Journal of Invertebrate Pathology **74,** 120–126 (1999)
Article ID jipa.1999.4865, available online at http://www.idealibrary.com on IDEAL®

Impact of *Vairimorpha* sp. (Microsporidia: Burnellidae) on *Trichogramma chilonis* (Hymenoptera, Trichogrammatidae), a Hymenopteran Parasitoid of the Cabbage Moth, *Plutella xylostella* (Lepidoptera, Yponomeutidae)

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Received July 13, 1998; accepted March 25, 1999

A multi-generation mass breeding colony of the cabbage moth, *Plutella xylostella,* was found to be infected with a microsporidium, *Vairimorpha* sp., which is passed transovarially between generations. The microsporidian infection had little impact on the fitness of this lepidopteran pest. However, when *Trichogramma chilonis* parasitized such infected host eggs, the offspring of this parasitoid species suffered from severe deficiencies. Microsporidian spores, ingested by parasitoid larvae together with the host egg nutrients, gave rise to stages which developed in various tissues of the parasitoid, such as the flight muscle and the nervous system. This infection resulted in a significantly increased rate of metamorphosis failure (related to host age) and reduced longevity and reproductive performance of the parasitoids. There are two main consequences arising from our findings if *T. chilonis* is to be used in an integrated control strategy against *P. xylostella:* (1) *T. chilonis* must be raised on *Vairimorpha*-free host eggs to receive viable and efficaceous parasitoids for release and (2) if natural populations of the cabbage moth in cruciferous crops are infected with *Vairimorpha* to a significant extent, the parasitoid must be released repeatedly within infested crop areas.

Key Words: **biological control; cabbage moth; *Plutella xylostella;* parasitoid; *Trichogramma chilonis;* pathogen; microsporidia; *Vairimorpha*.**

INTRODUCTION

The cabbage moth, *Plutella xylostella* (Lepidoptera, Yponomeutidae), is a serious pest insect in cruciferous crops. The annual costs for control of this pest species exceeds one million United States dollars (Talekar and Shelton, 1993). In tropical and subtropical climates, the cabbage moth produces up to 18–22 generations per year and, as a consequence, rapidly develops resistance against chemical control agents (Barroga, 1971; Barroga and Morallo-Rejesus, 1975/76; Talekar *et al.*, 1986; Lim, 1990; Wührer and Hassan, 1993; for review see Talekar and Griggs, 1986). Thus, an integrated pest control strategy is required to keep the cabbage moth below the economic damage threshold.

Parasitoids of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) have proved to be valuable tools in the biological control of agricultural pest species and have been successfully implemented in integrated pest management programs, including pest control in field crops (Wajnberg and Hassan, 1994; Smith, 1996). One promising candidate for the biological control of *P. xylostella* is the parasitoid *Trichogramma chilonis,* which preferentially parasitizes eggs of this target species (Klemm and Schmutterer, 1992; Wührer and Hassan, 1992, 1993).

The mass breeding of parasitoids should ideally be performed on the target pest species to increase host specificity of the released individuals. Recently, however, an infection of our multi-year mass breeding of *P. xylostella* with the microsporidium *Vairimorpha* sp. (Microsporidia: Burnellidae), an obligate intracellular parasite, was detected (Jungen, 1996). Although microsporidia have been used as biological control agents against lepidopteran pests (Kramer, 1965; Brooks, 1988), the moderately decreased vitality of *Vairimorpha*-infected individuals as reported by Jungen (1996) appears to be insufficient for control of cabbage moth populations. On the other hand, the simultaneous occurrence of intracellular parasites and parasitoids in the same host population may have negative implications for pest control, since infection of parasitoids with host microsporidia has frequently been reported (e.g., Thomson, 1958; Issi and Maslennikova, 1996; Brooks and Cranford, 1972; Andreadis, 1980; Brooks, 1993). More recently, Norton *et al.* (1988) reported a microsporidian infection of *Diadegma semiclausum* (Hymenoptera, Ichneumonidae), a larval parasitoid of *P. xylostella*. The infection of *D. semiclausum* with *Vairimorpha* sp. prevented a proper propagation of this

0022-2011/99 $30.00

parasitoid and thus decreased its control efficacy below the economically required level.

