The percentages of successfully parasitized larvae were significantly lower in the natural than in the unnatural host, whether exposed to *in vivo*- or to *in vitro*-reared females. Moreover, puparial weight was not significantly affected by the host effect, notwithstanding the fact that *L. dispar* larvae are larger then *G. mellonella* larvae. According to our findings, therefore, the natural host would appear to be less suitable for *E. larvarum*, regardless of whether produced *in vitro* or *in vivo*, than the unnatural host.

KEY WORDS: *Exorista larvarum*, artificial diets, quality control, *Galleria mellonella*, *Lymantria dispar*.

Valutazione in laboratorio del parassitismo di *Galleria mellonella* L. e di *Lymantria dispar* (L.) da parte di *Exorista larvarum* (L.) allevata *in vivo* e *in vitro*

RIASSUNTO

È stata effettuata una valutazione in laboratorio del parassitismo dell'ospite di sostituzione *Galleria mellonella* e di quello naturale *Lymantria dispar* da parte del tachinide *Exorista larvarum* allevato *in vivo* e *in vitro*. Gli allevamenti *in vivo* e *in vitro* sono stati rispettivamente effettuati su *G. mellonella* e su una dieta oligidica composta da latte scremato, tuorlo d'uovo, estratto di lievito di birra e saccarosio.

I risultati hanno indicato che, relativamente ai parametri presi in considerazione (precisamente le percentuali di larve in cui il parassitoide si è sviluppato fino allo stato adulto, i pesi dei pupari e i tempi di sviluppo del parassitoide stesso), non si sono manifestate sostanziali differenze tra i tachinidi allevati *in vitro* e *in vivo*. Anzi, i pesi dei pupari femminili sono risultati significativamente maggiori nelle larve parassitizzate da femmine prodotte *in vitro* piuttosto che *in vivo*.

Indipendentemente dalla tecnica di allevamento con cui erano stati ottenuti i parassitoidi, le percentuali di parassitizzazione sono state significativamente più elevate nell'ospite di sostituzione piuttosto che in quello naturale. Inoltre il peso dei pupari non è stato significativamente influenzato dal fattore ospite, anche se le larve di *L. dispar* sono di dimensioni maggiori rispetto a quelle di *G. mellonella*. In questo studio, pertanto, l'ospite naturale si è dimostrato meno idoneo di quello di sostituzione per *E. larvarum* allevata sia *in vitro* che *in vivo*.

PAROLE CHIAVE: *Exorista larvarum*, diete artificiali, controllo di qualità, *Galleria mellonella*, *Lymantria dispar*.

REFERENCES CITED

BARONIO P., VANCINI D., CAMPADELLI G., CAVICCHI S., 1982.- Variabilità megetica intraspecifica di *Gonia cinerascens* Rond. (Diptera Tachinidae) in relazione allo stadio di contaminazione dell'ospite *Galleria mellonella* l. (Lep. Galleriidae).- *Boll. Ist. Ent. Univ. Bologna*, 36: 27-35.

BRATTI A., CAMPADELLI G., MARIANI M., 1995.- *In vitro* rearing of *Exorista larvarum* (L.) on diet without insect components.- *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 49: 225-236.

CAMPADELLI G., 1973.- Allevamento di *Galleria mellonella* L. (Lepidoptera Galleriidae) con dieta semiartificiale.- *Boll. Ist. Ent. Univ. Bologna*, 32: 11-25.

COHEN A.C., 1985.- Simple method for rearing the insect predator *Geocoris punctipes* (Heteroptera : Lygeidae) on a meat diet.- *J. Econ. Entomol.*, 78: 1173-1175.

COHEN A.C., NORDLUND D.A., SMITH R.A., 1999.- Mass rearing of entomophagous insects and

predaceous mites: are the bottlenecks biological, engineering, economic, or cultural?- *Biocont. News and inform.*, 20: 85-90.

De Clercq P., and Degheele D., 1992.- A meat-based diet for rearing the predatory stinkbugs *Podisus maculiventis* and *Podisus sagitta*. *Entomophaga*, 37: 194-195.

Delrio G., Luciano P., Floris I., 1988.- I parassiti di *Tortrix viridana* L. in Sardegna.- *Atti XIV Congr. Naz. It. Entomologia*, pp.407-414.

