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DO SOME HOST LARVAE (*Spodoptera littoralis boisd.*) RETALIATE AGAINST ITS ENDOPARASITOID (*Microplitis rufiventris* Kok.)

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ABSRACT

Some parasitized larvae attack the parasitoid cocoons or the pupal stage, other attack the larvae. In all cases the parasitized host attacks only the parasitoid produced from its body. This phenomenon occurred in consistency with a 6% and independent from the controlling factors (season, temperature, light or feeding). The total body proteins were compared in parasitized and control larvae and it was found that parasitism affected the protein pattern and this effect was characteristic in those larvae which attack diet's produced parasite. This phenomenon is interpreted as being a "retaliation" of host larvae against the endoparasitoid.

INTRODUCTION

Successful parasitic relationships require that the endoparasitoid be adapted to the physiological conditions of the host and utilize host nutrients in a manner compatible with the host survival (Smyth, 1976). To fulfil such goal, alterations of host's physiology are necessary (Vinson, 1984). This alteration of host's physiology is multidiminensional phenomenon usually accompanied by observable changes in growth, development and behavior (Thompson, 1990; Eid *et al.*, 2001). Regulation of the host physiology is initiated in response to several entities, which may interact to produce synergistic effects (Stoltz, 1986; Tanaka and Vinson, 1991). The most notable are the polydnavirsuses (Stoltz *et al.*, 1990; Whitfield, 1990); virus like particles (Federici, 1991).

By association of "third-party" elements (e.g., polydnaviruses, and toxins associated with the endoparasitoid), those complex systems appear to have delicate counterbalance of parasite offense versus host defense, which presumably requires a similar strategy of gene-for-gene coevolution. The parasite may be considered the partner with driver genes directing the host toward evolution of resistance-related behavior (Sayles and Wasson, 1988). Behavioral modification caused by parasitism can be interpreted to have arisen: (1) through natural selection and beneficial to the parasite; (2) through natural selection and beneficial to the host; and (3) as a consequence of pathology or stress, which may or may not benefit either party (Minchella, 1985).

Monitoring the effects of parasitism on host protein synthesis of novel proteins in the host, now constitutes some documentable changes occurring during insect endoparasitism and poses questions about the nature of their potential biological significance and impact upon the host-parasite relationship. Several species of *lepidotrans* (Cook *et al.*, 1984; Beckage *et al.*, 1987&1989; Soldevila and Jones, 1991 & 1993) and *dipterans* (Lawrence,

1990) parasitized by endoparasitic wasps have been shown to synthesize parasitism specific proteins inducing modifications in host production and transport. Additionally, factors secreted or associated with the parasites, such as teratocytes (Dahlman and Vinson, 1993) and polydnaviruses (Stoltz, 1990; Fleming, 1992) may have also a regulatory effect. This regulatory effect may explain the adaptive significance of many of the immunological, metabolic and behavioral changes observed in host organism during parasitism.

The goal of this paper are to view the insect parasite host interaction as a product of "host regulation" and to discuss the major lines of evidence demonstrating that the endoparasitoid *M. rufiventris* regulates its host *S. littoralis* exhibiting major changes involving proteins and pose questions about their potential impact upon altering host behavior.

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.

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). Host larvae were thus transferred individually to clean and sterilized plastic pots covered with muslin cloth and supplied daily with fresh castor leaves. When the parasitoid's pre-pupa emerges care was given to inspect the reaction of the host larvae towards the parasitoid.

Electrophoretic analysis was carried aiming to identify the general protein pattern in the whole body of the late fourth instar larvae from which the third instar larvae of the parasitoid had emerged (PI) and those who attacked the emerged parasitoid (PII) as well as the control larvae. Sampling, stock solutions, protein extraction by SDS polyacrylamide gel electrophoresis (SDS PAGE) and detection of the proteins were carried according to the method of Al-Akkad (1997).

