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In vitro rearing of *Exorista sorbillans* (Wiedemann) and
Meigenia simplex Tschorsnig & Herting
(Diptera Tachinidae): preliminary results. (*)

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INTRODUCTION

The development of artificial diets for the *in vitro* rearing of entomophagous insects is considered a promising technique for mass-producing parasitoids and predators more easily and less expensively. For this purpose, the diets employed should be economical and easy to prepare (Mellini, 1975; Grenier *et al.*, 1994; Thompson, 1986; Vinson, 1986, 1994).

Mellini and Campadelli (1995a, b; 1996) and Dindo *et al.* (1997) respectively reared the tachinid *Exorista larvarum* (L.) and the chalcidid *Brachymeria intermedia* (Nees) from egg to adult on artificial media composed of a few, easy to find and quite inexpensive ingredients. Adult yields were close to being satisfactory for the economical mass production of both species.

In the present study, attempts were made to rear two tachinid parasitoids, *Exorista sorbillans* (Wiedemann) and *Meigenia simplex* Tschorsnig & Herting, on artificial diets based on the best media previously tested for *E. larvarum* and *B. intermedia*. *E. sorbillans* is a polyphagous, gregarious larval endoparasitoid of Lepidoptera, including *Lymantria dispar* (L.) and *Hyphantria cunea* Drury (Mesnil, 1965). Females lay macrotype eggs on the host body (Manjunatha and Puttaraju, 1993). Parasitoid development is dependent on host physiology, as first instar larvae only moult to second instar after the host has moulted to last larval instar (Oshiki and Nakazawa, 1987).

M. simplex is a solitary larval endoparasitoid of Chrysomelidae, mostly included in the genera *Melasoma* (= *Chrysomela*) and *Crioceris* (Tschorsnig & Herting, 1998).

The development of *M. simplex* also depends on host physiology, as the parasitoid larvae only moult to second instar when the host has reached larval maturity. The biology of this tachinid can be compared to that of *Meigenia mutabilis* Fallén described by Mellini (1954).

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Because of their synchronised development with the host, the *in vitro* rearing of both *E. sorbillans* and *M. simplex* appeared difficult.

MATERIALS AND METHODS

1. Biological materials

Adults of both species were kept in Plexiglas cages (40x30x30 cm) in a rearing chamber at $26\pm 1^\circ\text{C}$, $65\pm 5\%$ RH, and a 16:8 L:D photoperiod. Flies were fed on lump sugar and cotton balls soaked in a honey and water solution. Watering was provided with cotton plugs moistened with the distilled water contained in a small plastic reservoir.

A colony of *E. sorbillans* was not reared *in vivo*. Adults were obtained from *Smerinthus ocellatus* L. larvae collected in the field.

A colony of *Meigenia simplex* was established from adults which had emerged from *Chrysomela populi* L. larvae collected in nature on *Populus nigra* L. leaves. The colony was maintained on *C. populi* larvae collected in the field. Parasitization was performed placing second or early-third instar host larvae in the cages containing the flies (4 larvae per fly on average). Depending on female oviposition activity, the larvae were removed after 2 to 4 hours when 2-3 eggs had been laid on each. The parasitized larvae were maintained in plastic rearing boxes (30x25x20 cm) and supplied with fresh poplar leaves. Upon being formed, the puparia were removed from the host remains, rinsed in distilled water and placed on filter paper on the bottom of a 5-cm diameter glass Petri dish. The Petri dishes were left in the same rearing chamber as the adult cages.

C. populi larvae and adults were kept at $25\pm 5^\circ\text{C}$, $65\pm 5\%$ RH and a 16:8 L:D photoperiod in the previously described rearing boxes and fed on fresh poplar leaves. To induce oviposition, adults were transferred into special wooden cages with walls made of plastic net (40x20x20 cm). They were supplied with fresh poplar leaves, which were changed daily. Leaves with eggs were removed from the cages, deposited on filter paper placed on the bottom of 20-cm diameter glass Petri dishes and covered with fresh poplar leaves. After hatching, the larvae were transferred into the rearing boxes.

