Boll. Ist. Ent. "G. Grandi" Univ. Bologna, 48: 93-100, 1993.

ALESSANDRO BRATTI
Istituto di Entomologia "Guido Grandi," Università di Bologna.

In Vitro Rearing of *Lydella thompsoni* Herting and *Archytas marmoratus* (Town.) (Dipt.Tachinidae) Larval Stages: Preliminary Results. (*) (1)

Introduction

Lydella thompsoni Hert. is one of the main parasitoid of *Ostrinia nubilalis* Hübner in Italy (Maini and Burgio, 1990). In Europe it ranges from Spain to southern Russia, being especially common in Italy, Yugoslavia, Rumania and Hungary (Galichet *et al.*, 1985).In the United States it was imported from 1920 to 1940, although it had to be re-introduced thereafter in the northern part of the country (Burbutis *et al.*, 1981). The rearing techniques *in vivo* perfected for this tachinid by Wood and Rodriguez (1989) have resulted in yields of sufficient number to enable inundative releases against *O. nubilalis* in Texas.

Archytas marmoratus (Town.), an important larval-pupal parasitoid of many Noctuid species in the southern United States (Arnaud, 1978), is of considerable practical interest because it can be mass-reared, whether on the natural (*Helicoverpa zea* Boddie) or on the factitious host *Galleria mellonella* L. (Gross and Johnson, 1985; Gross, 1993; Bratti and Costantini, 1991). Because the fecundity of both of these tachinids is high (an average of 500 maggots/female for *L.thompsoni* and 4000 for *A.marmoratus*) they are particulary suited to *in vitro* rearing (Bratti, 1993). However, while the development cycle of *L. thompsoni* is partially host-dependent, that of *A. marmoratus* is dependent on the physiology of its host (Mellini, 1990). This last behaviour might be a limit for its eventual rearing on artificial diet (Mellini, 1975; Bratti, 1993). The present study reports and discusses the results of biological tests run to monitor the growing-capability of *L. thompsoni* and *A. marmoratus* on insect-component (mainly of hemolymph and pupal extracts) diets and meridic ones based on that developed by Nettles (1986) for *E. bryani*.

Materials and Methods

Lydella thompsoni.

Insect rearing. *O.nubilalis* and *L.thompsoni* were reared after Wood and

(*) Accepted for publication November 26, 1993.

(1) Research supported by C.N.R. Bilateral-Project N° 92.01586.CT06.

Rodriguez (1989).

In vitro first-instar rearing. The maggots were extracted from the uterus of fecund females. The uterus was disinfected by a 70% ethanol solution for a few seconds, placed it on a concave glass slide and rinsed in a 0.8% NaCl solution. The newly hatched maggots were then placed on another glass slide by a pipette and, after blotting any excess liquid with small pieces of filter paper, dipped in several types of pupal extracts, which had been taken from the chrysalides of the three Lepidoptera species *O. nubilalis*, *Manduca sexta* L. and *H. zea* using the method conceived by Bratti (1990). Small balls of absorbent cotton in sealed Petri dishes (35 mm diam x 10 mm high) were used as breathing supports for the maggots; cotton was also used to absorb Nettles's (1986) meridic diet. Each of the media was tested on 40-50 *L. thompsoni* first instars and all the material stored in a growth chamber 27-28°C and 100% r.h.

In vitro second-instar rearing. The parasitoids were collected by dissecting the penultimate-instar larvae of *O. nubilalis* that had been parasitized about 48 h beforehand after Wood and Rodriguez (1989) and then disinfected in 70% ethanol. About 300 maggots, which were removed from the uterus of a number of females, were put in aqueous solution and placed by pipette in a Petri dish (147 mm diam) containing respectively 2, 5, 10 and 20 host larvae. This was done to determine if it was possible to superparasitize the *O. nubilalis* larvae and collect by dissection the largest possible number of parasitoids. The tested diets were those of Nettles (DN) (1986) and its following modified versions: (a) DN without soybean flour (DNWS); (b) DNWS with egg yolk (1.6%) (DNWSEY); (c) DN with egg yolk (1.6%) (DNEY); (d) DNEY soaked into cotton without agar (DNEYC); (e) and DNEY at 0.75% agar (DNEYHA). The diets were sterilized and poured into MicroWell 96 F containers, which were kept for 24 h at room temperature and r.h. After the introduction of the biological material on the diet surface, the containers were stored in growth chambers at 27-28°C and 90% r.h.; approximately one hundred maggots were used in all tests. The biological parameters recorded were the percentage of L3, puparia and adults (including those partially emerged, calculated on the number of initial maggot placed in the media).

