Boll. Ist. Ent. "G.Grandi" Univ. Bologna, 47: 141-149, 1993.

ALESSANDRO BRATTI°, GIUSEPPE GARDENGHI°°, VALENTINA MIGLIOLI°°°
° Istituto di Entomologia "Guido Grandi", University of Bologna, Italy.
°° Dipartimento di Biologia Evoluzionistica Sperimentale, University of Bologna, Italy.
°°° Holder of post-graduate research grant from the Cassa di Risparmio di Cento (Ferrara), Italy.

Behavior and growth rate of *Archytas marmoratus* (Town.)(Diptera Tachinidae) planidia in larvae of *Galleria mellonella* L. (Lepidoptera Galleriidae)(*)

(Research supported by M.U.R.S.T. 40%)

INTRODUCTION

The larval-pupal parasitoid *Archytas marmoratus* (Town.), a tachinid important in agriculture as an antagonist of many Noctuid species (Arnaud, 1978), has recently been reared on the factitious host *Galleria mellonella* L. (Bratti and Costantini, 1991; Gross, 1992), and attempts are now under way to test its viability for *in vitro* rearing (Bratti, 1993). The eggs laid in the enviroment by the females hatch immediately, freeing planidium-type maggots that penetrate the host larva and molt to second instar only after host pupation. The maggots enter under the pupa wing pads where they induce a secondary respiratory funnel and complete development, killing the host (Hughes, 1975). The first-instar maggots of the tachinid species that lay microtype eggs escape host reactions by avoiding contact with the host hemocoele, by temporarily inhabiting such organs as muscles, salivary glands and the digestive tract (Clausen, 1940; Salt, 1968; Mellini, 1990; Vinson, 1990). Very little is known about the ethology of first-instar maggots of planidium-laying species. Bratti *et al.* (1992) argued that the complex behavior of *A. marmoratus* maggots allows them to evade host defences and grow to a size that enables the parasitoid to avoid encapsulation when it finally enters the host hemocoele.

The present study determined the position and the growth rhythm of planidia in parasitizing *G. mellonella* penultimate and last instars.

MATERIAL AND METHODS

A two-part trial was conducted on the host *G. mellonella* and its parasitoid *A. marmoratus*, which were reared as reported in Bratti and Costantini (1991). The

---

(*) Accepted for publication January 28, 1993.

objective of the first trial was to determine the maggots' position inside the body of last instars host. Penultimate instars were isolated in a 12x12x10 cm container and allowed to molt. Thereafter the last instars were removed, parasitized with an average batch of three maggots hatched from eggs laid within 24 h and placed in other, identically sized containers supplied with abundant food. Three larvae were removed at one-day intervals and placed in freezer at -10°C for about 15 min to prevent shifting of the planidium; thereafter samples of integument, whose transparency made the parasitoid visible, were taken from randomly selected host larvae for the histological tests. The latter were conducted under standard procedures, i.e. fixing in Bouin's liquid of the plugs of integument containing the planidium, paraffin embedding, serial cutting in 8μ slices and staining with Mayer's hemallume and eosin. The observations continued up to the prepupal stage.

The second part measured the growth of the parasitoid maggots in the penultimate and last host instars. Groups of host larvae were selected in fifth instar and parasitized in the sixth (L6)(penultimate) and seventh (L7)(last) instars as reported *supra*. Maggot growth was measured by optical microscope with micrometer eyepiece, calculating length and width by volume after Bouletreau (1968); the first-instar maggots were killed with 5% nitric acid to prevent body contraction. Depending on the time elapsed between parasitization and planidia removal, six groups of *A. marmoratus* maggots, ranging in number from 15 to 32, were determined: LP, one-day-old (hatched on substrate) planidia; L6 1-2, maggots extracted after 1 and 2 days; L7 1-2, maggots extracted after 1 and 2 days; L7 3-4, maggots extracted after 3 and 4 days; L7 6-7, maggots extracted after 6 and 7 days; and prepupae, maggots extracted from prepupae after 7-8 days. The data were processed by once-criterion analysis of variance after Kruskal and Wallis and, for means comparison, by non parametric multiple comparison test (Zar, 1984).

