J. Agric. Sci. Mansoura Univ., 28 (3): 2221 - 2229, 2003

DOES REGULATIVE ENDOPARASITOID *MICROPLITIS Rufiventris* ALSO ANNIHILATE ONTOGONY IN *Spodoptera littoralis* LARVAE

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ABSRACT

Comparative histology of parasitized and unparasitized hosts was used to evaluate proposed mechanisms of host regulation by endoparasitoid. Parasitization of *Spodoptera littoralis* larvae by the endoparasitoid *Microplitis rufiventris* was found to cause the destruction of female imaginable gonads and pathological changes in the nervous system. These histopathological changes were reflected on the locomotory and feeding activities. This regulative endoparasitoid not only distract the nervous system, as a juvenile structure, but also annihilate the female gonads as an imaginable structure.

INTRODUCTION

Solitary endoparasitoids often alter the growth of their hosts in a dramatic fashion (Smilowitz and Iwantsch, 1973; Strand *et al*., 1988). Host endocrine and neuroendocrine functions are disrupted (Beckage, 1985 ; Beckage and Kanost, 1993; Marianne and Beckage, 1997; Zitnan *et al*., 1995), feeding and locomotory activities are inhibited (Beckage and Riddiford, 1978; Beckage and Templeton, 1986; Adamo *et al.*, 1997).

Parasitic effects on the reproductive tissue development and fecundity of adult male hosts are well-documented (Hurd, 1993; Hurd and Webb, 1996). Parasitic castration or destruction of reproductive tissue of immature male insects within host/parasitoid systems now is receiving increased attention (Junnikkala, 1985; Reed-Larsen and Brown, 1990; Yagi and Tanaka, 1992; Hurd, 1993, Tanaka *et al*., 1994; Brown and Friedlander, 1995; Brown and Reed, 1997). Both male and female parasitized hosts fail to develop gonads (Reed-Larsen and Brown, 1990; Brown and Kainoh, 1992; Brown *et al*., 1993).

The nervous system received our attention to study the pathological effects of parasitism reflected on the locomotory and feeding activities of *S. littoralis* larvae. Since development of the female reproductive imaginable discs occurs primarily following pupation in the larvae of *S. littoralis* we restricted our observations to these discs in the parasitized larvae. The regulative effect of the endoparasitoid *M. rufiventris* on *S. littoralis* larvae was thus studied on a juvenile organ, the central nervous system, and on an imaginable organ, i.e. the female reproductive disc.

MATERIALS AND METHODS

Insect cultures

Host rearing

Fertilized *S. littoralis* females were confined for oviposition in glass cages. In each cage, castor leaves were used as oviposition sites together with a cotton piece moistened with 10% sucrose solution as food. Egg masses were transferred individually to glass jars for breeding. Castor leaves were introduced daily to the hatched larvae. Starting from the second instar, the density in each jar (10x10x15cm) was decreased to 100-150 larvae., then to 75-100 in the third instar, decreased to 40-50 for the fourth instar and to 30-35 larvae for the fifth instar. In the last instar the number of larvae in each jar become 25 and prior to pupation a layer of clean and fine sawdust was placed on the bottom of the jar to provide a site for pupation, rearing was carried out in the laboratory under normal conditions.

Parasitoid rearing

Rearing of the parasitoid *M. rufiventris* was carried out according to the method of Ibrahim (1973) with some modifications. The fertilized females were initially obtained from the Biological control laboratory, faculty of agriculture, University of Alexandria. The rearing process took place under controlled conditions to provide the wasps with 9 hours of light and 15 darkness, and a temperature of 25 C° $\pm$ 3 and a humidity of 60-70% RH. The parasitoid rearing unit consisted of a plastic pot about 10 cm in diameter and a glass chimney 12 cm high and 10 cm in diameter. The glass chimney was fixed over the pot.

Host parasitization protocol

Early third instar larvae were introduced to the fertilized females of the parasitoid (15-20 larvae/female) every 24 hours. Fresh castor leaves were introduced as food for the larvae and honey droplets were scattered on the wall of the glass chimney to provide food for the adult parasitoids. Host larvae were then transferred to clean and sterilized plastic pots covered with muslin and supplied daily with fresh castor leaves until the parasitoid prepupa emerges and pupates.