Archytas marmoratus.

Insect rearing. The host *H. zea* and the parasitoid *A. marmoratus* were reared after Bratti *et al.* (1992).

In vitro first-instar rearing. One-day-old planidia, which had been laid by fecund females on a filter-paper support inside the rearing cages, were removed by special needle, disinfected in a formaldehyde solution (0.4%) and rinsed in sterilized distilled water on concave glass slides. The aqueous solution was then blotted up with filter paper and replaced by liquid media (both hemolymph than Nettles meridic diet). Each maggot was transferred by Gilson dispenser in 10-20 µl drop into a cell of a Microwell 96 F dishes. All the diets tested were based on last-instar haemolymph of *M. sexta* and *H. zea* extracted and prepared after Xie *et al.* (1986); the exact diets composition is shown in table 2. Approximately twenty maggots were reared per diet; no physical support to aid insect breathing was used

since the objective was not so much full parasitoid rearing as the start of feeding. The growth indicators employed were mid-gut colour and direct visual monitoring of planidium size (volumteric growth was measured only in one case).

In vitro second-instar rearing. The maggots were removed by dissection from pupae of *H. zea* that had formed 24 h earlier and were transferred on the diets by using a special spatula. Each host-larva was parasitized in last instar (full-grown larva) by placing about 10 planidia on its body surface. Then the newly-formed chrysalides were disinfected in a 70% ethanol solution for ten seconds and then placed on Petri capsules containing paraffin for dissection. The second instars, distinguishable by their mouth apparatus, were disinfected in a formaldehyde solution and rinsed in a nutrient solution similar to the diet. The two tested media were based on DNEY supplemented respectively with 20% homogenate of last instar (DNEYLH) and pupae (DNEYPH) of *H. zea*. The *H. zea* larval homogenate was prepared by coddling the larvae in water at 60°C for 10 min and blending by weight with a Virtis homogenizer 1 part larva to 10 parts sterile distilled-water; the pupae were homogenized after Bratti and Monti (1988). The diets were mixed, poured into plastic Petri dishes (55 diam x 15 hgt mm.) and left at room temperature and r.h. for 24 h; about 10 maggots were used per diet in as many Petri dishes (2-3 ml per maggot), and the percentages of puparia formed were calculated. All the material (glassware, scissors, etc.) was autoclaved at 121°C for 15 min, and all operations were carried out under laminar-flow hood.

Results and Discussion

Lydella thompsoni. No significant results emerged from the first instar rearing: the maggots moved along the walls of the dishes and died within 24-48 h. There may be several reasons for this failure, including the fact that the insect-component employed were collected from a different growth stage and, in two cases, different species than those attacked by the parasitoid in nature. Other tachinids have been successfully reared on diets containing insect materials differing from those of the natural host (Bratti and Campadelli, 1993). If, as reported by Grenier (1986) for the planidia of *Lixophaga diatreae* Town., the ingestion of food is stimulated by the kind of free aminoacids and their ratio, the Nettles diet is unsuitable for this goal.