RESULTS AND DISCUSSION

1-Behavioral reaction of some parasitized larvae (PII) towards their parasitoid:

The larvae of the endoparasitoid *M. rufiventris* egress's from its host through an exit hole that the third instar larvae opens in the cuticle of the fifth abdominal segment and then the prepupa starts to spin the cocoon which in most cases remains attached to the host by a silky filament (Fig. 1). The cocoon of the endoparasitoid is spindle shaped with one end broader than the other (Fig. 2). When the adult parasitoid emerges, it opens an operculum at the wide end (Fig. 3). The parasitized larvae were generally different from the unparasitized in their reduced activity, faint tactile responses, loosing behavior of feeding and paralyzed as C shape. After egression of the parasitoid prepupa, the host larvae continued its motionless life for one or two days and died without molting.

It was observed that some larvae of the host (PII) attacked the egress parasitoid in different stages. In some cases as larvae during egression from the body of the host and bit them causing wounds leading to death (Fig. 4). In other cases the pre-pupal stage of the parasitoid was attacked while spinning its cocoon (Fig. 5) and the pre-pupa was vanished (Fig. 5). Sometimes the host larvae attacked the pupal stage by cutting the silk of the cocoon and bit the body causing fatal damage to it (Fig. 6) or leaving the remains of the cocoon after consuming the pupae (Figs. 7&8).

The attack was fulfilled in a little while, tensive, violent and effective by quick twist backward, while the parasitoid larvae was egression. The mouth parts which was not used long ago were used in biting the parasitoid larvae. If this stage of the parasitoid were missed the parasitized larvae would attack the other stages. In all cases these "resuscitated" larvae return, again immobile remaining alive for few days. It died without apolysis. These attacking larvae (PII) were looking more healthy than those who never attack the parasite (PI). They have also brighter cuticle colors and larger size and longer life after the events of attack, as they remained alive two days more than their counter parts(PI).

It was noticed during two years of the experimental work that (PII) larvae always attack their own parasitoid only without attacking any other parasitoids. In an experiment to verify this observation, the host larvae were exposed individually to the fertilized female of the parasitoid to ensure the occurrence of parasitism. The parasitized larvae were then reared individually until the parasitoid's egression. After that the (PII) larvae received full-formed parasitoid cocoons, which were not attacked. Consequently, it is believed that the (PII) only attack the parasitoids produced from their own bodies.

This phenomenon was studied during two years and was found to happen individually under the standard conditions of light, food, temperature and humidity. The results presented in Table 1 show that: 1-In the first year the percentages ranged from 1.9 in June to 13.6 in March. 2-In the second year, the phenomenon was present all year round with a range from 1.0 in January to 12.2 in August. 3-In both years, the average percentage was 6.0 and the phenomenon was nearly consistent in all months.

Figure (1): Cocoon of the parasitoid *M. rufiventris* attached to its host larva at the point of emergence

Figure (2): Normal Cocoon of the parasitoid *M. rufiventris*

Figure (3): Normal Cocoon after the emergence of the adult parasitoid *M. rufiventris*

Figure (4): Third instar larva of the parasitoid *M. rufiventris* attacked by the host larva in the abdominal region

Figure (5): The pre-pupa of the parasitoid *M. rufiventris* attacked by the host larva before completing spinning the Cocoon

Figure (6): The pupa of the parasitoid *M. rufiventris* attacked by the host larva after cutting the Cocoon

Figure (7): Cocoons of the parasitoid *M. rufiventris* attacked the host larva and pupal stage is missing

Figure (8): Cocoons of the parasitoid *M. rufiventris* attacked the host larva and pupal stage is missing

Table (1). Monthly distribution of the number of individuals of the parasitoid *Microplitis rufiventris* attacked by the larvae of its host *Spodoptera littoralis*

Month	Total Number of Parasitoid Larvae Emerging from Host		Total Number of Parasitoid Individuals attacked by Their Host		Percentage of Attacked Individuals	
	1997	1998	1997	1998	1997	1998
January	18	90	-	1	0.0	1.0
February	19	80	-	1	0.0	1.3
March	22	82	3	7	13.6	8.6
April	56	104	6	12	10.7	11.5
May	72	117	5	9	6.9	7.6
June	107	150	2	7	1.9	4.6
July	159	130	10	10	6.3	7.7
August	132	189	12	23	9.1	12.2
September	169	173	8	15	4.7	8.6
October	192	184	17	15	8.9	8.2
November	119	161	10	10	8.4	6.2
December	100	153	7	5	7.0	3.3