2. *In vitro*-rearing procedures

The two parasitoids were reared *in vitro* on two basic diets, both devoid of hormones (I and II) and supplemented with $2\mu\text{g/ml}$ 20-hydroxyecdysone (20-HE) (I+ecd* and II+ecd*). *E. sorbillans* was also reared on the same diets I and II integrated with $4\mu\text{g/ml}$ 20-HE (I+ecd** and II+ecd**). The $2\mu\text{g/ml}$ concentration was based on previous tests performed with another tachinid, *Pseudogonia rufifrons* (Wied.), a parasitoid dependent on host hormonal balance which only moults from the first to the second larval instar when it is triggered by the host ecdysteroids (Baronio and Sehnal, 1980). Fanti (1990) demonstrated that, *in vitro*, the first instar larvae of the parasitoid only moulted to second instar when the diet was added with $1\mu\text{g/ml}$ 20-HE. Further studies showed that, when the diet was integrated with $2\mu\text{g/ml}$ 20-HE the percent moulting to second instar was similar or even

higher than that obtained with 1µg/ml. (Farneti, 1998).

M. simplex was also reared on a sub-natural medium composed of homogenate of mature larvae of *C. populi* (diet III).

A. Preparation of diets

Diet (I) was prepared by mixing 7.7 g skimmed milk and 0.2 g saccharose in a 25 ml sterile beaker. An agar-water suspension (6% of agar) was prepared separately by dissolving 1.5 g agar in 25 ml distilled water in a 50 ml flask. The suspension was sterilised at 120°C for 15 minutes and left to cool for about 5 minutes at room temperature. To give a 1.2% (w/w) final agar concentration in the diet, 2.65 ml agar-water suspension were removed using a sterile syringe and added to 0.7 g yeast extract (Sigma Chemical Co. USA, cod. Y-0550) in a second 25 ml sterile beaker. Both beakers were then covered with tinfoil and heated at 120 °C for 10 minutes. Separately, a chicken egg was surface sterilised with 60% ethanol and rinsed with distilled water. The shell was broken using a sterile glass stirrer. The yolk was carefully placed in the bottom of a Petri dish. Then, 1.4 ml yolk were removed using a 1 ml sterile syringe and added to the content of the first beaker, which had been allowed to cool at room temperature for about 10 minutes. A 10 mg/ml solution of Gentamicin (Sigma Chemical Co. USA) was mixed into the beaker's content at the rate of 0.01 ml/ml using a 1 ml sterile syringe, to obtain a 0.005% (w/w) final concentration in the diet. The second beaker was removed from the autoclave. After cooling to 55°C, the contents of the two beakers were finally mixed.

Diet (II) was prepared by mixing 8.5 ml commercial veal homogenate (Gerber®, by Gerber Products Co., USA) and 1 ml egg yolk obtained as described for diet (I). The homogenate was removed from the pot using a 10 ml sterile syringe. Gentamicin solution was then added, according to the method and at the same rate as described for diet (I). Separately, 0.5 g yeast extract (the same used in diet (I)) were mixed in a second 25 ml sterile beaker, with 2.12 ml agar-water suspension prepared as described for diet (I). The final agar concentration in the diet was 1% (w/v). The beaker's content was heated as described for diet (I), left to cool and finally added to the first beaker's content.

To prepare diets (I+ecd*), (I+ecd**), (II+ecd*) and (II+ecd**), the above-mentioned diets (I) and (II) were integrated with 20-HE (Sigma Chemical Co., USA - cod H5142). The hormone was added to the diets at the rates of 2µg/ml (diets I+ecd* and II+ecd*) and 4µg/ml (diets I+ecd** and II+ecd**) before pipetting the media into the rearing plates.

Diet (III) was composed of larval homogenate of *C. populi*. By means of tweezers, the larvae were induced to secrete salicylic aldehyde drops which were then absorbed with filter paper. After rinsing with distilled water they were heated in water at 60 °C for 12 minutes and homogenized at 20,000 revolutions for 3 minutes.

All diets were pipetted into the wells of 24-well plastic rearing plates (Nunclon, Denmark) (0.4-0.5 ml per well). Each well had a diameter of 1.6 cm.

Tab. 1: Nutrient content (%) of the basic artificial diets utilized for the *in vitro* rearing of *Exorista sorbillans* and *Meigenia simplex*. Percentages were calculated on the total diet weight (diet I) or volume (diet II), Gentamicin solution and agar suspension excepted. The diets were tested both devoid and supplemented with 20-hydroxyecdysone.