RESULTS

Maggot position. The histological observations showed that after one day the maggot is encased in a capsule-like "pouch" situated between the cuticle and the epidermis. The latter seamlessly wraps up the parasitoid. The individual epidermal cells appear disordered, being arranged in two or three layers, and of irregular shape in comparison to the others, far from the maggot. These cells are so arranged as to form a regular, single-layered cube-like epithelium. Outside the pouch and next to the epidermal cells are other cells arranged in masses which are larger around the penetration point. These massed cells at times look just like the epidermal cells and are continuous with them and at others differ in appearance and seem to form filler tissue of hemocytic origin. The entry hole is open to act as a direct air intake. Internally, along the neck of the pouch containing the planidium, there are brown-coloured clumps of acellular coagulate, which remained through day 2 (fig.I,1).

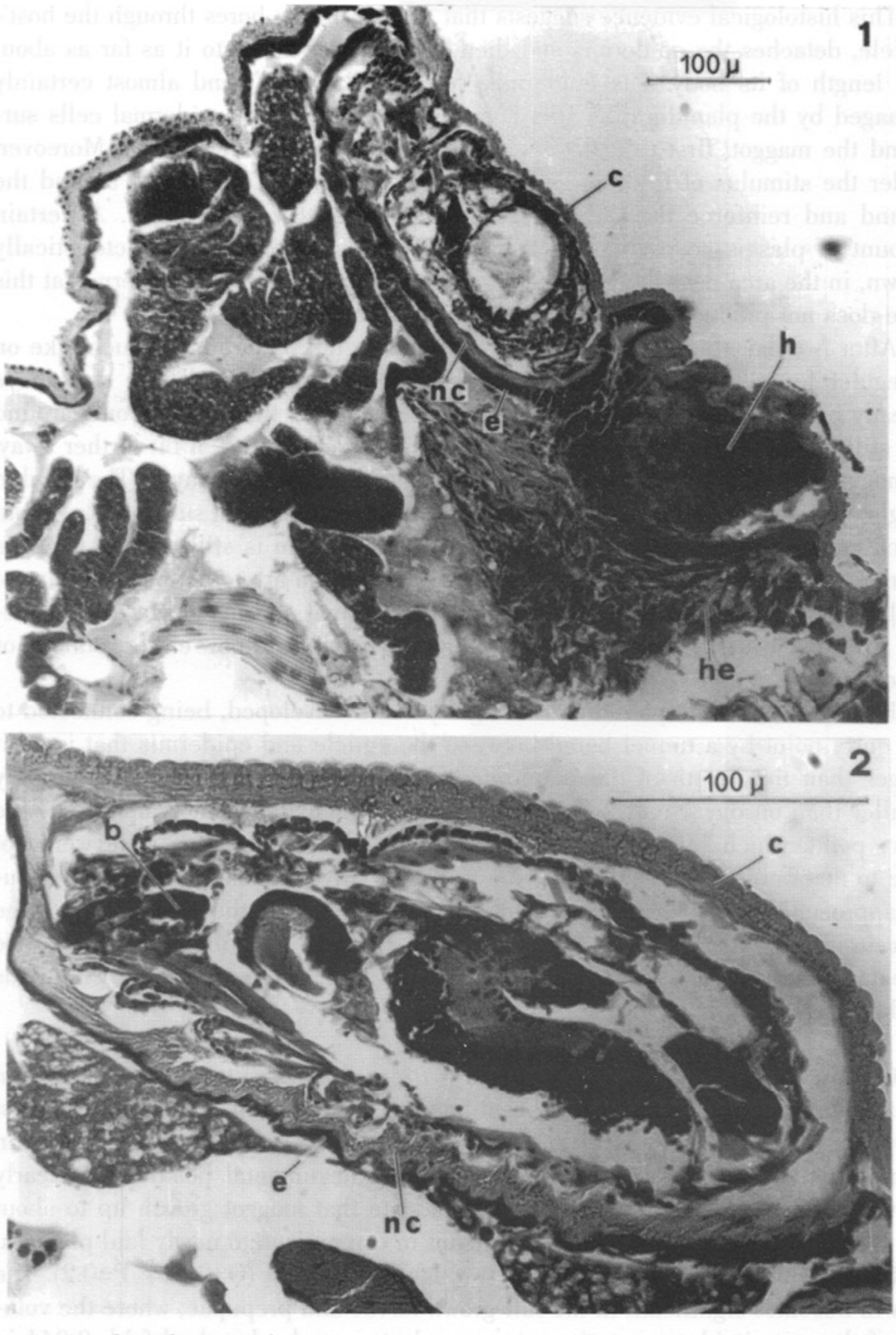

Fig. I - Histological section showing an *A.marmoratus* maggot between the old and the newly formed cuticle of *G. mellonella* larvae 2 days (1) and 4 days (2) after parasitization. The histological cut partially slashes across the entry hole (1).

c, cuticle; *e*, epidermis; *h*, entry hole; *nc*, new cuticle, *he*, hemocytes; *b*, buccopharyngeal apparatus.

This histological evidence suggests that the planidium bores through the host's cuticle, detaches the epidermis and then slides under and into it as far as about one length of its body. The epidermis, forcibly "unglued" and almost certainly damaged by the planidium, is stimulated to proliferate. The epidermal cells surround the maggot, first in a disorderly and then in a regular manner. Moreover, under the stimulus of the foreign body, hemocytes collect in masses around the wound and reinforce the planidium's pouch walls at various point. A certain amount of plasma seeps into the pouch and oxidizes, turning characteristically brown, in the area near the wound. Unglued from the cuticle, the epidermis at this time does not produce, as it will later, new cuticle.

