SHORT NOTE

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Impact of *Vairimorpha ephestiae* (Microsporidia: Nosematidae) on *Bracon hebetor* (Hymenoptera: Braconidae), an external parasite of the American bollworm, *Heliothis armigera* (Lepidoptera: Noctuidae)

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Microsporidia, *Vairimorpha ephestiae*, Braconidae, *Bracon hebetor*, Noctuidae, *Heliothis armigera*, pathogen-parasitoid interaction

Abstract. The impact of the microsporidium *Vairimorpha ephestiae* on the parasitoid *Bracon* (= *Habrobracon*) *hebetor* increased by increasing the concentration of spores to which its host, *Heliothis armigera* was exposed. Infecting parasitized hosts with the microsporidia did not significantly affect either the egg or larval duration of the parasitoid, while it caused significant prolongation in pupal duration of *B. hebetor*. However, infected parasitized hosts produced female parasitoids with reduced longevity, oviposition period and fecundity.

It was concluded that the detrimental impact on the parasitoid might be related to nutritional deficiency caused by the accumulation of nondigestable spores in the parasitoid's gut lumen.

Introduction

Pathogenicity of the microsporidian, *Vairimorpha* (= *Nosema*) *ephestiae* to lepidopterous pests has been proved in many research studies (Weiser, 1957, 1977; Maddox & Sprenkel, 1978; Lewis et al., 1982; Weiser & Purrini, 1985; Moawed et al., 1987). Moawed et al. (1991) found that early instars of the cotton bollworm, *Heliothis armigera* (Say) were highly susceptible to the microsporidian, showing very high mortality levels while several adverse effects as sluggish movements, reduced food consumption and retardation of growth were demonstrated in late instars sublethaly infected with the pathogen. Hence, the microsporidian could be considered as a potential biological control for this pest.

The ectoparasitoid *Bracon hebetor* (Say) was also reported as an efficient biological control agent of *H. armigera* (Davletshina, 1972) and other lepidopterous larvae (Soliman, 1940; Harakly, 1969; Nasrallah, 1969; Reinert & King, 1971; Beard, 1972; Press et al., 1982; Marei, 1985). However, information on the effects of microsporidia on parasitic insects is not available.

The objective of this study is to explore the possible interactions between both bioagents, the parasitoid *B. hebetor* and the microsporidian *V. ephestiae*, when acting together on the host pest, *H. armigera*.

Material and methods

Host insect

A laboratory colony of the cotton bollworm, *H. armigera*, reared on artificial diet (Shorey & Hale, 1965) and maintained under sterile conditions (Hassan & Moawed, 1974) was used as the source of experimental insects.

Pathogen

Spores of *V. ephestiae* were obtained from diseased *Ephestia kuehniella* larvae. The method of Weiser & Purrini (1985) for isolation of the microsporidia was adopted. The spores of *V. ephestiae* were propagated in the original host larvae (*E. kuehniella*) when a stock culture was reared.

Parasitoid

A colony of *B. hebetor* was established under laboratory conditions according to Marei, 1985. Adult parasitoids were confined in pairs in small test tubes (1.5 cm width and 5 cm length). Adults were provided with 25% honey solution in the form of droplets on the walls of the test tubes, which were stoppered with balls of cotton wool. Adults were provided daily with *H. armigera* larvae (one larva/tube) on which parasitoid eggs were deposited. Host larvae externally harbouring parasitoid eggs were removed daily and maintained in other tubes till parasitoid emergence; they were replaced by fresh larvae.

Assessment of pathogen parasitoid interaction

Small rectangular pieces of synthetic host diet (1 × 2 × 3.5 cm each) and two suspensions of the microsporidia in water at the concentration levels of 20×10^6 and 2.5×10^6 spores/ml were prepared. The spores of the microsporidia were incorporated into the larval diet using the method of Moawed (1980) as follows: 0.5 ml of the desired concentration was distributed on the surfaces and sides of each piece of diet, previously perforated by means of a thin needle, and left until the diet surface dries. The rectangular pieces of contaminated diet were placed in plastic cups of 7 × 7 × 7 cm with fifty first instar larvae of *H. armigera*, at the rate of 5 pieces/50 larvae/cup. Four cups were used for each concentration level. All cups were incubated at 27°C and 60–70% R.H. for five days, during which time the larvae reached the third instar. A control group of 50 *H. armigera* first instar larvae were offered uncontaminated pieces of diet; the surface was treated with 0.5 ml distilled water each and incubated under the same conditions. Samples of dead and alive larvae were examined for the presence of the pathogen under a phase contrast microscope.

After incubation, twenty survivors of *H. armigera* larvae from each treatment group as well as control group, were collected and distributed individually into test tubes that confined one pair of adult *B. hebetor* per each and kept for 24 h to allow enough time for parasitisation. Parasitized larvae that stopped feeding and development were transferred to rearing tubes: 4 replicates and 5 larvae/replicate were used for each concentration level, as well as for the control group.

The latent effects of the microsporidia on unparasitized and parasitized *H. armigera* larvae exemplified by cumulative percent mortalities in different developmental stages were evaluated.