	Diet I	Diet II
Skimmed milk	77 (w/w)	-
Veal homogenate (Gerber®)	-	85 (v/v)
Chicken egg yolk	14 (w/w)	10 (v/v)
Yeast extract	7 (w/w)	5 (w/v)
Saccharose	2 (w/w)	-

B. Collection of parasitoid eggs or larvae and *in vitro* incubation conditions

E. sorbillans

The parasitoid eggs were collected from superparasitized *Calliphora vomitoria* (L.) mature larvae, which had been exposed to females for about 1 hour (about 2 larvae per female). The larvae were introduced in the tachinid rearing cages every 5-6 days. It should be noted that *C. vomitoria* is a non-host species of *E. sorbillans*. Nevertheless, as the larvae were accepted by the parasitoid females and as they were also easily available at a low price in fishing tackle shops, they were selected as an oviposition substrate for the parasitoid. No more than 10 eggs were deposited on each larva, the mean being 5-6 eggs per larva. The eggs were loosely attached so that they easily came off the integument when the larvae were dipped in distilled water. The eggs were surface sterilized for 3 minutes in 60% ethanol and rinsed three times with sterile distilled water in glass Petri dishes. They were then placed on the diets contained in the multiwell plates (one egg per well) using sterile spatulae.

Meigenia simplex

This parasitoid was reared *in vitro* starting either from macrotypic eggs collected from the host body surface (test 1) or from first instar larvae obtained by dissecting the host larvae (test 2).

Test 1. The eggs were collected from 2-3 previously superparasitized *C. populi* third instar larvae, which were exposed to parasitoid females for 4-5 hours. The parasitized larvae were rinsed for 1 minute with 60% ethanol and then with sterile distilled water, and finally dried with filter paper. The eggs were removed from the host integument using spatulae, after having pinned the host larva to a paraffin support. The eggs were surface sterilized for 2 minutes in 60% ethanol, rinsed three times with distilled water and transferred individually into the wells of multiwell plates containing the diet.

Test 2. Parasitoid first instar larvae were obtained by exposing host first instar or early-second instar larvae to *M. simplex* females in the tachinid rearing cages. The host larvae were removed from the cages as soon as one egg had been laid on their surface, placed in the rearing boxes described in section A and fed with poplar leaves. They were dissected with a sterile bistoury about 20-24 hours after

parasitoid egg hatching, when they were in the second stage. Before dissection the larvae were rinsed for 1 minute with 60% ethanol and then with sterile distilled water, and finally dried with filter paper. The parasitoid larvae were removed using sterile spatulae, briefly rinsed with distilled water, and transferred individually into the rearing plates.

All plates were sealed with Parafilm® and kept in darkness at 26 ± 1 °C throughout the experiment except when they were removed for daily examinations.

Instruments and glassware were sterilized by autoclaving for 20 minutes at 120°C. All operations, including visual examinations, were performed in a laminar flow hood.

3. Experimental design

E. sorbillans

Several replicates were carried out, each comprising a different number of eggs. The total number of eggs placed on each diet is given in table 2. Results were assessed in terms of the number and percentage of (1) live first instar larvae (LI) observed 4 days after placing the eggs on the media; (2) second instar larvae (LII); (3) third instar larvae (LIII); (4) puparia and (5) adults. Calculation of the percentages of live first instar larvae and adults was based on the original number of eggs placed on the media. The percentages of second instar larvae, third instar larvae and puparia were determined on the basis of the number of first, second and third instar larvae respectively.

Meigenia simplex

Three replicates were carried out each comprising a different number of eggs or first instar larvae. The total number of individuals placed on each diet is shown in table 3. Only one replicate comprising 12 first instar larvae was performed for diet III. Results were assessed in terms of the number of (1) LI recorded 2 days after placing the eggs on the media; (2) LII; (3) LIII; (4) puparia.

The data were not sufficient for statistical analysis to be made.

RESULTS

E. sorbillans

Given that empty egg shells were difficult to distinguish from non-hatched eggs and that newly-hatched larvae were very hard to see, actual hatching rate on the media was difficult to assess. It was therefore decided to calculate the percentages of LI observed 4 days after placing the eggs on the media instead of those of hatched eggs. These percentages were found to range from 50 to 81.3 (table 2). It should be noted, however, that the lowest percentage was found on diet (II+ecd**), which was contaminated by moulds in a number of wells 3 days after placing the eggs. In all diets a number of individuals reached the third larval stage. The percentages of LII ranged from 14.8 on diet (II) to 44.4 on diet (I+ecd*) while those of LIII (calculated on the number of LII) ranged from 33.3 on diet (II+ecd*) to 100 on diet (I+ecd**). Only a few puparia developed on diets (I), (I+ecd**)

and (II+ecd**), pupation rates being between 25-50%. The only puparium which developed on diet (II+ecd**) led to the emergence of one adult, development time from egg to adult being 54 days. Development times for the other individuals were observed to be extremely variable: some larvae died within two days after being placed on the media, while others survived on the diet for more than 60 days.

