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SQUEESING THE MID GUT OF PARASITIZED *Spodoptera Littoralis* LARVAE BY PARASITOGENIC TRACHEOLS

Eid, M.A.A.; G. Elsayed; S.A.S. El-Mamsrrawy and Hanan M. Abdel-Samad

Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT

The mid gut of the parasitized larvae was decreased in diameter compared with unparasitized ones. Th epithelial cells were loose, full with vacuoles, turned to folds more thick and faintly stained. The tracheols occupied a considerable space of the perivesceral protein of the body cavity of the parasitized larvae. The parasitized *Spodoptera littoralis* larvae showed parasitogenic degeneration in their fat bodies.

INTRODUCTION

Microplitis rufiventris, a solitary endoparasitoid, is considered an important and efficient agent in controlling the population of the cotton leaf worm. This braconid parasitoid can also attack other Lepidopterous hosts, but hosts from genus *Spodoptera* are the most preferred. *Hymenopterous* endoparasitoids are known to cause many physiological changes in their hosts in action known as "host regulation". This regulation alters many aspects of host physiology and ontogeny (Harvey, 1996). In some host parasite system it includes an acceleration in host growth rate/or assimilation efficiency by the host (Slansk, 1978, Cloutier and Mackauer, 1979 & 1980, Alleyne and Beckage, 1997).

In contrast with other systems; feeding and locomotary activities are inhibited (Adamo *et al.*, 1997; Beckage and Riddiford, 1978; Beckage and Templeton, 1986; Eid *et al.*, 2001), novel virally encoded haemolymph protein are produced (Harwood and Beckage, 1994; Harwood *et al.*, 1994), the relative amounts of several endogenous host haemolymph protein are altered (Beckage and Kanost, 1993), and pathological changes in the host fat body are found (Dahlman and Vinson, 1980; Lardner and Hales, 1990; Zhang *et al.*, 1991).

However, the changes in host tissues associated with the parasitoid's development have seldom been documented. In addition, the cause of such changes is poorly understood. This work describes the histopathological changes observed in the parasitized *S. littoralis* larvae and discusses these changes. Relatively few studies have focused on the effects of parasitoid on feeding and growth of hosts, particularly in those koinobiontic species.

MATERIALS AND METHODS

Larvae of the host *Spodoptera littoralis* were reared in glass jars (15x15x20cm.), at room temperature. The larvae were fed fresh castor leaves washed and sterilized by formaldehyde 0.25-0.5%.

The parasitoid *M. rufiventris* was reared on early third instar larvae of the host in glass rearing units, where droplets of diluted honey were scattered

on the inner walls as a source of food for the adult parasitoids. Twenty early third instar larvae were introduced daily to one fertilized female wasp every 24 hours.

Parasitized larvae were then transferred to clean pots and reared on sterilized castor leaves till parasitoid egression. The rearing process of adult parasitoid was carried out in an incubator under controlled conditions of temperature (20±5C°), humidity (60-70% R.H.) and a 9:15 L/D photoperiod regime.

The parasitized larvae were histologically studied to investigate the effect of the endoparasitoid on hosts integument and tracheal system. It was observed that some parasitized larvae attack their parasitoid and these individuals were considered as a category from the normal parasitized (PI) and are referred to as (PII). Three replicates were taken for each category (unparasitized, PI and PII) and longitudinal sections were made in the thoracic and abdominal regions after fixing in aqueous buin and then drying in ascending alcohol concentrations. The sections were then double embedded in celloidin and parafin wax. The 7u sections were stained with Relish's heamatoxylin for staining the nucleus and Eosin for staining the cytoplasm.

RESULTS AND DISCUSSIONS

1- Effect on the mid gut:

The parasitized larvae become sluggish and loses its appetite gradually until it finally stop feeding about two days before egression of the parasite. However, it was not easy to differentiate between the parasitized and nonparasitized larvae on basis of size as no obvious differences were detected.

The histological inspection showed that the mid gut in the parasitized larvae was less in diameter than in the unparasitized ones. It's lumen was nearly empty of food material or residue (Figs. 1 & 2). In the unparasitized larvae (Fig. 1), it is noticed that the epithelium is consisted of a single layer of columnar cells as reported by House (1965), with microvilli forming a striated border (Chapman, 1973), and lined with an inner layer of circular muscles and outer longitudinal one (Wigglesworth, 1972). The epithelial cells were deeply stained without vacuoles. Some granules are seen in the cytoplasm of the cells and others were liberated in the lumen close to the inner border of the epithelial cells and considered as digestive enzymes.