Histological Studies

The longitudinal and transverse sections were made in the late fourth instar larvae, parasitized and non parasitized, after the emergency of the parasitoid. The sections were made according to the method described by Kamel, 1969.

RESULTS AND DISCUSSION

Effects on the nervous system

When examining an abdominal ganglion in healthy larvae of *S. littoralis* (Fig. 1), it was noticed that it was clothed by a nerve sheath differentiated into an outer lamella and an inner perineurum. The nerve cells were completely surrounded by glial cells. The body wall, the nucleus and the cytoplasm are clearly seen. No spaces or vacuoles were found in this layer. At the center of the ganglion, the neuro pile was found and seemed homogenous without gaps or vacuoles.

The nerve chord was also surrounded by the nerve sheath and the axons were seen clearly bounded together (Fig. 2). By examining the ganglion of parasitized larvae shown in Fig. 3, it is noticed that the nerve sheath completely disappeared. The nuclei of the nerve cells and the glial cells were not clearly seen and cells seemed to be loose and separated from each other to small groups. The neuro pile was no more homogonous and interspaces were clearly abundant. The nerve cord in the parasitized larvae lost its nerve sheath and the axons were loose (Fig. 4). These physical damages found in the central nervous system were associated with observed symptoms. The larvae ceased all feeding behavior activities, standing still curved on themselres in C shape, paralyzed and having faint tactile response.

Effects on the female imaginable reproductive discs

The gonads of the *lepidopterous* larvae are found at the dorsal side of the fifth abdominal segment along both sides of the heart and directly under the integument. The female gonads are spindle-shaped while the male ones are kidney-shaped. In the unparasitized larvae, the female gonads were clearly noticed as shown in Fig. 5, while in the parasitized larvae they vanished completely. Thus the postembryonic development of the female germ cells of the parasitized larvae were annihilated. These effect goes beyond the irreversible destruction of the nervous system presented in this work and the testicular atrophy reported by many authors.

Parasites and pathogens play a major role as regulators of host life histories (Dobson, 1988; May and Anderson, 1990). It is our intention to confine the regulatory effect *of M. rufiventris to S. littoralis* larvae, not as a lethal agent, but by causing behavioral changes and blocking reproductive output on ontogony level. The entomological literature emphasizes the effects of parasitism on host physiology; effects on host behavior are less well-described (Mc-Allister and Roiberg, 1987; Broduer and Mc-Noil, 1989). Nonethless, behavioral changes caused by parasitoid include among many changes; activity level and feeding behavior (Molyneux and Jeffris, 1986).

Behavioral modifications shown in the present work can be interpreted to have arisen as a consequence of the pathological effect of parasitism on the nervous system. Parasitism cause hosts to become sluggish (Henry and Oma, 1981; Johnson, 1989); others produce paralysis (Kulincevic *et al.*, 1970; Reinganum *et al.*, 1970) or reduced activity (Webber et al., 1987). These behavioral changes, however, were present only fortuitously as consequence of pathological effects of parasitism (Moore and Gotelli, 1990).

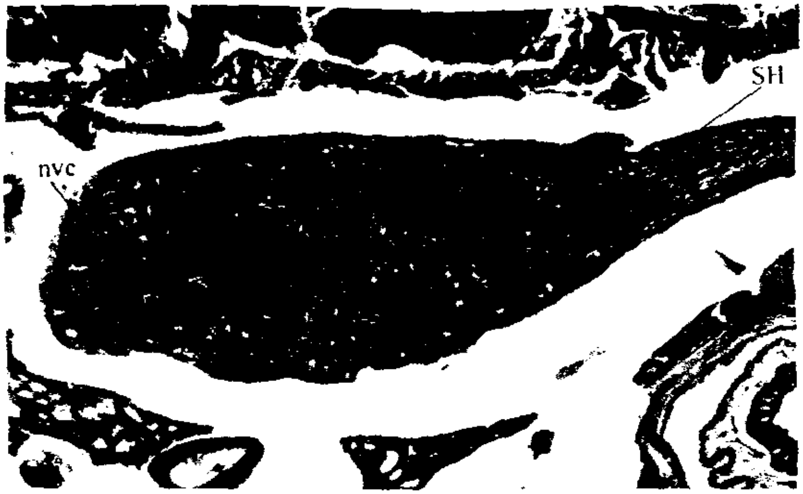

Figure (1): Longitudinal section in an abdominal ganglion of a healthy larva of *S. littoralis* (40x)
nvc: nerve cells SH: nerve sheath
nc: nerve chord np : neuro pile