The rearing of the second instars was hampered by the failure to superparasitize the host and the resulting short supply of the former. It was found that if more than a dozen larvae had penetrated *Ostrinia nubilalis* after the first 24 h, the host died, succumbing to a kind of rotting away, and that the surving host larvae contained only 5-6 alive parasitoids at best, the average being about 3 per host larva. This rather scant number is a serious drawback to the applicaton of the *in vitro* technique (Bratti, 1993). By contrast, the situation is altogehter different for such other tachinids as *E. bryani*, *P. rufifrons* and *Palexorista laxa* (Curran). For these species by artificially superparasitizing the host larvae it is possible to collect by dissection 150-200, 20-30 and 40-50 maggots\host-larva, respectively (Nettles *et al.*, 1980; Bratti and Monti, 1988; Bratti and Nettles, unpublished data). The precocious mortality induced by the parasitoids in the larvae of *O. nubilalis* may be

ascribed to the marked reaction in response to the formation of the tracheal funnel, i.e. the debilitated larvae are undermined by bacteria-induced degenerative processes that eventually cause their demise.

Of the diets employed to rear the second instars, only DNWS failed to promote growth; all the others, albeit in varying percentages, enabled development to the puparium and DNEY even to the adult stage (Tab.1). Note too that in many of the dissected puparia the adult was found almost fully formed.

The tested diets are definitely not the optimum pabulum for this tachinid even though from the second instar on many maggots successfully grow and develop *in vitro*. A similar response has also been found in other parasitoids which develop only after spending a certain amount of time inside the host (Nettles *et al.*, 1980; Bratti and Benini, 1991; Volkoff *et al.*,1992). No practical advantage is offered by superparasitization and subsequent dissection as a way of obtaining biological material for i*n vitro* rearing purposes; by contrast, egg yolk and soy flour seem to have a beneficial effect on the growth and development of *L. thompsoni* maggots.

Tab. 1 - *In vitro* rearing of *Lydella thompsoni* second instar maggots.

Diet	Third instar	Puparia	Adults partially emerged	Adults
DNWS	0/20	0/20	0/20	0/20
%	0.0	0.0	0.0	0.0
DNEY	8/22	3/22	2/22	1/22
%	36.3	13.6	9.1	4.5
DNWSEY	4/19	3/19	1/19	0/19
%	21.1	15.8	5.2	0.0
DNEYC	11/23	5/23	3/23	0/23
%	47.8	21.7	13.0	0.0
DNEYHA	8/24	2/24	1/24	0/24
%	33.3	8.3	4.1	0.0

DNWS - Nettles' diet without soy flour.
DNEY - Nettles' diet with egg yolk.
DNWSEY - Nettles' diet without soy flour and with egg yolk.
DNEYC - Nettles' diet with egg yolk, without agar and with cotton as phisical support.
DNEYHA - Nettles' diet with egg yolk and with half agar concentration.

Archytas marmoratus. The planidia, despite their being immersed in a liquid environment, are able to breathe and survive, at least over the first 72 h, by placing their rear tracheal spiracles above the liquid surface. Table 2 shows that the few fully developed planidia fed on media containing egg yolk, cholesterol and the hemolymph of *H. zea* pupae. All these diets presented a certain amount of sterols. The insects used the sterols both as structural cell components and in ecdysteroid synthesis (Reinecke, 1985). While these substances have proved to be indispensable for several parasitoid species when added to their diets (Thompson, 1986), the limited data available for *A. marmoratus* make it impossible to establish a

direct correlation between cholesterol and the onset of feeding in this species. However it is indicative that, *in vivo* in the factitous host *G. mellonella*, *A. marmoratus* first instars register peak growth in the prepupal phase before host metamorphosis (Bratti *et al.*, 1993), developmental stage characterized by high ecdysteroids level (Sehnal *et al.*, 1988). Parasitoid dependence on host hormones is a phenomenon common to several parasite symbioses (Mellini, 1975, 1983; Beckage, 1985; Lawrence, 1986): for example, *P. rufifrons*, a tachinid with a biological cycle very similar to *A. marmoratus*, evinces *in vitro* the effect of ecdysone on the first-second instar moult (Fanti and Bratti, 1991). Table 2 shows that only three of the second instars reared reached the puparium stage although more than half developed to third instar on both diets. The second instars were reared individually because they evinced cannibalism a negative trait where mass rearing is concerned. Here, too, as in the case of *L. thompsoni*, the parasitoid grows, albeit partially, only after a certain period spent feeding in the host. The Nettles diet is, even when supplemented with host material, unsatisfactory for the growth and development of *A. marmoratus*.