Delrio G., Luciano P., Prota R., 1983.- I parassiti di *Malacosoma neustria* (L.) in Sardegna.- *Atti XIII Congr. Naz. It. Entomologia,* pp. 237-244.

Dindo M.L., Farneti R., Scapolatempo M., Gardenghi G., 1999.- *In Vitro* Rearing of the Parasitoid *Exorista larvarum* (L.) (Diptera : Tachinidae) on Meat Homogenate-Based Diets.- *Biol. Control*, 16 (3) : 258-266.

Doutt R.L., Annecke D.P., Tremblay E., 1976.- Biology and host relationship of parasitoids. In: "Theory and practice of biological control" (C.B. Huffaker and P.S. Messenger eds.), pp. 143-168. Academic Press, New York.

Farneti R., Dindo M.L., Fanti P., 1998.- *In vitro* rearing of *Exorista sorbillans* (Wiedemann) and *Meigenia simplex* Tschorsnig & Herting (Diptera: Tachinidae): preliminary results.- *Boll. Ist. Ent. Univ. Bologna*, 52: 75-84.

Fraval A., Graf P., Hamdaoui M., Kadiri Z., Ramzi H., Villemant C., 1989.- "*Lymantria dispar*" (A.Fraval ed.), 220 pp.

Grenier S., 1994.- Rearing of *Trichogramma* and other egg parasitoids on artificial diets.- In "Biological Control with egg parasitoids" (E. Wajnberg and S.A. Hassan eds.), pp. 73-92. CAB Int., Wallingford, UK.

Grenier S., Greany P.D., Cohen A.C., 1994.- Potential for mass release of insect parasitoids and predators through development of artificial culture techniques. In "Pest Management in the Subtropics Biological Control - A Florida Perspective" (D. Rosen, F. D. Bennett and J. L. Capinera eds.), pp.181-205. Intercept Ltd, Andover.

Hafez M., 1953.- Studies on *Tachina larvarum* L. (Diptera, Tachinidae). III. Biology and Life - history.- *Boll. Soc. Fouad Entom.*, 37, 305-335.

Hokkanen H., 1983.- The effect of 200 year association of coevolution between the green stink bug, *Nezara viridula* (Hemiptera, Pentatomidae) and the parasite *Trichopoda pennipes* (Diptera, Tachinidae) on the host-parasite relationship.- In: H. Hokkanen "Interspecific homeostasis, pest problems and the principles of classical biological control pest control". Ph.D Thesis, Cornell University, Ithaca, NY, 157 pp.

Hokkanen H., Pimentel D., 1984.- New approach for selecting biological control agents.- *Can. Ent.*, 116: 1109-1121.

Kraaijeveld A.R., Adriaanse i.C.T., Bergh B. van den, 1999.- Parasitoid size as a function of host sex: potential for different sex allocation strategies.- *Ent. Exp. et Appl.*, 92: 289-294.

Li L.Y., Liu W.H., Chen C.S., Han S.T., Shin J.C., Du H.S., Feng S.Y., 1988.- *In vitro* rearing of *Trichogramma* spp. and *Anastatus* sp. in artificial eggs and methods of mass production.- In "*Trichogramma* and other egg parasites" , Les Colloques de l'INRA, 43 (J. Voegele, J. Waage and J. van Lenteren eds.), pp. 339-352.

Liu J.F., Liu Z.C., Wang C.X, Yang W.H., Li D.S., 1995.- Study on rearing of *Trichogramma minutum* on artificial host eggs.- In "*Trichogramma* and other egg parasitoids", Proc. of the 4th intren. Symp., Cairo Egypt, 4-7 October 1994 (E. Wajnberg ed.), pp. 161-162. INRA, Paris.

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.

Mackauer M., 1980.- Some aspects of quality and quality control of biological control agents during insectary propagation.- *Proc. V. Int. Symp. Biol. Contr. Weeds,* pp.207-220.

Mellini E., 1984.- Importanza dello stadio dell'ospite, al momento della parassitizzazione, per la biologia dei ditteri larvevoridi.- *Frustula Entomologica*, 7-8: 395-419.