Figure (2) shows the mid gut of parasitized larvae where the epithelial cells are faintly stained, compared with those of the unparasitized larvae, due to the decrease in the digestive enzymes. The epithelial cells appear to be loose and separated and the layer is full with gaps and vacuoles. In the mid gut of the parasitized larvae; the circular and longitudinal muscles are detached from the epithelial layer, the epithelial layer turns into adjacent folds. By measuring the thickness of the epithelial layer in parasitized and nonparasitized larvae (Table, 1), it was 122.1 µ and 119.4µ, respectively. The differences were not significant (Table,1).

Figure (1) Longitudinal section in the mid gut of a healthy larva of *S. littoralis* (10x)

Epth: Epithelial cells em : Epithelial muscles
L : Mid gut lumen g : Ganglion

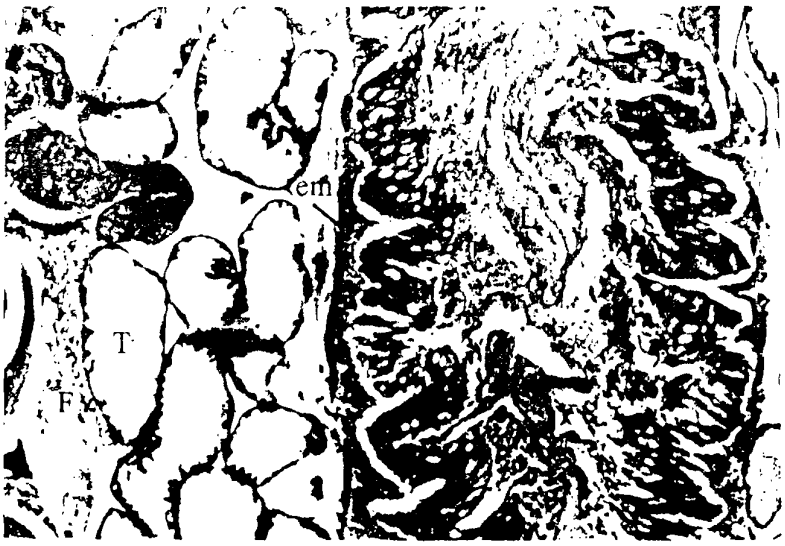

Figure (2) Longitudinal section in the mid gut of a parasitized larva of *S. littoralis* (10x)

Epth: Epithelial cells em : Epithelial muscles T : trachea
L : Mid gut lumen g : Ganglion

Figure (3) Longitudinal section in the fat body of a healthy larva of *S. littoralis* (40x)

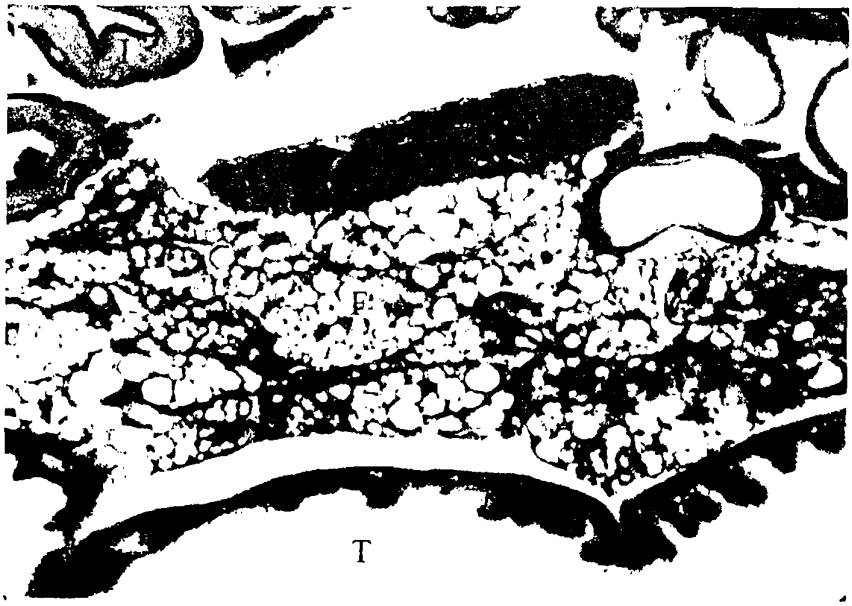

Figure (4) Longitudinal section in the fat body of a parasitized larva of *S. littoralis* (40x)