After two days the epidermal cells surrounding the planidium's pouch take on a regular form like that of the cells far from the planidium. These cells have already produced a new cuticle, which is turned towards the parasite; only around the still open entry wound are there masses of epidermal and, a bit farther away, hemocyte cells (fig.I,1). The situation is not much changed by day 3. The regular, single epidermal layer has already secreted the new cuticle that surrounds, without touching the pouch walls, the planidium. The entry hole is still open. Along the segment entering the chamber containing the planidium are to be found clumps of acellular coagulate. However, the masses of epidermal and hemocyte cells detected after day 1 and 2 around the pouch and entry point are now either reduced or absent.

By day 4 and 5 the planidium is larger yet still enveloped, being connected to the entry point by a tunnel bored between the cuticle and epidermis that is a bit longer than the length of the planidium's body. The tunnel diameter is slightly smaller than on day 1 and 2 and carpeted with coagulated hemolymph up to the entry point, which seldom is occluded (Fig.I,2). In these few cases we were not able to determine whether the planidia were still alive. By day 6, with the prepupa approaching, the situation remains unchanged, and at the host prepupa, the parasitoid's first instars are still enveloped by the host's old cuticle yet about to be rejected. They are outside the host's body and ready to bore under the wing pads of the newly formed pupa.

Growth rate of first instar parasitoids. Planidium volume increases progressively and significantly along with the advance of the host larva's physiological stage (T=106.873; P<0.0001). This growth, denoting a certain trophic activity of the planidium even in intra-integumental position, is clearly shown in figure II. The recorded values indicate that maggot growth up to about day 3 of post-parasitization is not significant in comparison to newly laid planidia, 0.004 vs 0.008 mm^3 of maggots after two days in the host (Q=2.464; P=0.2). The growth becomes significant in the full-grown larvae and prepupae, where the volume of the parasitoid maggots increases nearly ten- and a hundred-fold: 0.044 in the former and 0.61 mm^3 in the latter (Q=6.23 and P<0.001; Q=8.34 and P<0.001). Significant too is the difference between the maggots extracted at L7 3-4 days and at L7 6-7 days and the prepupa: Q=3.67; P<0.001 and Q=4.512;

P<0.001. In the full grown host larvae many planidia are in the ventral area ready to bore into the wing pads of the newly formed pupa.