That both tested species evince high fecundity and provide, via their relative easy hatching, a ready supply of maggots underscores the need to identify which factors (nutritional and otherwise) stimulate the onset of trophic activity. While it would appear from the limited number of available data that cholesterol induces in *A. marmoratus* the taking and ingestion of food, further and more detailed studies are required fully to elucidate this question.

Tab. 2 - *In vitro* rearing of *Archytas marmoratus* larval stages.

Diet composition	Stage reared	Results
M.sexta larval HEM 10 µl each drop.	First instar	No growth
M. sexta larval HEM, egg yolk (5, 10, 20 and 40%) 10 µl each drop.	First instar	Slight growth of 4 maggots on diets with 5 and 10% of egg yolk.
M. sexta larval HEM, cholesterol (0.10%), Tween 80 (1/15 ratio) 10 µl each drop.	First instar	Slight growth of 3 maggots.
M. sexta larval HEM, cholesterol (0.005, 0.01, 0.02. 0.04%), 1-15 µl each drop.	First instar	Slight growth of 8 maggots in 0.01 and 0.02% cholesterol.
M. sexta larval HEM, cholesterol (0.01%), Tween 80 (ratio with cholesterol: 1/1, 1/5, 1/10, 1/15). 10-15 µl each drop.	First instar	Growth of 6 maggots: 7.56 mm^3 (mean vol.) vs. 3.2 mm^3 of laid planidia. Ratio Tween 80/cholesterol: 1/10, 1/15
H. zea HEM. 10 µl each drop.	First instar	Slight growth of 3 maggots.
DNEYLH	Second instar	6 third instars and 3 puparia.
DNEYPH	Second instar	8 third instars and 4 puparia.

DNEYLH - Nettles' diet, egg yolk (1.6%) and larval homogenate.
DNEYPH - Nettles' diet, egg yolk (1.6%) and pupal homogenate.

SUMMARY

Lydella thompsoni Hert. and *Archytas marmoratus* (Town.) maggots were reared in two sets of artifical diets, those featuring insect components and meridic ones modelled on that developed by Nettles for *Eucelatoria bryani* Sab. (1986).

Lydella thompsoni. First instars failed to grow on the diets based on pupal extract of *Ostrinia nubilalis* Hübner, *Helicoverpa zea* Boddie and *Manduca sexta* L. While second instars fed on the meridic diets without insect material evinced puparium rates ranging from 8.3 to 21.7%, only the Nettles' diet that was supplemented with 1.6% egg yolk yielded 1 viable adult.

Archytas marmoratus. The planidia reared on the diets supplemented with cholesterol (0.01 and 0.02% concentration range), egg yolk (5 and 10%) and *H. zea* pupal haemolymph registered slight growth, although they did not moult . The second instars reared on the two meridic diets supplemented with *H.zea* larval and pupal homogenate reached the puparium stage but not developed into viable adults.

Key words: *Ostrinia nubilalis*, *Helicoverpa zea*, parasitoids, artificial diets.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Lita Wood, Mission (Tx), who supplied the *Lydella thompsoni* colonies, and Dr. W. C. Nettles, in whose laboratory part of the research was performed.

Allevamento *in vitro* degli stadi larvali di *Lydella thompsoni* Hert. e *Archytas marmoratus* (Towns.) (Dipt.Tachinidae): risultati preliminari.

RIASSUNTO

Gli stadi larvali di *Lydella thompsoni* Hert. e *Archytas marmoratus* (Towns.) sono stati allevati su diete artificiali a base di componenti di insetto e meridiche basate su quella ideata da Nettles per *Eucelatoria bryani* Sab.(1986).