All data were subjected to statistical analysis of variance. Means were compared with Student's t-test and Duncan's (1955) multiple range test.

Results and discussion

Effect of *V. ephestiae* and *B. hebetor* on *H. armigera*

Normal mortalities of hosts in the control groups were 5.00, 3.51 and 8.33% during larval, pupal and larval-pupal stages, respectively. Corrected percentages of mortalities among *V. ephestiae*-treated *H. armigera* larvae at the concentration levels 2.5×10^6 and 20×10^6 during the whole larval stage were 21.1 and 52.6%, respectively (Table 1). Subsequent mortalities of 67.8 and 74.0% were evaluated during the pupal stage for the same concentrations, respectively.

However, the corresponding cumulative mortalities reached 74.6 and 87.3%, respectively. Mortalities due to parasitism were 95 and 100% in the larval and pupal stages, respectively.

TABLE 1. Effect of *Vairimorpha ephestiae* and *Bracon hebetor* on the survival in *Heliothis armigera*.

Treatment	Mortality (%)		
	Larvae	Pupae	Cumulative
V. ephestiae 20×10^6	(52.6)* 55.00	(74.0)* 74.07	(87.3)* 88.33
V. ephestiae 2.5×10^6	(21.1)* 25.00	(67.8)* 68.89	(74.6)* 76.76
B. hebetor	95.00	100	100

* Corrected mortalities.

Effect of *V. ephestiae* on the immature stages of *B. hebetor*

Neither the incubation period of the egg stage nor the larval duration of the parasitoid reared on *V. ephestiae* infected *H. armigera* larvae differed significantly from the corresponding measures when the parasitoid was reared on untreated larvae. However, the microsporidia significantly increased the pupal duration ($P < 0.01$) (Table 2).

TABLE 2. Duration of different immature stages of *B. hebetor* parasitizing non-infected and *V. ephestiae*-infected *H. armigera* larvae (in days).

Stage of *B. hebetor*	Infected hosts	Non-infected hosts
Egg	1.50 ± 0.354a*	1.25 ± 0.250a
Larva	3.75 ± 0.250a	2.88 ± 0.217a
Pupa	12.63 ± 0.960a	8.88 ± 0.739b

* Means within rows followed by the same letter are not significantly different according to Student's t-test means based on 5 replicates/treatment, 10 larvae/replicate; b – different at 5% level.

Effect of host treatment with *V. ephestiae* on the biological activities of *B. hebetor* adults

The results presented in Table 3 showed that the host disease had greatly affected emerged parasitoid females. Adult females produced from *V. ephestiae* diseased hosts had significantly reduced longevity, oviposition period and fecundity, while hatchability was not significantly affected. On the other hand, the average longevity of adult males of *B. hebetor* produced from diseased hosts was similar to that of adults resulting from healthy ones.

TABLE 3. Biological activity of *B. hebetor* produced from non-infected and *V. ephestiae*-infected *H. armigera* larvae.

Aspect	Adults produced from	
	infected hosts	non-infected hosts
Preoviposition period (days)	1.38 ± 0.42a*	1.63 ± 0.42a
Oviposition period (days)	18.50 ± 4.39a	21.75 ± 4.87b
Post-oviposition period (days)	1.74 ± 0.65a	1.92 ± 0.54a
Longevity of males (days)	20.25 ± 6.22a	22.75 ± 6.18a
Longevity of females (days)	21.75 ± 3.34a	28.50 ± 5.59c
No. of eggs/female	197.75 ± 45.29a	262.56 ± 45.35c
No. of eggs/host larva	8.50 ± 1.80a	13.50 ± 4.03c
Hatchability (%)	86.75a	87.24a

* Means within rows followed by the same letter are not significantly different according to Student's t-test, means based on 10 pairs/treatment; b – different at 5% level; c – different at 1% level.

These results are in agreement with those obtained by Wilson, 1978; Fuxa, 1979; Moawed, 1980 and Moawed et al., 1987, 1991. The microsporidian, *V. ephestiae* usually kills the host during the larval stage when the fat bodies are filled with spores (Moawed, 1980; Weiser & Purrini, 1985). Moreover, the infection with microsporidia increases the metabolic activity of the insect (Kučera & Weiser, 1985). These interactions may be the reason for the high mortality among the pupal and adult stages of *H. armigera* in the present investigation.

Concerning the effect of *V. ephestiae* on *B. hebetor*, the results obtained agree with those of Cossentine & Lewis (1986) and Siegel et al. (1986) who stated that the lower percentage of parasitoid pupation from microsporidian-infected host probably was not a result of the host dying before the parasitoid development was completed, but, instead, was a direct effect of the microsporidia on the parasitoid.

Previous research findings, as reviewed by Brooks (1973), have suggested the accumulation of large numbers of undigestible microsporidian spores in the midgut of the parasitoid larva may have resulted in a nutritional imbalance that may be fatal to the parasitoid.

In conclusion, this study indicates that *V. ephestiae* exerts detrimental effect on the parasitoid *B. hebetor*, and future research should concentrate on the interplay between the pathogen and the parasitoid.

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