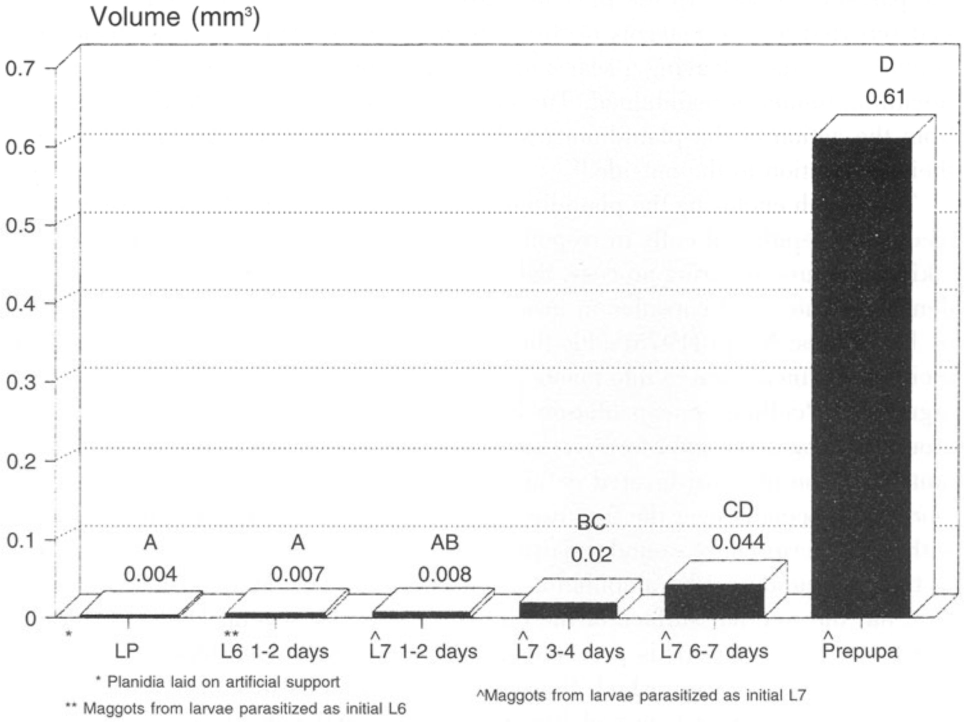

Fig. II - Growth of *A. marmoratus* maggots collected from penultimate and last instar of *G. mellonella* larvae. Means with the same letter above the columns do not differ significantly (Non parametric multiple comparisons test; $P < 0.01$).

CONCLUSIONS

The histological evidence clearly reveals that the planidium does not come into contact with the hemocoele via the cuticle but detaches the epidermis from the rest of the integument, thereby mechanically and/or chemically inducing a proliferation of the epithelial cells that secrete a new cuticle. The parasitoid remains wrapped in a pouch external to the host's body, evincing in this instar an ectoparasitoid behavior similar to that reported by Gerling *et al.* (1991) for the larva of *Eretmocerus* spp., a parasitoid of *Bemisia tabaci* L. The Aphelinid induces the formation in the host of an epidermal capsule. The capsule allows the larva parasitoid to exploit the host by isolating itself from the host's lacunoma.