I : Integument
m : Muscles
T : trachea
f : fat body

The most striking feature in the sections of the parasitized larvae is numerous sections of tracheols which occupy a considerable space of the section approximating that of the mid gut. It may be of concern to note that the sections of the unparasitized larvae are devoid of these sections of tracheols and the mid gut consequently occupies, its normal size with a lumen full of foodstuff (Figs. 1 & 2). The diameter of the mid gut in the unparasitized larvae may thus exceed two folds the diameter of the parasitized ones.

Endoparasitoids induce physiological and behavioural changes in their hosts, many of which can be considered as regulation of host environment to meet the needs of the parasitoids (Horton and Moore, 1993; Godfray, 1994). In *S. littoralis/M. rufiventris* system; known effects of parasitism include growth inhibition (Tawfik et al., 1977; Hegazi et al., 1991), behavioural changes such as the pre-eggression cessation of feeding and motor activity (Eid et al., 2001). These effects are induced by the polydnavirus of endoparasitoid females injected into hosts along with their eggs, other factors such as teratocytes and venom may also be important (Beckage, 1993a & b; Dahlman and Vinson, 1993; Lavine and Beckage, 1995 & 1996; Jones and Coudron, 1993; Wani *et al.*, 1993; Beckage *et al.*, 1994; Strand and Pech, 1995).

In the *S. littoralis / M. rufiventris* system the host continues to feed after parasitization. But it gradually lose appetite and the decline in food uptake reach's its maximum two or three days before egression of the parasitoid. Similar to many parasitized insects larvae of *S. littoralis* cease feeding prior to parasite emergence untill their death (Smilowitz et al., 1976; Hegazi et al., 2001). As reported by Hegazi *et al.*, (1988) "The developmental stress of the parasite has an effect on the muscles of the host's gut and its muscles lose the ability to process food. Indeed some food remained inside the alimentary canal of these parasitized hosts following cessation of food consumption". But the findings of this work show other view.

We suggest that the mechanical effect of the growing mass of trachoels in the mid gut is responsible for the gradual loss of appetite. The perivesceral protein of the body cavity available to mid gut extension by food injection become occupied by these trachoeles or air sacs. This immense increase in the respiratory mass developed as consequence of the growing need for oxygen owing to parasitism as shown by Alleyne *et al.*, (1997).

The lack of mid gut tissues damage makes such a suggestion acceptable. The alterations of the tracheale system in the parasitized larvae are not only an association but also a causal phenomenon. It may be of concern to point out the probable effect of the polydnavirus, teratocytes and venom in participating in losing appetite.

Table 1: Average values of the thickness of the epithelial layer of the mid gut of *S. littoralis* larvae parasitized by *M. rufiventris*.

Treatment	Thickness of the epithelial layer (in microns)	Analysis of variance		
		F.	P. value	F. critical
Control larvae	119.8			
		0.39	0.54	3.97
Parasitized larvae	122.1			

Each figure is the average of three replicates, each of 24 reading
The differences were not significant.

2- Effect on the Fat body:

In the healthy larvae, the fat bodies (Fig. 3) were numerous and contained large cells with dense granular cytoplasm, indistinct cell margins, and a small rounded nucleus. The cytoplasm was deeply stained and lightly vacuoles.

The fat bodies of parasitized larvae were less in their mass (Fig. 4) and the staining was faint. The cytoplam of the cells became less dense and contained numerous clear patches, probably representing fat droplets. In the parasitized larvae the fat bodies showed parasitogenic degeneration and increased vacuolization although it kept existing.

For assessing the pathological consequences of parasitism on the host, the histological measure of parasitism cost on the fat body level may, in our view, provide a parameter for the evaluation of the overall metabolic stress induced by parasitism. The parasitized *S. littoralis* larvae showed parasitogenic degeneration through: faint staining, less dense cytoplasm, clear patches in the cytoplasm, increased vacuolisation, and less mass of fat bodies (Fig.4).