Lydella thompsoni. Le larve di prima età non sono cresciute su nessuna delle diete a base di estratto pupale di *Ostrinia nubilalis* Hübner, *Helicoverpa zea* Boddie, e *Manduca sexta* L. Mettendo in coltura larve di seconda età si sono ottenute, in diete meridiche prive di componenti d'insetto, percentuali di pupari oscillanti dall'8.3 al 21.7%, mentre solo da quella di Nettles, addizionata con tuorlo d'uovo (1.6%), si è formato un adulto vitale.

Archytas marmoratus. Dei planidi allevati, solo quelli nelle diete liquide contenenti colesterolo (in concentrazioni dello 0.01 e 0.02%), tuorlo d'uovo (5 e 10%) ed emolinfa di pupe di *H.zea*, si sono leggermente accresciuti, senza, per altro, mai mutare. Nelle due diete meridiche, addizionate con omogeneizzato di larve e di pupe di *H.zea*, ponendo in coltura le larve di seconda età, si sono formati alcuni pupari ma nessun adulto vitale.

REFERENCES CITED

ARNAUD P.H. Jr., 1978. A host-parasite catalog of North American Tachinidae(Diptera). *U.S. Dep. Agric. Misc. Publ.* N° 1319, 860 pp.

BECKAGE N.E., 1985. Endocrine interactions between endoparasitic insects and their hosts. - *Ann. Rev. Entomol.*, 30:371-413.

BRATTI A., 1990. - Allevamento *in vitro* di *Pseudogonia rufifrons* Wied. in estratti di omogeneizzato di *Galleria mellonella* L. e su diete meridiche. - *Boll.Ist.Entom."G. Grandi" Univ.Bologna*, 44:11-22.

BRATTI A., 1993. - Principi generali per l'allestimento di diete artificiali per gli stadi larvali dei Ditteri Tachinidi e nuovi contributi nel campo della sperimentazione. - Collana M.A.F, in press.

BRATTI A., CAMPADELLI G., 1993. - Comparison of Insect-material in a Meridic Diet for *Exorista larvarum* L. (Dipt.Tachinidae) *in Vitro* Rearing. -*Boll.Ist.Entom."G. Grandi" Univ.Bologna*, 48: 59-65.

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

BRATTI A., BENINI S., 1991. - Allevamento *in vitro* delle larve di *Pseudogonia rufifrons* Wied. (Dipt.Tachinidae). Prove su diete subnaturali e meridiche .- *Boll.Ist.Entom."Guido Grandi" Univ.Bologna*, 46:71-85.

BRATTI A., COSTANTINI W., 1991. - Effects of new artificial diets on the host-parasitoid system *Galleria mellonella* L. (Lepidoptera Galleriidae)-*Archytas marmoratus* Town. (Diptera Tachinidae)-*REDIA* , 74:445-448.

BRATTI A., GARDENGHI G., MIGLIOLI V., 1993. - Behavior and Growth Rate of *Archytas marmoratus* (Town.)(Diptera Tachinidae) Planidia in Larvae of *Galleria mellonella* L. (Lepidoptera Galleriidae). - *Boll.Ist.Entom."G. Grandi" Univ.Bologna*, 47: 141-150.

BRATTI A., NETTLES W.C.Jr., FANTI P., 1992. - Influence of *Helicoverpa zea* (Lepidoptera: Noctuidae) Age During the Last Instar on Rates of Parasitization by the Larval-Pupal Parasitoid, *Archytas marmoratus* (Diptera:Tachinidae).- *Environ. Entomol.*, 21: 1196-1201.

BURBUTIS P., ERWIN N., ERTLE L., 1981. - Reintroduction and Establishment of *Lydella thompsoni* and Notes on Other Parasites of the European Corn Borer in Delaware.- *Environ. Entomol.*, 10: 779-781.

FANTI P., BRATTI A., 1991.- *In vitro* rearing of the larval stages of the parasitoid *Pseudogonia rufifrons* Wied. (Diptera Tachinidae): preliminary results.- REDIA 74: 449-452.