The situation found in the *G. mellonella-A. marmoratus* system does not exactly match the one for *Heliothis virescens F.-Archytas marmoratus* reported by Hughes (1975), who found the first-instar maggots in a sub-integumental position via the induction of primary respiratory funnels. According to Mellini (1965), the respiratory funnel is a structure deriving from processes of integumental scar-tissue formation in the proximal area and from a hemocyte defence reaction in the distal area; there is also a very thin membrane of hemocytic nature encapsulating the parasitoid's body. In the present study, by contrast, the situation is similar to that reported for the maggots of *Archytas analis* F. by Allen (1926): "penetration is effected without leaving a scar and no surface breathing pore with the attached breathing funnel is maintained. The detachment occurring inside the integument from the action of the planidium results only in tunnels that are mostly open in their connection to the outside."

The pouch enclosing the planidium is not so much a capsule as a structure formed by the epithelial cells in response to their damaging and hence generated by a kind of wound reparing process. Salt (1970) notes that much of the available evidence indicates that capsules in insects are formed principally, if not exclusively, by hemocytes; Nappi (1975) adds that "other cells and tissues of the host may be incidentally incorporated into hemocytal capsules;" and Smith and Ratcliff (1977) argue that "cellular encapsulation involves the aggregation of host hemocytes around a parasite or other foreign tissues present in the hemocoele, with the resultant formation of multi-layered cellular sheath." Yet our observations on *A. marmoratus*, especially over the first two days, coincide less with those just cited than with the description of wound repairing reported by Bohn (1975): "an open wound in the skin produced by amputation or cutting is immediately sealed by a blood clot, but the internal surface of the clot is followed by several layers of flattened and living hemocytes. This provisional wound closure is soon followed by definite wound closure during which time the epidermal cells in highly flattened form migrate through these layers of living hemocytes and join up again." The same author also notes that "wound sealing and encapsulation evince similar histological traits."

The planidium remains in an "external" position up to host pupa formation, during which time it significantly increases in size, reaching peak growth rates close to pupation. This growth, even during occupation of a host organ, is also reported in other tachinids. Tothill (1922) found a certain size increase in first-instar maggots of *Compsilura concinnata* (Meigen), which first feed and grow between the peritrophic membrane and the intestinal wall of the host and then penetrate the body cavity by inducing the formation of tracheal funnels. Mellini *et al.* (1986) report that after "the maggots of *Pseudogonia rufifrons* Wied., hatched from microtype eggs in the gut of *G. mellonella*, entered in the host's muscle tissue, and grew mainly near the end of the larval-pupal moult". In *A. marmoratus*, the peak weight increase coincides with the approach of the host pupal molt, suggesting a likely effect on parasitoid growth of the host's ecdysteroids, which in this ontogenetic stage reach very high concentrations (Sehnal *et al.*, 1986). Yet this rapid weight increase might also be explained by the fact that parasitoids attacking late larval and prepupal insects may need high levels of lipids and proteins. Supplied to late larval and pupal hosts these chemicals probably enable first instar parasitoids to feed faster, grow to an optimal size for molting and initiate rapid development at the onset of their hosts' metamorphosis (Lawrence, 1990).

The taking of provisional refuge by first-instar maggots in an organ of the host is a phenomenon typical of tachinids that lay microtype eggs in the enviroment (Mellini, 1990). The newly hatched maggots of these species are very small: with a length usually in the 0.02-0.2 mm range and a width of about 2/3 of length (Thompson, 1924), they would, once free in the hemocoele, surely face encapsulation. While the initial size of the planidia of *A. marmoratus*, about 0.5 mm in length, is much larger than that of the maggots hatched from microtype eggs, it is still not enough to counter the host's hemocyte reactions (Bratti *et al.*, 1992). The findings of the present study indicate that the intra-integumental position taken up by the planidia of *A. marmoratus* and their subsequent growth are in all likelihood linked to strategies enacted by the parasitoid to circumvent the host's immune system. Vinson (1990) lists five approaches employed by parasitoids to counter the immune system's response: avoidance, evasion, destruction, suppression and subversion. The present investigation into the behavior of first-instar maggots in host larvae is an example of "avoidance," the ability of the parasitoid to counter the immune system simply by avoiding it: the planidia temporarily occupy the integument until reaching a size enabling them to bore into the pupa. In this stage of *G. mellonella*'s development the *A. marmoratus* maggot bores with the front of its body into the host's hemocoele by inducing the formation of a secondary respiratory funnel (Hughes, 1975). Although this structure is determined by the parasitoid, it is largely a response to the reaction of the host's hemocytes (Mellini, 1965) and can thus be defined in Vinson's terms as a case of "subversion," wherein the host's immune system is exploited by the parasitoid to complete its growth.