Tab. 2: *In vitro* rearing of *E. sorbillans* on diets devoid and supplemented with 2µg/ml (*) or 4µg/ml (**) 20-HE (ecd). Number and percentages (in brackets) of eggs, of live first instar larvae observed on the media 4 days after placing the eggs (LI), of second instar larvae (LII), of third instar larvae (LIII), of puparia (P), and of adults (A).

Diet	n. eggs (%)	n. LI (%)	n. LII (%)	n. LIII (%)	n. P (%)	n. A (%)
I	32	26 (81.3)	9 (34.6)	4 (44.4)	1 (25)	-
II	33	27 (81.2)	4 (14.8)	2 (50)	-	-
I+ecd*	13	9 (69.2)	4 (44.4)	2 (50)	-	-
I+ecd**	14	11 (78.6)	4 (36.4)	4 (100)	2 (50)	-
II+ecd*	12	8 (66.7)	3 (37.5)	1 (33.3)	-	-
II+ecd**	14	7 (50)	3 (42.9)	2 (66.7)	1 (50)	1 (7.1)

Tab. 3: *In vitro* rearing of *M. simplex* on diets devoid and supplemented with 20-HE (ecd*), performed starting from eggs (test 1) or first instar larvae (test 2). LI = number of live first instar larvae observed on the media 2 days after placing the eggs (1) or removed from the host and transferred onto the diets (2); LII = number of second instar larvae; LIII = number of third instar larvae; P = number of puparia.

Diet	test	eggs	LI	LII	LIII	P
I	1	33	29	-	-	-
	2	/	10	-	-	-
II	1	36	33	-	-	-
	2	/	11	-	-	-
I+ecd*	1	32	23	2	-	-
	2	/	11	4	1	-
II+ecd*	1	38	35	4	-	-
	2	/	10	5	2	1

M. simplex

The results of tests 1 and 2 are reported in table 3. In both tests, no parasitoid moulted to second larval instar on the diets devoid of 20-HE, whereas a few larvae

managed to do so on the hormone integrated ones. A few LIII developed on the same diets in test 2. One parasitoid pupated on diet (II+ecd*), but failed to emerge as an adult. Development time from egg to puparium lasted 11 days.

The larvae placed on diet (III), made up of mature *C. populi* larvae homogenate, died almost immediately, probably because of the salicylic aldehyde contained in the substrate. Although most secretion was absorbed before homogenizing the larvae its concentration in host tissue remained apparently too high to permit parasitoid life.

DISCUSSION

The complete development of *E. sorbillans* from egg to adult was previously obtained by Watanabe and Mitsuhashi (1995) on sub-natural media composed of *B. mori* hemolymph, and by Mitsuhashi (1996) on diets based on tissue culture media integrated with host hemolymph and other components (i. e. melon fly artificial diet, wheat bran, cotton, toilet paper, banana or apple) which improved diet consistence and prevented the parasitoid larvae from being submerged. *E. sorbillans* larvae were observed to survive for a long time on diets devoid of host material, composed of insect cell culture medium with 10% foetal bovine serum, moulting however to the second instar only sporadically. By adding 20-HE at the final concentration of 1 µg/ml to the diet many larvae moulted to the second instar but only a few reached the third stage and none of these pupated (Mitsuhashi and Oshiki, 1993; Mitsuhashi, 1996). Parasitoid pupation (Mitsuhashi and Oshiki, 1993) and development to the adult stage (Watanabe and Mitsuhashi, 1995; Mitsuhashi, 1996) have been observed only on media supplemented with host components.

The results obtained in the present study however show that *E. sorbillans*, despite its dependence on host physiology, may be reared *in vitro* from egg to adult on host material-free diets supplemented with 20-HE. *In vivo*, host physiology especially affects the first larval moulting of the parasitoid, after which the growth and development of *E. sorbillans* are rapid (Oshiki and Nakazawa, 1987).