GALICHET P.F., RIANY M., AGOUNKE D., 1985. - Bioecology of *Lydella thompsoni* Herting, (Dipt.Tachinidae) within the Rhone delta in southern France. - *Entomophaga*, 30:315-328.

GRENIER S., 1986. - Biologie et physiologie des relations hôtes-parasïtoides chez 3 tachinaires (Diptera, Tachinidae) d'intérêt agronomique. Developpement en milieux artificiels. Lutte biologique. - These présentée davant l'I.N.S.A de Lyon et L'Universieé Claude Bernard Lyon. 156 pp.

GROSS H.R. Jr., 1993. - Advances in Mass Propagation of *Archytas marmoratus* (Diptera Tachinidae), A Primary Parasitoid of *Helicoverpa zea* and *Spodoptera frugiperda* (Lepidoptera Noctuidae). - *Environ. Entomol.*, in press.

GROSS H.R. Jr., 1985. - *Archytas marmoratus* (Diptera: Tachinidae): advances in large-scale rearing and associated biological studies. - *J. Econ.Entomol.*, 78: 1350-1353.

LAWRENCE P.O., 1986. - Host-parasite hormonal interactions: an overview. - *J. Insect Physiol.*, 32: 295-298.

MAINI S., BURGIO G., 1990. - I parassitoidi di *Ostrinia nubilalis* (Hb.) in Emilia Romagna. - *Inf.tore Fitopat.*, 9: 19-28.

MELLINI E., 1975. - Studi sui Ditteri Larvevoridi.XXV. Sul determinismo ormonale delle influenze esercitate dagli ospiti sui loro parassiti. - *Boll.Ist.Entom."G. Grandi" Univ.Bologna*, 31: 165-203.

MELLINI E., 1983. - L'ipotesi della dominanza ormonale, esercitata dagli ospiti sui parassitoidi, alla luce delle recenti scoperte nella endocrinologia degli insetti. - *Boll.Ist.Entom."G. Grandi" Univ.Bologna*, 38: 135-166.

MELLINI 1990. - Sinossi di biologia dei Ditteri Larvevoridi.-*Boll.Ist.Entom."G. Grandi" Univ.Bologna*, 45: 1-38.

NETTLES W.C. Jr., 1986. - Effects of soy flour, bovine serum albumine, and three amino acid mixtures on growth and development of *Eucelatoria bryani* (Diptera:Tachinidae) reared on artificial diet. - *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.

NETTLES W.C. Jr., WILSON C.M., ZISER S.W., 1980. - A diet and methods for the *in vitro* rearing of the tachinid *Eucelatoria bryani* sp.- *Ann. Entomol. Soc. Am.*, 71: 383-388.

REINECKE J.P., 1985. - Nutrition:Artificial Diets. - In: Comprehnsive Insect Physiol. Pharm., 4: 391-419. Ed.Kerkut G.A. and Gilbert L.I. Pergamon Press.

SEHNAL F., FONAGY A., AKAI H., KALLEDORNS H.G., 1988. - Prothoracic glands and ecdysteroid titre in *Galleria mellonella* larvae.- *J. Insect Physiol.*, 7: 609-614.

THOMPSON S.N., 1986. - Nutrition and in vitro culture of insect parasitoids. - *Ann. Rev. Entomol.*, 31: 197-219.

VOLKOFF N., VINSON S.B., WU Z.X., NETTLES W.C. Jr., 1992. - *In vitro* rearing of *Trissolcus basalis* (Hym.,Scelionidae) an egg parasitoid of *Nezara viridula* (Hem., Pemtatomidae).- *Entomophaga* 37 (1): 141-148.

WOOD L., RODRIGUEZ J.M., 1989. - Techniques for mass production of *Lydella thompsoni* a parasitoid of european corn borer. - USDA., 1989. International Symposium on Biological Control Implementation. Abstracts. April 4-6, 1989, McAllen, Texas, USA.

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