2-Differences of body protein of (PI) and (PII) larvae:

These differences were studies using the gel electrophoresis technique (SDS-PAGE) with a low molecular weight protein marker of 66.45 and 24 KD. By examining the protein bands for the healthy larvae and the parasitized (PI and PII) shown in Table 2 and Fig. 9, it can be concluded that control lane contains 16 bands ranging from 28.6 to 21.4 KD, while the PI and PII lanes contain 16 bands ranging from 54.3 to 21.4KD. It is noticed that 9 protein bands are constant in the three lanes which means that parasitism did not cause to them any changes. The molecular weight of these bands are 52.0, 48.7, 46.7, 34.2,31.5,30.0, 26.1, 24.9 and 21.4 KD.

Some protein bands (82.6,43.3,42.4,40.8,28.7 and 23.0 KD) are found only in the control lane, while others (54.3, 45.6,44.2,33.0,27.0 and22.2 KD) are characterizing the parasitized larvae. As shown in Fig. 9 the PII lane is different from the PI lane as well as the control lane. It is evident also, as shown in Table 2, that the PII are different from PI in containing a protein band (23.9 KD) while PI contained a 23.6 KD protein.

The parasitized larvae of *S. littoralis* regulated by the endoparasitoid *M. rufiventris* were suffering from the pathological effects of parasitism on the nervous system. These effects were associated with reduced activity, ceased feeding and faint tactile responses (Eid *et al.*, 2001). Behavioral modifications caused by parasitism were shown by many authors: host larvae become sluggish (Henry and Oma, 1981; Johnson, 1989); others show reduced activity (Webber *et al.*, 1987); or altering the pollination behavior; altering host dispersal behavior (Goulson, 1997); or leading to precocious expression of premetamorphic behavior (Wani *et al.*, 1997).

Table (2): Molecular weight of the protein bands in the different treatments of *Spodoptera littoralis* larvae parasitized by the parasitoid *Microplitis rufiventris*

Band No.	Treatment		
	Control Larvae	Parasitized Larvae	Parasitized Larvae Attacking Their Parasitoids
1	82.6	-	-
2	-	54.3	54.3
3	52.0	52.0	52.0
4	48.8	48.8	48.8
5	46.7	46.7	46.7
6	-	45.6	45.6
7	-	44.2	44.2
8	43.4	-	-
9	42.4	-	-
10	40.8	-	-
11	34.2	34.2	34.2
12	-	33.0	33.0
13	31.5	31.5	31.5
14	30.0	30.0	30.0
15	28.7	-	-
16	-	27.0	27.0
17	26.1	26.1	26.1
18	24.9	24.9	24.9
19	23.9	-	23.9
20	-	23.6	-
21	23.0	-	-
22	-	22.2	22.2
23	21.4	21.4	21.4

In the running work some parasitized larvae of *S. littoralis* submitted to the endoparasitoid *M.rufiventris* displayed a novel behavior, after being in "pathological quiescence" they were "resuscitated" to attack its own endoparasitoid. This novel behavior comprises a behavioral program, which goes beyond a "simple reflex" after egression of the endoparasitoid. It needs physiologically its innate releasing mechanism (IRM) which when stimulated it had to evoke a fixed action pattern (FAP) or a novel behavioral program. These larvae, after this flash rebound, return again to its quiescent state for two or three days before death. This dramatic behavior was processed by injured nervous system and could be interpreted according to Minchella (1985) as have been arisen as a consequence of pathology or stress and may benefit the host because it increase the survival of kin. Our interpretation for this novel behavior is that it is a "retaliate" behavior against the parasitoid beneficial for the host kin. This evaluation as "retaliation" against the parasitoid seems to be objective in light of the host suicidal hypothesis shown by many authors (Latta and Tomlinson, 1987; Mcallister and Roitberg, 1988 and Mcallister *et al.*, 1990).