In this study, we examined the pathogenicity of *Vairimorpha* sp. on the *Plutella* egg parasitoid *T. chilonis* in an effort to contribute to the potential for using *T. chilonis* as a biological control agent against *P. xylostella*.

MATERIALS AND METHODS

Culturing of Cabbage Moth and Parasitoids

Two groups of *P. xylostella* were cultured separately, one infected with *Vairimorpha* and another free of this microsporidium. *Vairimorpha*-free host cultures were acquired by storage of freshly laid host eggs at 4°C for 1 week over two or three generations. Each new generation was carefully checked for microsporidian infection by selection of 10 caterpillars at random which were subsequently examined in wet-mount tissue preparations under a microscope. Once the *Vairimorpha*-free stock culture had been established, wet-mount tissue checks for the presence of *Vairimorpha* were performed weekly. Eggs of *P. xylostella* were harvested from pieces of folded parafilm which were readily accepted by *Plutella* females for egg deposition.

A stock culture of *T. chilonis* was purchased from Dr. S. A. Hassan (Federal Biological Agency, Darmstadt, Germany). The parasitoid was raised subsequently on eggs of the grain moth, *Sitotroga cerealella* (Lepidoptera, Gelechiidae) in small glass tubes (14.5 cm high × 2.5 cm in diameter) which contained fresh host eggs at a host-to-parasitoid ratio of about 3:1. Host eggs were glued with Traganth (Merck AG, Darmstadt) onto a paper strip in three discrete circles each containing about 1000 eggs. Adult *T. chilonis* were fed with an aqueous honey solution (200 g honey, 100 ml water, and 3 g gelatine) applied onto the host egg-containing paper strips. Host-egg strips were replaced daily and the parasitized host eggs stored separately in fresh glass tubes (hatching chambers). For our experiments, adults were harvested by emptying a hatching chamber over a white paper sheet. The emergent wasps were then confined with small glass tubes whose walls they rapidly ascended. From these trapping tubes they were directly passed to the experimental tubes. Unless stated otherwise, all experiments were performed at constant climatic conditions of 70% RH (±5%), 26°C (±1°C), and a 16L:8D photoperiod.

Impact of Vairimorpha *on the Emergence Rate of* T. chilonis *Offspring from Host Eggs*

Infected and *Vairimorpha*-free host eggs were sampled from the respective stock culture and stored at room temperature for 0–24 h (egg class I), 24–48 h (egg class II), and 48–72 h (egg class III) prior to use (at 20–23°C, *Plutella* completes embryonic development within 96 h; see Happe, 1991). Parasitoids used for this experiment were between 0 and 24 h old and approached their first reproductive cycle.

The parasitoids were inserted into glass tubes which contained one strip of either infected or noninfected host eggs of the same egg age class (host egg-to-parasitoid ratio of about 3:1). After 24 h, host eggs were removed and separately stored in snap glasses (14.5 cm high × 2.5 cm in diameter) for a 10-day period to allow all parasitoid offspring to emerge from the host eggs. All *T. chilonis* that emerged were counted and removed. Then, host eggs were inspected under a binocular scope to determine the number of parasitized host eggs in which the parasitoid failed to complete the metamorphosis (no hatch openings). Parasitized host eggs are easy to identify by their black coloration. For each egg age class, 24 egg strips each containing about 100 host eggs were examined. Twelve of these strips contained *Vairimorpha*-free host eggs; the other 12, infected host eggs. For our evaluation it was assumed that all host eggs from the *Vairimorpha*-infected stock culture were infected by this microsporidium. This assumption is supported by Jungen (1996), who found *Vairimorpha* spores in >90% of the host eggs from this stock culture. Since potentially a maximum of 10% of the host eggs considered to be infected may have been *Vairimorpha*-free, our observed impact of this microsporidium on the parasitoid emergence rate may be a slight underestimate of the real impact. This may slightly affect the quantitative but not the qualitative conclusions from our test results.