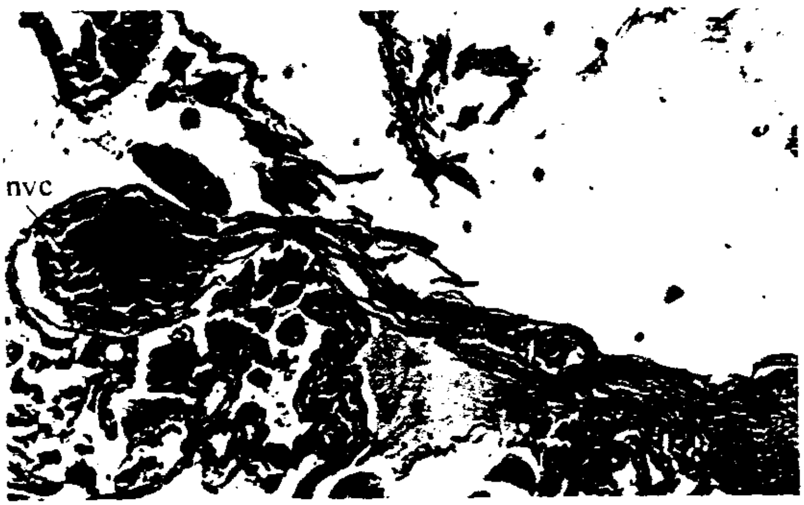

Figure (2):Longitudinal section in an abdominal ganglion of a parasitized larva of *S. littoralis* (40x)
nvc: nerve cells nc: nerve chord np : neuro pile

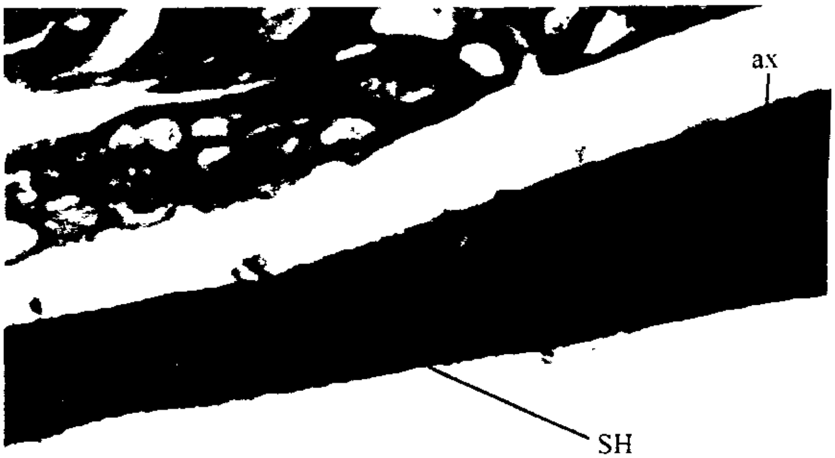

Figure (3): Longitudinal section in the nerve chord of a healthy larva of *S. littoralis* (40x)