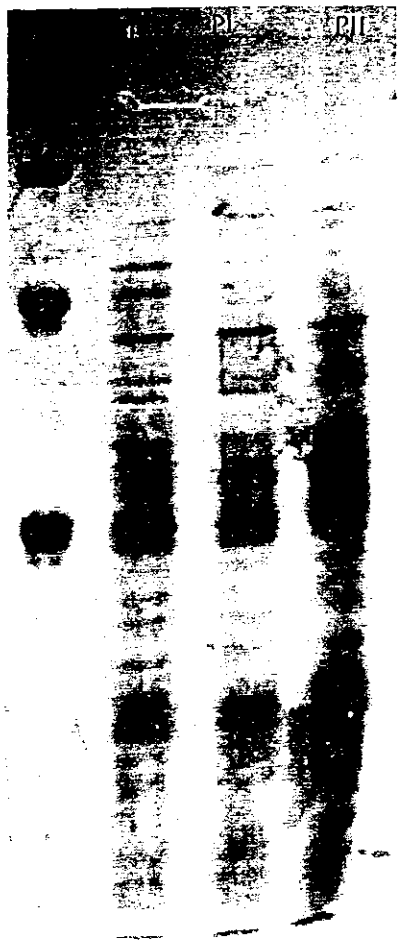

Figure (9): SDS-PAGE electrophoresis of the total body protein contents for the healthy and parasitized larvae of the host *S. littoralis* parasitized by the endoparasitoid *M. rufiventris*

M : Low molecular weight marker
I : Control lane
PI : Parasitized larvae
PII: Parasitized larvae attacking their parasitoid

To determine whether parasitism had affected the protein pattern of the body of host larvae in the same way, electrophoretic analysis was done. It was revealed that in parasitized larvae some proteins disappeared and in those attacking its parasitoid an extra protein disappeared, when compared with healthy larvae.

Several studies demonstrated qualitative and quantitative changes in protein composition in parasitized insects as evidenced by electrophoretic pattern (Vinson and Iwantsch, 1980; Beckage *et al.*, 1987; Jones, 1989 and Solulu *et al.*, 1998).

Several authors studying insect/parasite systems have identified "parasitism-specific" or "parasitism-induced" proteins in insect host of *lepidoptrans* (Cook *et al.*, 1984; Beckage *et al.*, 1987 & 1989; Soldvila and Jones, 1991&1993) and *dipterans* (Lawrence, 1990), a state probably comparable with the host larvae which attack its parasitoid. According to Thompson (1990), the simple presence of the parasite within the body cavity of the host may be sufficient to affect the rates of transcription or translation of host genes, whereby having an impact on host protein synthesis. Additionally, factors secreted or otherwise associated with the parasites, such as teratocytes (Dahlman and Vinson, 1993) and polydnaviruses (Stoltz, 1990; Fleming, 1992) may also have a regulatory influence.

This physiological alteration arises by natural selection through evolutionary process. This evolution was described by Barnard (1990) as an "arm race" during which there occurs constant action and reaction by both host and parasite. Lastly, we suggest as Jones, 1985; Moore and Gotelli, 1990 that a comparative approach may be necessary to fully understand the "retaliation" of host *S. littoralis* against its endoparasitoid *M. rufiventris*.

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هل بعض يرقات عوائل الطفيل الداخلـــي *Microplitis rufiventris* تـــثأر لنفسها من الطفيل؟

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قسم الحشرات الاقتصادية والمبيدات – كلية الزراعة – جامعه القاهرة

يوضح البحث أن بعض يرقات دودة ورق القطن المتطفل عليها تهاجم شرانق أو طـور العذراء وأحيانا الطور اليرقي للطفيل الداخلي الخارج منها. وهذه الظاهرة حدثت بنسبة ثابتة قدرها ٦%. تم تقدير البروتين الكلي لليرقات المتطفل واليرقات غير المتطفل عليها ولوحظ أن اليرقـــات المتطفل عليها تأثرت من حيث كمية ونوعية البروتين. وهذه الظاهرة توضح علاقة جديـــدة بيـــن العوائل والطفيليات الداخلية.