The successful parasitism may be linked with synthesis of "early" proteins (Harwood, 1993; Harwood et al., 1993) produced by the fat body and haemocytes (Dunn, 1985; Harwood and Beckage, 1993). Several studies suggested that qualitative and quantitative changes in lipid metabolites accompany parasitism (Barras *et al.*, 1970; Thompson, 1982; Hayakaw, 1986&1987).

The fat body of parasitized larvae almost glycogen depleted (Dahlman and Vinson, 1980). Altered host tissue carbohydrate levels have also been repotted during many insect-parasite host relationships (Fuhrer, 1972; Hawlitzky and Boulay, 1986; Thompson *et al.*, 1990). The host fat body curtailment may be due to parasite induced nutrient deprivation resulting in increased fat body catabolism and decreased anabolism (Tomalak *et al.*, 1990 and Lieutier, 1984 a and b). It could be suggested that these effects are consequences of the parasitic-induced stresses on the mid gut and losing appetite.

REFERENCES

Adamo S.A.; C.E. Linn C.E. and N.E. Beckage (1997). Correlation betweenchanges in host behaviour and octopamine levels in the tobacco horn worm, *Manduca sexta*, parasitized by the gregarious braconid parasitoid wasp *Cotesia congragata*. J.Exp. Biol., 200:117-127.

Alleyne M. and N.E. Beckage (1997). Parasitism induced effects on host growth and metabolic efficiency in tobacco horn worm larvae parasitized by *Cotesia congragata*. Insect Physiol., 43(4): 407-424.

Alleyne M., M.A. Chappell; D.B. Gelman and N.E. Beckage (1997). Effects of parasitism by *Cotesia congragata* in the metabolic rate of host larvae of the tobacco horn worm, *Manduca sexta*. Insect Physiol., 43:143-154.

Barras D.J.; R.L. Joiner and S.B. Vinson (1970). Neutral lipid composition of the tobacco bud worm, *Heliothis virescens* (Fab.) as affected by its habitual parasite, *Cardiochiles nigriceps* Viereck. Comp. Biochem. Physiol. 36:775-783.

Beckage N.E. (1993a). Endocrine and neuroendocrine host parasite relationships. Receptor, 3:233-245.

Beckage N.E. (1993b). Games parasites play: the dynamic roles of proteins and peptides in the relationship between parasite and host. In parasites and pathogens of insects (Eds Beckage N.E., Thompson S.N. and Frederici B.A.). Vcl. 1, pp. 25-27, Academic press, San Diego, CA.

Beckage N.E. and M.R. Kanost (1993). Effects of parasitism by the braconid wasp *Cotesia congregata* on host haemolymph proteins of the tobacco horn worm Manduca sexta. Insect Biochem. Molec. Biol., 23:643-653.

Beckage N.E. and Riddiford I.M. (1978). Developmental interactions between the tobacco horn worm *Manduca sexta* and its braconid parasite *Apenteles congregatus*. Ent. Exp.Appl., 32:139-151.

Beckage N.E. and Templeton (1985). Temporal synchronization of emergence of *Hyposotor exiguae* and *H. fugirivus* (*Hymenoptera: Ichneumonidae*) with apolysis preceiding larval molting in *Manduca sexta* (*Lepidoptera: Sphingidae*). Ann. Ent. Soc. Amer., 78:775-782.

Beckage, N.E., Tan, F.F., Schleifer, K.W., Lane, K.D. and Cherubin, L.L. (1994). Characterization and biological effects of *Cotesia congregata* polyaena virus on host larvae of the tobacco horn worm, *Manduca sexta*, Arch insect Biochem. Physiol., 7: 234-247.

Chapman R.F. (1973). The insects: Structure and function. The English Universities Press Ltd., London.

Cloutier C. and Mackauer M. (1979).The effect of parasitism by *Aphidius smithi (Hymenoptera: Aphididae*) on the food budget of the pea aphid, *Acyrthosiphon pisum (Homoptera: Aphididae*). Can. J. 2001-57, 1605-1611.