SUMMARY

Archytas marmoratus (Town.) is a solitary larval-pupal parasitoid that attacks many Noctuid species. It has been reared on the factitious host, *Galleria mellonella* L. for several years. The females lay eggs that hatch immediately. The maggots, recognized as planidium type, penetrate the host larva and molt to second instar only after host pupation.

The present study determined the position and the growth rhythm of planidia in parasitizing *G. mellonella* penultimate and last instars.

The findings indicate that the first instars of *A. marmoratus* live, in the host-larva, in an intra-tegumental position. The maggots remain in the host larva in an "external position", between the old cuticle and the newly formed cuticle secreted by the damaged epidermal layer, up to host pupa formation. During this time they significantly increase in size, reaching peak growth rate close to pupation. The intra-tegumental position taken up by the planidia and their growth are in all likelihood linked to strategies enacted by the parasitoid to circumvent the host's immune system.

Key words: Parasitoid, Tachinids, Parasitoid behavior, Host defence-reaction.

ACKNOWLEDGEMENTS

We thank H. R. Gross Jr. (USDA-ARS, Tifton, GA) and W.C. Nettles Jr. (USDA-ARS, Weslaco,TX) for the insects used to establish the *A.marmoratus* colony; E. Mellini and P.Fanti for reviewing the manuscript. We are also indebted with the Cassa di Risparmio di Cento for providing Miglioli's scholarship.

Comportamento e crescita dei planidi di *Archytas marmoratus* (Town.)(Diptera Tachinidae) nelle larve dell'ospite *Galleria mellonella* L.(Lepidoptera Galleriidae).

RIASSUNTO

Archytas marmoratus (Town.) è un parassitoide solitario, a sviluppo larva-pupale, di varie specie di Nottuidi. Nei nostri laboratori, da alcuni anni, questo tachinide viene allevato sull'ospite di sostituzione *Galleria mellonella* L.

Le femmine dell'entomofago depongono uova che schiudono immediatamente liberando delle larvette di tipo planidio. Una volta penetrate nelle larve ospiti, esse mutano nello stadio successivo solo dopo la metamorfosi del simbionte.

Nel presente lavoro abbiamo determinato la posizione e il ritmo di accrescimento del planidio, all'interno delle larve di penultima ed ultima età dell' ospite.

I risultati indicano che le L1 di *A. marmoratus* si trovano in posizione intrategumentale e quindi non in diretto contatto con l'emolinfa del simbionte. Le larvette si accrescono, durante la loro permanenza nella larva, raggiungendo un picco di crescita in prossimità dell'impupamento dell'ospite.

La posizione intrategumentale dei planidi e la loro crescita nella larva del lepidottero sono collegate, molto probabilmente, con le strategie utilizzate dal parassitoide per evitare le reazioni emocitarie messe in atto dall'ospite.

REFERENCES CITED

Allen W.H., 1926. - Life history of the variegated cutworm tachina fly, *Archytas analis*.- *J. Agric. Res.*, 32:417-435.

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

Bohn H., 1975. - Growth-Promoting Effect of Haemocytes on Insect Epidermis *In Vitro*.- *J. Insect Physiol.*, 21:1283-1293.