Mellini E., 1986.- Importanza dello stadio postembrionale degli ospiti olometabolici, al momento dell'attacco, per la biologia degli imenotteri parassiti.- *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 40: 13-49.

Mellini, E., Campadelli, G., Dindo, M.L., 1993a.- Artificial culture of the parasitoid *Exorista larvarum* L. (Dipt. Tachinidae) on bovine serum-based diets.- *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 47: 223-231.

Mellini, E., Campadelli, G., 1994.- Qualitative improvements in the composition of oligidic diets for the parasitoid *Exorista larvarum* (L.).- *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 49: 187-196.

MELLINI, E., CAMPADELLI, G., 1995.- Formulas for "inexpensive" artificial diets for the parasitoid *Exorista larvarum* (L.).- *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 50: 95-106.

MELLINI, E., CAMPADELLI, G., 1996a.- A first overall comparison between the *in vitro* and *in vivo* production of the parasitoid *Exorista larvarum* (L.).- *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 50: 183-199.

MELLINI, E., CAMPADELLI, G., 1996b.- Analisi del superparassitoidismo di *Exorista larvarum* (L.) nell'ospite di sostituzione *Galleria mellonella* L.- *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 51: 1-11.

MENDEL Z., 1986.- Hymenopterous parasitoids of bark beetles (Scolytidae) in Israel: relationships between host and parasitoid size, and sex ratio.- *Entomophaga*, 31: 127-136.

MORALES-RAMOS J.A., ROJAS M.G., COLEMAN R.J., GREENBERG S.M., SUMMY K.R., KING, E.G., 1996.- Comparison of in vivo versus in vitro-reared *Catolaccus grandis* in the field.- *Proc. Beltwide Cotton Conf.*, 2: 1099-1104.

MORATORIO M.S., 1987.- Effect of host species on the parasitoids *Anagrus mutans* and *Anagrus silwoodensis* Walker (Hymenoptera: Mymaridae).- *Environ. Entomol.*, 16: 825-827.

MOSTELLER, F.M., YOUTZ, C. 1961.- Tables of the Freeman-Tukey transformations for the binomial and Poisson distributions.- *Biometrika*, 48: 433-440.

NORDLUND D.A., WU Z. X., GREENBERG S. M., 1997.- *In vitro* rearing of *Trichogramma minutum* Riley (Hymenoptera : Trichogrammatidae) for ten generations, with quality assessment comparisons of *in vitro* and *in vivo* reared adults.- *Biol. Control*, 9: 201-207.

PIMENTEL D., 1963.- Introducing parasites and predators to control native pests.- *Can. Ent.*, 95: 785-792.

RIDET J.M., 1972.- Etude des conditions optimales d'elevage et d'alimentation de *Lymantria dispar* L.- *Ann. Soc. Entomol. France*, 8: 653-668.

ROJAS M. G., MORALES-RAMOS J. A., KING E. G., 1996.- In vitro rearing of the boll weevil (Coleoptera : Curculionidae) ectoparasitoid *Catolaccus grandis* (Hymenoptera : Pteromalidae) on meridic diets.- *J. Econ. Entomol.*, 89 (5): 1095-1104.

SEHNAL F., 1966. - Kritisches Studium der Bionomie und Biometrik der in verschiedenen lebensbedingungen gezuchteten Wachsmotte, *Galleria mellonella* L. (Lepidoptera). – *Zeit. fur Wissensch. Zool.*, 174: 53-82.

STATISTICA for WINDOWS, 1995.- Statistics II. StatSoft, Inc., Tulsa, OK.

THOMPSON S.N., 1999.- Nutrition and culture of entomophagous insects.- *Ann. Rev. Entomol.*, 44: 561-592.

THOMPSON S.N., HAGEN K.S., 1999.- Nutrition of entomophagous insects and other arthropods.- In: "Handbook of Biological Control" (T. Bellows, T.W. Fisher *et al.* eds.). Chapter 22. Academic Press, San Diego, pp. 594-652.

ZAR, J.K., 1984.- "Biostatistical analysis", 718pp. Prentice Hall, Englewood Cliffs.

Correspondent author's address: Dr. Maria Luisa Dindo, Istituto di Entomologia "G. Grandi", via F. Re, 6, I-40126 Bologna, Italy
E-mail: ldindo@agrsci.unibo.it.