Cloutier C. and M. Mackauer (1980).The effect of superparasitism *by Aphidius smithi (Hymenoptera: Aphididae)* on the food budget of the

pea aphid, *Acyrthosiphon pisum (Homoptera: Aphididae)*. Can. J. 2001-58, 214--244.

Dahlman D.L. and Vinson S.B. (1980). Glycogen content in *Heliothis virescens* parasitized by *Microplitis croceips*. Comp. Biochem. Physiol. 66A, 625-630.

Dahlman D.L. and Vinson S.B. (1993). Teratocytes: Developmental and biochemical characteristics. In parasites and pathogens of insects (Eds. Beckage N.E., Thompson S.N. and Frederici B.A.). Vol. 1, pp. 145-156., Academic press, San Diego, CA.

Eid M.A.A., Elsayed G. and Abdel-Samad H.M. (2001). Does regulative endoparasitoid *Microplitis rufiventris* also annihilate ontogeny in *Spodoptera littoralis* larvae. Under publication.

Fuhrer E. (1972). Abnorme glycogenspeicherung in larvan von *Pieris brassica* L. als folge des parasitismus von *Apenteles glomeratus* L.Z.Angew. Entomol. 70, 370-374.

Godfray H.C.J. (1994). Parasitoids: Behavioural and Evolutionary Ecology. Princeton University Press. Princeton.

Harvey J.A. (1996). Venturia canescens parasitizing *Galleria mellonella* and *Anagasta kuehniella*: is the parasitoid a conformer or regulator. Insect Physiol., 42: 1017-1025.

Harwood S.H. and Beckage N.E. (1994). Purification and characterization of an early-expressed polydnavirus-induced protein from the haemolymph of *Manduca sexta* larvae parasitized by *Cotesia cengregata*. Insect Biochem. Molec. Biol. 24:685-698.

Harwood S.H., Grosovsky A.J., Cowles E.A., Davis J.W. and Beckage N.E. (1994). An abundantly expressed haemolymph glycoprotein isolated from newly parasitized *Manduca sexta* larvae is a polydnavirus gene product. Virol. 205:381-392.

Harwood S.H., McElfresh J.S., Nguyen T.Q. and Beckage N.E.C. (1993). Pattern of expression of a Cotesia congregata polydnavirus gene transcript in different sphingid hosts: Correlation with successful parasitism.

Hawlitzky N. and Boulay C. (1986). Effects of the egg-larval parasite, *Phanerotoma flavitestacea Fisch (Hymenoptera, Brachonidae)* on dry weight and chemical composition of its host *Anagasta kuehniella Zell. (Lepidoptera, Pyralidae*). Insect Physiol., 32:269-274.

Hayakawa Y. (1986). Inhibition of lipid transport in insects by a factor secreted by the parasite, *Blepharipa sericariae*. FEBS Lett., 195:122-124.

Hayakawa Y. (1987). Inhibition of lipid transport by a parasitic factor Comp. Biochem. Physiol., B87B: 279-283.

Hegazi E.M.. A.M. El-Minshawy and Hammad S.M. (1977). Mass rearing of the Egyptian cotton leaf-worm *Spodoptera littoralis* (Boisd.) on semi-artificial diet. Proc. 2 nd Arab pesticide Conf., Tanta University.

Hegazi E.M.; M.A. Shaaban and N.R. El-Singaby (1991). Development of *Microplitis rufiventris (Hymenoptera: Brachonidae)* in superparasitized

Spodoptera littoralis (Lepidoptera: Noctuidae) Ann. Ent. Soc. Am. 84:571-574.

Hegazi, E.M., Schopf, A., Fuhrer, E. and Fouad, S.H. (1988). Developmental synchrony between *Spodoptera littoralis* and its parasite *Micrplitis rufiventris*. Insect Physiology, 34:773-778.

Horton D.R. and Moore J. (1993). Behavioral effects of parasites and pathogens in insect hosts. In parasites and pathogens of insects (Eds. N.E. Beckage S.N. Thompson and B.A. Federici) pp. 107-124. Academic press, San diego, CA.

House H.L. (1965). Digestion. The physiology of insects II ed. M. Rockstein, Academic Press, New York.