Bouletreau M., 1968. - Premiers résultats de l'élevage des larves d'un Hyménoptère Chalcidien (*Pteromalus puparum* L.) sur hémolymphe de Lépidoptère. - *Entomophaga*, 13 (3): 223-228.

Bratti A., 1993. - Preliminary results on *in vitro* rearing of *Lydella thompsoni* Hert. and *Archytas marmoratus* (Town.) (Dipt. Tachinidae) larval stages. - *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 48: (in press).

Bratti A., Costantini W., 1991. - Effetti di nuove diete artificiali dell'ospite sulla coppia ospite-parassita *Galleria mellonella* L. (Lep.Galleriidae)- *Archytas marmoratus* (Town.) (Dip. Tachinidae). - *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 46: 49-62.

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). - *Env. Entomol.*, 21:1196-1201.

Clausen C.P., 1940. - Entomophagous insects. - McGraw-Hill Book Co., 688 pp.

Gerling D., Tremblay E., Orion T., 1991. - Initial Stages of The Vital Capsule Formation in the *Eretmocerus-Bemisia tabaci* Association. - *REDIA*, 74 (3) 411-415.

Gross H.R., 1992. - Advances in Mass Propagation of *Archytas marmoratus* (Diptera:Tachinidae), A Primary Parasitoid of *Helicoverpa zea* and *Spodoptera frugiperda* (Lepidoptera:Noctuidae).- *Env. Entomol.*, in press.

Hughes P.S., 1975. - The biology of *Archytas marmoratus* (Townsend).- *Ann. Entomol. Soc. Am.*, 68: 759-767.

Lawrence P.O., 1990. - The Biochemical and Physiological Effects of Insect Hosts on the Development and Ecology of Their Insect Parasites: An Overview. - *Arch. Insect Biochem. Physiol.*,13: 217-228.

Mellini E., 1965. - L'imbuto repiratorio negli ospiti dei Ditteri Larvevoridi. - *Atti Accademia Naz. Ital. Entomologia*, Anno XII, 47-62.

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

MELLINI E., CAMPADELLI G., DINDO M.L., 1986. - Ritmo di accrescimento delle larve di I età del parassita in relazione allo stadio dell'ospite al momento della contaminazione nel sistema *Galleria mellonella* L.- *Pseudogonia rufifrons* Wied.- *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 40: 221-237.

NAPPI A.J., 1975. - Parasite encapsulation in insects. - In: Invertebrate Immunity, Edited by K. Maramorosch and R.E. Shopa. Academic Press, 293-326.

SALT G., 1968. - The resistance of insect parasitoids to the defence reactions of their hosts.- *Biol Rev.*, 43: 200-232.

SALT G., 1970. - The Cellular Defence Reactions of Insects.- Cambridge Monographs in Experimental Biology. Cambridge University Press, Great Britain, 118 pp.

SEHNAL F., DELBECQUE J.P., MAROY P., MALA J., 1986. - Ecdysteroid titres during larval life and metamorphosis of *Galleria mellonella*. - *Insect Biochem.*, 16: 157-162.

SMITH A.R., RATCLIFFE N.A., 1977. - The Encapsulation of Foreign Tissue Implants in *Galleria mellonella* Larvae. - *J. Insect Physiol.*, 23: 175-184.

THOMPSON W.R., 1924. - Les larves primaires des tachinaires a oeufs microtypes.- *Ann. de Parasitol. Humaine et Compar.*, 2:185-201, 279-306.

TOTHILL J.D., 1922. - The natural control of the fall webworm (*Hyphantria cunea* Drury) in Canada. - *Canada Dept. Agr. Tech. Bull.*, 3: 107 pp.

VINSON S.B., 1990. - How Parasitoids Deal With the Immune System of Their Host: An Overwiev. - *Arch.Insect Biochem. Physiol.*, 13:3-27.

ZAR J.H., 1984. - Biostatistical analysis. - Prentice-Hall, Englewood Cliffs, NJ., 718 pp.