ax: axons SH: nerve sheath

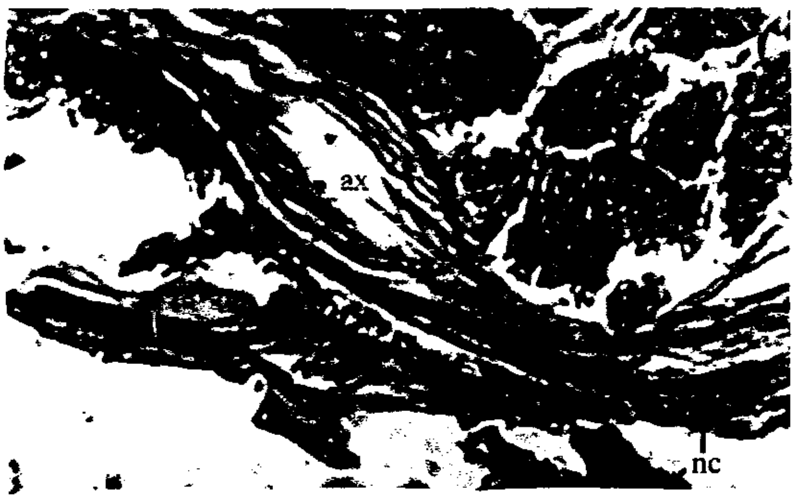

Figure (4): Longitudinal section in the nerve chord of a parasitized larva of *S. littoralis* (40x)

ax: axons nc: nerve chord

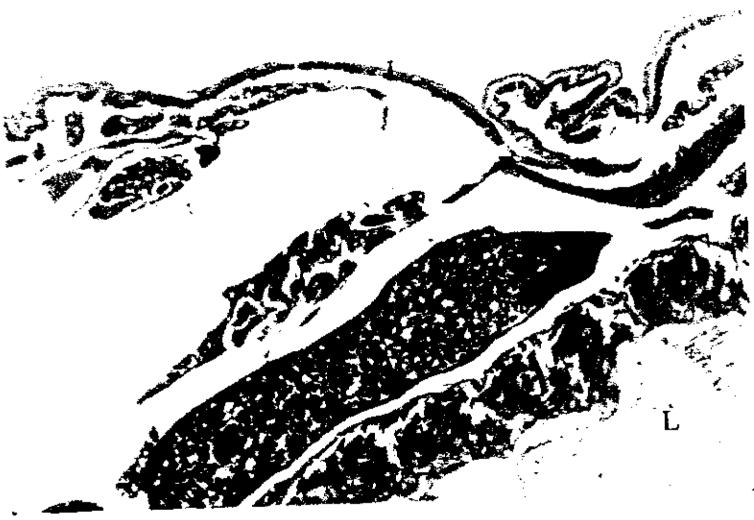

Figure (5): Longitudinal section in a gonad of a healthy larva of *S. littoralis* (40x)

Epth: Epithelial cells G : Female gonad L: Mid gut lumen
m: Muscles f : fat body I : Integument

Insect parasitoids usually attack immature insects (Kuris, 1974), inhibit testicular development (Keed and Beckage, 1997); and kill their hosts before they reach maturity. Host castration is thus rarely absorbed in insect / insect associations. A reduction in the volume of developing testes was found after parasitism (Junnikkala, 1985), the degree of the gonads damage depends on the time of attack (Reed-Larson and Brown, 1990; Darcy *et al.*, 1997).

Since growth and development of imaginable tissues during the postembryonic period are controlled and influenced by both juvenile hormone and ecdysone, hormonal mechanisms plausibly could be linked to blocking of female gonad ontogony in host – parasitoid relationship as in the present work. Our understanding of the mode of parasite action is rudimentary but in most associations it appears to involve a more subtle interplay than the simple scenario of parasite utilization of nutrients destined for development as claimed by Hanan (2000).

Indirect diversions of nutrients via alteration of fat body metabolism and/or perturbance of neuroendocrine control mechanisms are undoubtedly involved in some associations and putative parasite secretion of mediator substances which may alter the imaginable disc sensitivity and affect hormone metabolism. However, the studies of Darcey and Beckage, 1997, offer speculation on the possible combined effects of PDV, venom and the developing parasitoid on host germinal tissues development.

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هل يسبب الطفيل الداخلي *Microplitis rufiventris* اضمحلال عمليات تشكل الأعضاء في يرقات دودة ورق القطن المتطفل عليها؟

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استخدمت المقارنة بين التركيب الهستولوجي لليرقات المتطفل عليها واليرقات غير المتطفل عليها في تقدير إمكانية تنظيم العائل (دودة ورق القطن) بواسطة الطفيل الداخلي *Microplitis rufiventris*. الغدة التناسلية للإناث المتطفل عليها أتلفت وظهر أيضا تغيرات في الجهاز العصبي وهذا انعكس علي النظام الحركي والنشاط الغذائي لليرقات المتطفل عليها.