Parasitoids which emerged from *Vairimorpha*-infected host eggs as well as the larva/pupa inside host eggs were examined for microsporidian infection. Approximately 50 adult and 50 preimaginal *T. chilonis* were selected randomly from each of the 12 replicates of infected host egg batches. Adult parasitoids and preadult stages removed from parasitized host eggs were individually mashed within a droplet of distilled water on a glass slide and tissues of the parasitoid examined for microsporidian spores using a phase-contrast microscope (Leitz Dialux 22) at 400×. The sex ratio of all parasitoids emerging from host eggs (only age class I) was also determined to highlight sex-specific differences in parasitoid sensitivity. Sex of *T. chilonis* was determined by morphological differences of the antennae: the antennal tips of *T. chilonis* females are clavated and poorly fimbriciated. In contrast, male *T. chilonis* have nonclavated antennal tips and their antennae are densely fimbriciated.

Longevity and Fecundity of T. chilonis *Infected with* Vairimorpha *via Host Eggs*

T. chilonis females raised from either infected ($n = 25$) or *Vairimorpha*-free ($n = 30$) *P. xylostella* eggs (egg age

class I) were examined for their longevity and their lifetime reproductive performance. Individual females were maintained in glass tubes (5 cm high, 2 cm in diameter) and fed with a honey–water solution. Parasitization capacity was determined by providing about 200 eggs of *Sitotroga cerealella* glued onto paper strips (1 × 3 cm) which were replaced every 24 h until the female died. All parent females were postmortally examined for microsporidia to ensure that these females had been correctly allocated to the respective test group. Harvested host eggs were stored separately for a 10-day period, after which the number of parasitized eggs was determined.

Transovarial Passage of Vairimorpha *sp. in* T. chilonis

Jungen (1996) reported that in *P. xylostella* the infection with *Vairimorpha* is passed transovarially between generations. To highlight a potential transovarial transfer of *Vairimorpha* in *T. chilonis*, microsporidia-free eggs of *P. xylostella* were exposed to female wasps ($n = 20$) which had been raised from infected host eggs. From the offspring, 90 randomly selected individuals were then examined for *Vairimorpha* spores. For assurance that the examined offspring did unequivocally originate from infected parasitoids, the parent females were examined for *Vairimorpha* presence after they had parasitized the host eggs (wet-mount tissue preparations). If no spores were found, the respective host egg batch was discarded.

Histological Examinations

The temporal appearance of *Vairimorpha* inside preimaginal stages of *T. chilonis* was followed by histological examinations. *Vairimorpha*-infected *P. xylostella* eggs ($n = 150$; egg age class I) were exposed for 1 h to *T. chilonis* females ($n = 50$) raised on *S. cerealella* eggs. Then, host eggs were removed and stored over a 7-day period (parasitoids normally commenced hatching at day 7 under these storage conditions). On each of these 7 days 15–20 parasitized *P. xylostella* eggs were carefully sampled from the egg strip using a fine paint brush. On days 1 and 2 after exposure, eggs were sampled randomly, since the black discoloration of parasitized host eggs does not develop before day 3 following parasitization. The sampled host eggs were fixed according to the method of Zalokar and Erk (1977). Eggs were dehydrated by subjecting them to an ascending alcohol solution sequence followed by embedding them into paraplastics (Sherwood Medical). Sections of 7 µm thickness were prepared and stained with Heidenhains' ferric haematoxilin.

RESULTS

Impact of Vairimorpha *sp. on the Emergence Rate of* T. chilonis *Offspring from Host Eggs*

In class I host eggs of *P. xylostella* (0–24 h), the emergence rate of *T. chilonis* offspring was not impacted by a host egg infection with *Vairimorpha* ($P = 0.12$; Mann-Whitney U test; $U = 99$) (Table 1). In contrast, the emergence rate was significantly ($P < 0.001$; Mann-Whitney U test; $U = 141$) (24–48 h) and $U = 140$ (48–72 h)) reduced in *Vairimorpha*-infected host eggs of age class II (24–48 h) and III (48–72 h) compared with noninfected host eggs of the same age class. This host age-related impact of the microsporidium on the emergence rate correlated well-with the host age-related increase in the percentage of parasitoid offspring found to be infected with *Vairimorpha* (Table 2).