In all of the diets tested, both integrated and devoid of 20-HE, a few larvae developed up to the third stage. On diets (I), (I+ecd**) and (II+ecd**) some puparia formed. This result suggests that *E. sorbillans* may complete its larval development even on diets devoid of hormones. As already shown by Mitsuhashi and Oshiki (1993), however, the addition of 20-HE to the media may have increased the rate of first larval moulting. This agrees with the findings of Oshiki and Nakazawa (1987) according to which the growth and development of the parasitoid is accelerated by the exogenous application of 20-HE to abdomens of *B. mori* isolated from the thorax by a ligature. In the present study, the total percent yields of third instar larvae obtained on the diets supplemented and devoid of 20-HE were 64 and 46% respectively. Moulting to the second larval instar represented a critical moment in the development of the parasitoid, with larval mortality ranging from 56 (diet I+ecd*) to 85% (diet II).

The percentages of live first instar larvae (LI) reflected the actual hatching rate of *E. sorbillans in vitro*. These percentages were close to those of the hatched

eggs obtained *in vivo* in the host *B. mori* (Devaiah *et al.*, 1993). The time of the development of the only parasitoid which developed from egg to adult on the diet was 54 days, a time comparable to that observed in *B. mori* in the laboratory, which ranged from 25 to 60 days (Oshiki *et al.*, 1989). In the present study, the adult emerged from a puparium weighing 46.8 mg. The weights of the puparia obtained *in vivo* in the host *B. mori* ranged from 35 to 110 mg (Mitsuhashi and Oshiki, 1993), whereas the puparia formed in the natural host *S. ocellatus* (from which the adults utilized in the present study emerged) weighed 76 mg on average. As shown by Oshiki *et al.* (1989), the adult emergence from puparia obtained from the host *B. mori* was successful only in the case of the puparia weighing more than 30 mg.

M. simplex never moulted to the second instar on diets devoid of 20-HE, neither when the eggs nor when the first instar larvae were placed on the media (tab. 3). When parasitoid eggs were placed on diets integrated with hormones some larvae moulted to the second instar but none to the third (test 1, table 3). When first instar larvae were placed on the media a few reached the third stage (test 2, table 3). These larvae were removed from the host body 20-24 hours after egg hatching. It has already been demonstrated that on artificial diets the time of permanence of the parasitoid in the host body may affect the rate of moulting to the second instar. For the tachinid *P. rufifrons*, Fanti and Bratti (1991) showed that when first instar larvae were removed from the host body after more than 72 hours the rate of moulting to the second instar increased. Subsequent parasitoid development, however, was seemingly unaffected by parasitoid permanence time within the host. For another tachinid, *Eucelatoria bryani* (Sabr.), Nettles *et al.* (1980) showed that larval mortality rate was high unless the larvae were removed from the host and transferred onto the diet at least 6-8 hours after parasitization; the mortality rate considerably dropped when the time of permanence of the parasitoid within the host was 24 hours.

One parasitoid reached the pupal stage on diet (II+ecd*), without any adult, however, being formed within the puparium. Development time from egg to puparium was 11 days, a time comparable to those usually observed *in vivo* in the host *C. populi*.

Since only a few eggs and larvae were placed on the media it cannot be stated with certainty that the addition of 20-HE to the diet was indispensable to the larval development of *M. simplex in vitro*. On hormone-free media, however, no parasitoid moulted to the second instar whether from egg (test 1) or from first instar larva (test 2).

In conclusion, the diets tested in the present study need to be improved as regards the nutrient and hormone content, in order to increase the adult yields of *E. sorbillans*, which proved to be capable of developing to the adult stage even on host-material-free media, and to favour the complete *in vitro* development of *M. simplex*, which failed to develop beyond pupal stage.