Jones D. and Coudron T. (1993). Venom of parasitic *Hymenoptera* as investigatory tools. In parasites and pathogenes of insects (Eds N.E. Beckage S.N. Thompson and B.A. Federici) pp. 227-244. Academic press, San diego, CA.

Lardner R.M. and Hales D. (1990). Histopathology of an aphid, *Schoutedenia lurea*, parasitized by a gall mid gut, *Pseudendaphis sp.* Entomologia, 35: 557-567.

Lavine M.D. and N.E. Beckage (1995). Polydnaviruses: Potent mediators of host insect immune dysfunction. Parsitol. Today, 11: 368-378.

Lavine M.D. and Beckage N.E. (1996). Temporal pattern of parasitism induced immunosuppression in Manduca sexta larvae parasitized by Cotesia congregata. Insect Physiology., 42: 39-49.

Lieutier F. (1984a). Ovarian and fat body protein concentration on *Ips sexdentatus (Insecta: Scolytidae)* parasitized by nematodes. J. Inverteb. Pathol., 43:21-31.

Lieutier F. (1984b). Observation sur le parasitisme *d'Ips sexdentatus (Insecta: Scolytidae)* par *Parasitorhabditis ipsophila (Nematoda: Rhobditidae)*. Ann. Parasitol. Hum. Comp., 59: 507-520.

Slansky F. (1978). Utilization of energy and nitrogen by larvae of the imported cabbage worm, *Pieris rapae*, as affected by parasitism by *Apenteles glomeratus*. Env. Ent., 7:759-763.

Smilowitz Z., Martinka C.A. and Jowyk E.A. (1976). The influence of a juvenile hormone mimic (JHM) on the growth and development of the cabbage looper, *Trichoplusia ni (Lepidoptera: Noctuidae)* and the endoparasite *Hyposoter exiguae (Hymenoptera: Ichneumonidae)*. Envir. Ent. 5: 1778-1182.

Stran M.R. and Pech L.L. (1995). Immunological basis for compatibility in parasitoid-host relationships. Ann. Rev. Entomol. 40:31-56.

Tawfic M.F.S., Hafez M. and Ibrahim A.R. (1997). On the bionomics of *Microplitis rufiventris* (Kok) Bull. Soc. ent. Egypt, 6: 123-135.

Thompson S.N. (1982). Effects of parasitization by the insect parasite *Hyposoter exiguae* on the growth, development and physiology of its host *Trichoplusia ni*. Parasitology, 84: 419-510.

Tomalak M.; H. E. Welch and T.Galloway (1990). Pathogenicity of *alllantonematidae (Nematoda)* infecting brak bettles (*Coleoptera: Scolytidae*) in Manitoba. Can. J. 2001., 68: 89-100.

Varley G.C. and C.G.Butler (1993). The acceleration of development of insects by parasitism. Parasitology. 25:263-268.

Wani M.; S. Yagi and T.Tanaka (1990). Synegistic effect of venom, calyx and teratocytes of *Apenteles kariyai* on the inhibition of larval-pupal ecdysis of the host, *Pseudaletia separata* . Ent. Exp. Appl. 57:101-104.

Wigglesworth F.R.S. (1972). The principles of insect physiology. Chapman and Hall Ltd. Great Britain.

Zhang D., D.L. Dahlman and U.E. Jarlfors (1991). Effects of *Microplitis croceipes* teratocytes on host haemolymph protein content and fat body proliferation. Insect physiology., 43:577-585.

انكماش معدة يرقات دودة ورق القطن بسبب ضغط قصباتها الهوائية المتضخمـــة نتيجة وجود الطفيل الداخلي,*Microplitis rufiventris* .

محمد احمد عيد – جمال السيد – صلاح المعصراوى – حنان عبد الصمد
قسم الحشرات الاقتصادية والمبيدات – كلية الزراعة – جامعة القاهرة

أوضح الفحص الهستولوجي لمعدة يرقات دودة ورق القطن المتطفل عليها صغر حجمها بالمقارنة باليرقات غير المتطفل عليها وكانت خلاياها الطلائية مفككة ومنثنية وممتلئـــة بفجـــوات. القصبات الهوائية تشغل حيز كبير من الحجم الداخلي لليرقات المتطفل عليها. كانت الأجسام الدهنية لليرقات المتطفل عليها قليلة.