In addition to a *Vairimorpha* infection, host egg age itself appeared to affect the emergence rate of the parasitoid. Compared with noninfected class I and II eggs, the emergence rate from noninfected class III eggs (48–72 h) was significantly reduced ($P = 0.01$; Mann-Whitney U test; $U = 16$).

In class I host eggs, the sex ratio of emerging *T. chilonis* (Table 3; $P > 0.05$; χ^2 test; $\chi^2 = 1.03$) and the time to emergence (166–170 h) was found not to be affected by a host egg infection with *Vairimorpha*.

Fecundity and Longevity of T. Chilonis *Infected with* Vairimorpha *via Host Eggs*

T. chilonis females, infected with *Vairimorpha* via host eggs (0–24 h), revealed a significantly ($P < 0.001$;

TABLE 1

Impact of Host Egg Age and of a Host Egg Infection with *Vairimorpha* sp. on the Emergence Rate of *Trichogramma chilonis* Offspring[a]

	Emergence rate [% mean ± SD][b]		
Host eggs	0–24 h old host eggs	24–48 h old host eggs	48–72 h old host eggs
Noninfected	82.9 ± 10.5 a (1789)	82.3 ± 8.8 a (2498)	70.4 ± 7.2 a (1998)
Infected	76.2 ± 8.3 a (2617)	45.0 ± 13.3 b (1970)	46.3 ± 12.1 b (1789)

[a] Eggs of *Plutella xylostella* were used as host eggs. Freshly laid host eggs were sampled and stored for different periods before parasitization.

[b] Mean values and standard deviations refer to 12 replicates. Numbers in parenthesis give the total number of host eggs examined. Numbers in rows followed by a different letter were found to be significantly different ($P < 0.05$; Mann-Whitney U test).

TABLE 2

Percentage of Infected *T. chilonis* which Were Raised from Differently Aged *Vairimorpha*-Infected Host Eggs[a]

Stage of parasitoid	Infection rate [% mean ± SD][b]		
	0–24 h old host eggs	24–48 h old host eggs	48–72 h old host eggs
Larvae/pupae found dead within host egg	69.1 ± 10.1 a (494)	96.5 ± 4.7 b (595)	95.0 ± 6.0 b (588)
Emerged parasitoids	53.5 ± 5.7 a (611)	79.6 ± 11.0 b (578)	87.1 ± 4.4 b (555)

[a] All parasitoids emerging from the host eggs were trapped and examined. Parasitoids which died before emergence were removed from the host eggs before examination.

[b] Mean values and standard deviations refer to 12 test runs. Numbers in parenthesis give the total number of examined parasitoids. Numbers in lines followed by different letters were found to be significantly different ($P < 0.05$; Mann-Whitney *U* test).

Mann-Whitney *U* test; $U = 16.5$) reduced longevity compared with *Vairimorpha*-free females (Table 4). An infection with *Vairimorpha* also caused a strong decrease in the fitness of the females. The total lifetime reproductive performance was significantly ($P < 0.001$; Mann-Whitney *U* test; $U = 0$) reduced when the parasitoid females were infected with *Vairimorpha* (Table 4).

Temporal Pattern of Infection of Larval Parasitoids with Vairimorpha

As soon as 3 days after parasitization, microsporidian spores were found in the intestinal lumen of the larval parasitoid (Fig. 1). Three days later, spores were also found in other tissues (Fig. 2).

Is There Evidence for a Transovarial Passage of Vairimorpha *in* T. chilonis?

A final aspect addressed the question whether or not offspring of *Vairimorpha*-infected *T. chilonis* will also become infected via transovarial passage of the microsporidium. Spores of *Vairimorpha* were not found in any of the developmental stages examined.