SUMMARY

Attempts were made to rear two tachinids, *Exorista sorbillans*, a gregarious larval endoparasitoid of Lepidoptera, and *Meigenia simplex*, a solitary larval endoparasitoid of Chrysomelidae, on host material-free artificial media. Because of the synchronised development with the host, both *E. sorbillans* and *M. simplex* appeared to be difficult to rear *in vitro*. The two parasitoids were cultured on two basic diets, both devoid of hormones (I and II) and supplemented with 2µg/ml 20-HE (I+ecd* and II+ecd*). *E. sorbillans* was also reared on the same diets (I) and (II) integrated with 4µg/ml 20-HE (I+ecd** and II+ecd**). The basic diets (I) and (II) had been previously successfully utilized for two other parasitoids, the tachinid *Exorista larvarum* (I) and the chalcidid *Brachymeria intermedia* (II). The main ingredient of the diets was (I) skimmed milk and (II) commercial veal homogenate, respectively. Both diets were integrated with chicken egg yolk and yeast extract. Diet (I) also contained saccharose. *E. sorbillans* reached the third larval stage (though at low percentages) in all the diets tested. Only a few larvae pupated on diets (I), (I+ecd**) and (II+ecd**). The puparium formed on diet (II+ecd**) led to the emergence of an adult, thus proving that despite its dependence on host hormonal balance, *E. sorbillans* may be reared from egg to adult on host material-free diets, integrated with 20-HE. *M. simplex* only moulted to second larval instar on diets supplemented with 20-HE. A few larvae moulted to third instar on these diets and one puparium formed on diet (II+ecd*), which, however, did not contain an adult inside.

Allevamento *in vitro* di *Exorista sorbillans* (Wiedemann)
e *Meigenia simplex* Tschorsnig & Herting
(Diptera, Tachinidae): risultati preliminari

RIASSUNTO

Sono stati effettuati dei tentativi di allevamento *in vitro* di due tachinidi, *Exorista sorbillans*, endoparassitoide polifago gregario di larve di lepidotteri, e *Meigenia simplex*, endoparassitoide solitario di crisomelidi, su diete prive di materiale derivato dall'ospite. Essendo lo sviluppo di queste specie dipendente dalla fisiologia dell'ospite, esse si presentavano a priori come particolarmente difficoltose da allevare *in vitro*. I due parassitoidi sono stati allevati su due diete di base (I e II) sia prive che integrate con 20-idrossiecdisone (20-HE), alla concentrazione di 2µg/ml (diete I+ecd* e II+ecd*). Inoltre, *E. sorbillans* è stata anche allevata sulle stesse diete (I) e (II) integrate con 20-HE alla concentrazione di 4µg/ml (diete I+ecd** e II+ecd**). Le due diete di base (I) e (II) erano state già sperimentate con successo per due altri parassitoidi, il tachinide *Exorista larvarum* (I) e il calcidide *Brachymeria intermedia* (II). Come ingrediente principale, le due diete contenevano rispettivamente latte scremato (I) e omogeneizzato commerciale di carne di vitello (II). Entrambe sono state integrate con tuorlo d'uovo di gallina ed estratto di lievito di birra. La sola dieta I conteneva anche saccarosio. *E. sorbillans* ha raggiunto il terzo stadio larvale (sia pure in bassa percentuale) in tutte le diete saggiate. Sulle diete (I), (I+ecd**) e (II+ecd**), poche larve si sono impupate. Dal pupario formatosi sulla dieta (II+ecd**) è sfarfallato un adulto. È stato così provato che, nonostante la sua dipendenza dal bilancio ormonale della vittima, *E. sorbillans* può essere allevata da uovo ad adulto su diete prive di materiale derivato dall'ospite, integrate con 20-HE. *M. simplex* è mutata in seconda età solamente sulle diete integrate con 20-HE. Su queste diete, poche larve sono anche mutate in terza età e, sulla dieta (II+ecd*) una si è impupata, senza che però, all'interno del pupario, si formasse l'adulto.