TABLE 3

Influence of a Microsporidian Infection (*Vairimorpha* sp.) of Host Eggs (*Plutella xylostella*) on the Sex Ratio of Emerging Parasitoids (*Trichogramma chilonis*)[a]

	Number of parasitoids emerged from	
	Infected host eggs	Noninfected host eggs
Male wasps	214	251
Female wasps	1052	1112
Sex ratio (males:females)	1:4.9	1:4.4

[a] Host eggs were parasitized by *T. chilonis* within 0–24 h after egg deposition. For each host egg category (infected and noninfected), 12 egg strips with about 100 host eggs each were examined.

TABLE 4

Mean Longevity and Lifetime Reproductive Performance of *T. chilonis* Females which Were Either *Vairimorpha*-free or Infected via *Plutella xylostella* Eggs (0–24 h)[a]

Host eggs	Number of parasitoids examined	Longevity of parasitoids [days][b]	Number of host eggs parasitized over lifetime[b]
Noninfected	30	11.5 ± 3.3 a	131.5 ± 42.6 a
Infected	25	2.6 ± 1.4 b	12.3 ± 7.9 b

[a] Emergent females were maintained individually in small glass tubes and provided daily with *Sitotroga* eggs. These eggs were examined for parasitization after a storage period of 10 days. Infection of parasitoid females was assured by postmortal wet-mount tissue examinations.

[b] Numbers in rows followed by different letters were found to be significantly different ($P < 0.05$; Mann-Whitney *U* test).

DISCUSSION

Egg parasitoids of the genus *Trichogramma* are considered to be highly efficient biological control agents since they kill the host before the disastrous feeding stages can develop. For that reason, egg parasitoids are numerously established in integrated pest control programs for greenhouse and field crops (Hassan, 1989; for review see, e.g., Li-Ying, 1992, 1994; Smith, 1996). *T. chilonis* is a representative species of this genus, which is assumed to be a potential tool in an integrated control strategy against *P. xylostella* due to its high host specificity. For determining whether or not a parasitoid is suitable for control purposes, a number of host–parasitoid relationships have to be examined. In the present paper, we focused mainly on effects of host egg infection with the microsporidium *Vairimorpha* sp. on the parasitization efficacy of *T. chilonis*. Some general aspects of parasitization efficacy related to host egg age were also considered.

Generally, *Trichogramma* appears to prefer freshly deposited host eggs for parasitization (Schmidt, 1994). In our study, the emergence rate of *T. chilonis* offspring significantly decreased when host eggs were older than 48 h at the time of parasitization. This is assumed to be attributed to an advanced host embryo development which limits the amount of free nutrients for the parasitoid larva within the host egg.

An infection of host eggs with the microsporidium *Vairimorpha* sp. reduced the parasitization efficacy of *T. chilonis* offspring in an host age-related pattern. In infected class II and III eggs (24–72 h), the emergence rate of the parasitoid offspring was significantly reduced, whereas in class I eggs, no significant impact was observed relative to noninfected host eggs. The decrease of the emergence rate was well correlated with the percentage of emergent parasitoids exhibiting *Vairimorpha* spores in their tissues; i.e., once *Vairimorpha* started sporulation in the host eggs, only very few parasitoids are spared from an infection. In addition to

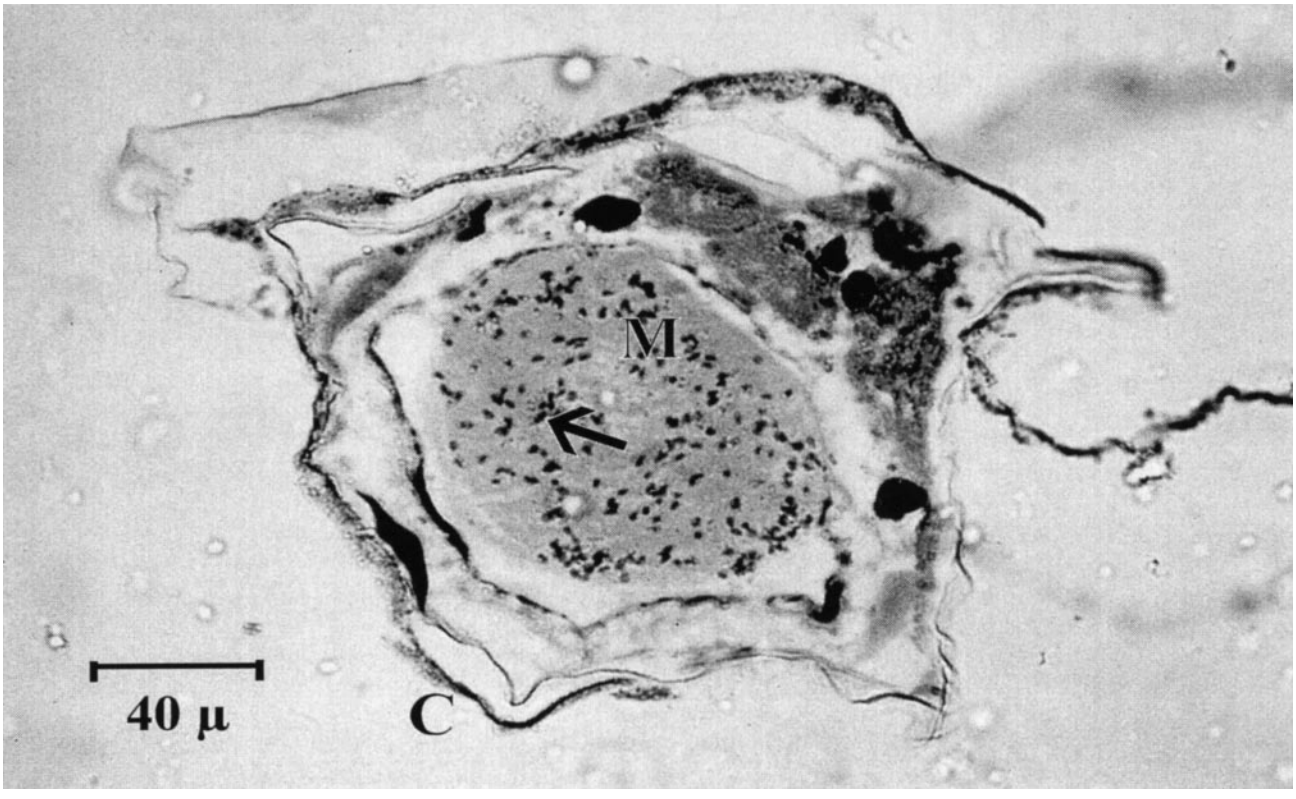

FIG. 1. Light micrograph. Cross section of host egg (C, chorion) showing *Vairimorpha* spores (arrow) in the midgut lumen (M) of the developing larva of *T. chilonis* (Heidenhains' ferric haematoxilin).