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REFERENCES

- BARONIO P., SEHNAL F., 1980. - Dependence of the parasitoid *Gonia cinerascens* on the hormones of its Lepidopterous hosts. - *J. Insect Physiol.*, 26: 619-626.
- DEVALIAH M.C., GOVINDAN R., NARAYANA SWAMY K.C., 1993. - Life cycle of uzi fly, *Exorista bombycis*. - In: Recent advances in uzi fly research, Proceed. National Seminar on Uzi fly and its control, 16-17 January, 1992, Bangalore, India (Dandin S.B. ed.) pp. 1-12.
- DINDO M. L., SAMA C., FANTI P., FARNETI R., 1997. - *In vitro* rearing of the pupal parasitoid *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae) on artificial diets with and without host components. - *Entomophaga* 42 (3): 445-453.
- FANTI P., 1990. - Fattori ormonali inducenti la prima muta larvale del parassitoide *Pseudogonia rufifrons* Wied. (Diptera: Tachinidae) in substrati di crescita *in vivo* e *in vitro*. - *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 45: 47-59.
- FANTI P., BRATTI A., 1991. - *In vitro* rearing of the larval stages of the parasitoid *Pseudogonia rufifrons* Wied. (Diptera: Tachinidae): preliminary results. - 4th European Workshop, Perugia 3-5 april 1991. *Redia*, vol. LXXIV, App. n. 3, 1991, pp. 449-452.
- FARNETI R., 1998. - Allevamento di insetti parassitoidi su diete artificiali. - Tesi di Dottorato di ricerca in Entomologia Agraria (X ciclo), 162 pp.
- GRENIER S., GREANY P.D., COHEN A.G., 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. Intercept Ltd., Andover, pp. 181-205.
- MANJUNATHA H. B., PUTTARAJU H. P., 1993. - The egg of Uzi Fly, *Exorista sorbillans* (? *E. bombycis* Louis) (Diptera: Tachinidae). - *Appl. Entomol. Zool.*, 28 (4): 574-577.
- MELLINI E., 1975. - Possibilità di allevamento di Insetti Entomofagi parassiti su diete artificiali. - *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 32: 257-290.
- MELLINI E., 1954. - Studi sui Ditteri Larvevoridi. II. *Meigenia mutabilis* Fall. su *Agelastica alni* L. (Coleoptera: Chrysomelidae). - *Riv. Paras.*, 15 (4): 489-512.
- MELLINI E., CAMPADELLI G., 1995a. - Further simplifications in the composition of oligidic diets for the parasitoids *Exorista larvarum* (L.). - *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 49: 211-223.
- MELLINI E., CAMPADELLI G., 1995b. - 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., 1996. - Latest results in the rearing of the parasitoid *Exorista larvarum* (L.) on oligidic diets. - *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 50: 143-153.
- MESNIL L., 1965. - Larvaevorinae (Tachininae). - In: Lindner E., Fliegen palaearktische Region, X (2): 555-864.
- MITSUHASHI J., 1996. - *In vitro* rearing of tachinid maggots on artificial diets. - Proceed. XI Intern. Conf. Invert. Cell Culture, San Francisco, California, U.S.A., June, 21-26, 1996, pp. 134-141.
- MITSUHASHI J., OSHIKI T., 1993. - Preliminary attempts to rear an endoparasitic fly, *Exorista sorbillans* (Diptera Tachinidae) *in vitro*. - *Jpn. J. Ent.*, 61 (3): 459-464.
- NETTLES W.C. JR., WILSON C.M., ZISER S.W., 1980. - A diet and methods for the *in vitro* rearing of the tachinid, *Eucelatoria* sp. - *Ann. Entomol. Soc. Am.*, 73: 180-184.
- OSHIKI T., NAKAZAWA K., 1987. - Growth and development of the larva of an endoparasitoid, *Exorista sorbillans* in the larva of *Bombyx mori*. - *J. Seric. Sci. Jpn.*, 56 (6): 494-499.
- OSHIKI T., NAKAZAWA K., YOKOYAMA T., NAKAYAMA F., SHIMADA J., 1989. - Parasitic habits of the tachinid fly, *Exorista sorbillans*. - *Bull. Farms Tokyo Univ. Agr. Tech.*, 13: 22-33.
- THOMPSON S.N., 1986. - Nutrition and *in vitro* culture of insect parasitoids. - *Ann. Rev. Entomol.*, 31: 197-219.
- TSCORSNIC H. P., HERTING B., 1998. - A new species of the genus *Meigenia* Robineau-Desvoidy (Diptera: Tachinidae). - *Stuttg. Beitr. Naturk. Ser. A*, nr. 569: 1-5.
- VINSON S.B., 1986. - New prospects for the augmentation of natural enemies of pests of the Pacific region. - *Agric. Ecosyst. Envir.*, 15: 167-173.
- VINSON S.B., 1994. - Parasitoid *in vitro* rearing: successes and challenges. - In "Techniques of Insect Rearing for the development of integrated pest and vector Management strategies" (J.P.R. Ochieng - Odera Ed.), pp. 49-108. ICIPE Science Press, Nairobi.
- WATANABE M., MITSUHASHI J., 1995. - *In vitro* rearing of an endoparasitic fly *Exorista sorbillans* (Diptera: Tachinidae). - *Appl. Entomol. Zool.*, 30 (2): 319-325.