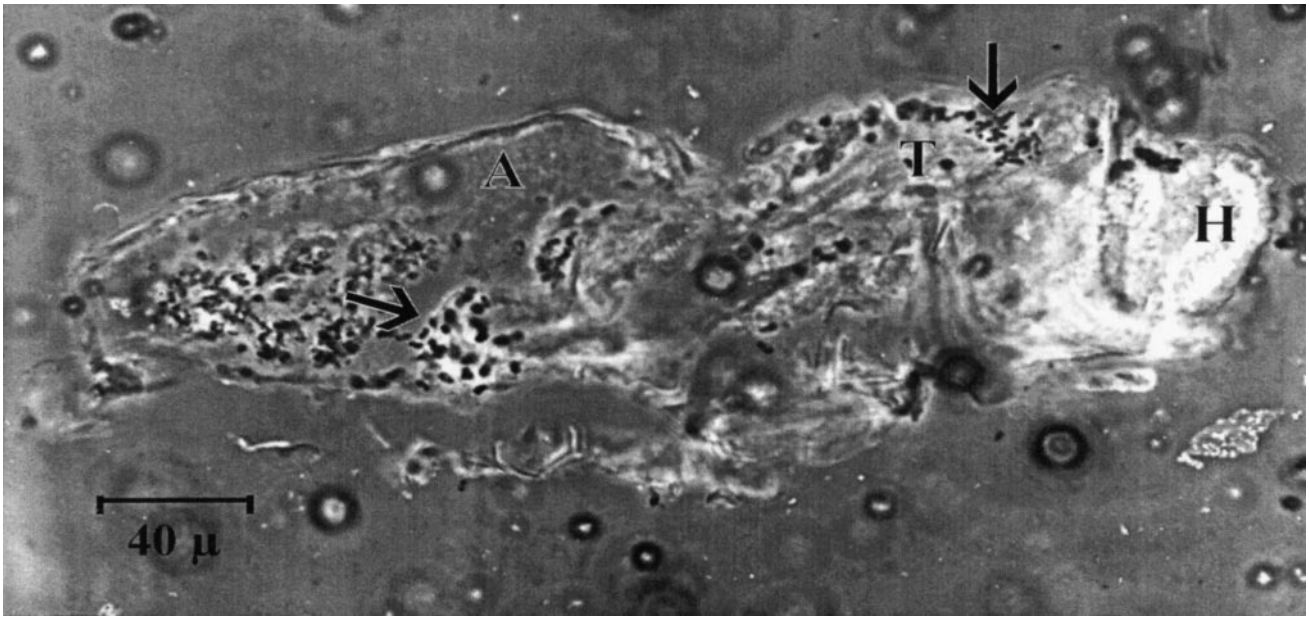

FIG. 2. Light micrograph. Longitudinal section of the pre-emergent stage of *T. chilonis* with microsporidia spores (arrows) in abdomen (A) and in the wing muscle tissue (T, thorax; H, head) (Heidenhains' ferric haematoxilin).

the reduced emergence rate, infected female parasitoids suffered from a strongly reduced longevity and lifetime reproductive performance. Similar results are described by other authors who examined such specific host–parasitoid–microsporidium interactions in *Campoletis sonorensis* (Brooks and Cranford, 1972), *Macrocentrus grandii* (Andreadis, 1980), and *Pediobius foveolatus* (Own and Brooks, 1986). For *Trichogramma nubilale,* Sajap and Lewis (1988) also found a significant reduction of the offspring emergence rates but reproductive performance was hardly impaired by a *N. pyrausta* infection. In contrast, *T. evanescens* suffered from a significantly reduced fitness but no adverse effects of microsporidia on offspring emergence rates were observed (Huger, 1984). It must be emphasized, however, that the authors of the last two studies examined the impacts of microsporidia only on 0- to 24-h-old host eggs.

Based on our histological examinations, we conclude that larval *T. chilonis* ingest the microsporidia together with the egg nutrients of the host egg. This conclusion is based on the finding that in the early phase of the infection microsporidian spores were exclusively detected in the intestine. Only in later phases of infection were spores found in other tissues of the parasitoid.

In our study, we found no indications of a transovarial passage of the microsporidium by *T. chilonis.* Although the wet-mount preparation technique does not enable detection of vegetative stages of *Vairimorpha,* presence of vegetative stages only is considered to be highly unlikely. This assumption is based on the consideration that microsporidia can amplify only via spores (infectious stage) and sporulation is the main endeavor of the microsporidia after successful host infection. Thus, if no spores can be detected as late as 7 days after infection, the host is most presumably noninfected. As with *T. chilonis*, no indications for a transovarial passage of microsporidia were also found for *T. evanescens* and *T. nubilale* (Huger, 1984; Sajap and Lewis, 1988).

Summarizing our results, we conclude that *T. chilonis* is susceptible to a host egg infection with *Vairimorpha* sp., which may limit its use as a biological control agent against *P. xylostella*. If *T. chilonis* is to be used for *Plutella* control, breeding must be conducted on microsporidia-free host eggs for efficient mass breeding. In addition, if the *Plutella* population to be controlled is infected with microsporidia to a significant extent, biological control would require more than one release of that parasitoid to counter the impacted propagation of *T. chilonis* on those host populations.

ACKNOWLEDGMENT

We thank Mr. Klaus-Dieter Stachulla very much for electronic processing of the light micrographs.

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