Some studies on *Nosema* infecting DBM in Malaysia

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Abstract

Field and laboratory studies on *Nosema bombycis* infecting diamondback moth (DBM), *Plutella xylostella* (L.), were conducted. Percent *N. bombycis* infection and mean infection intensity were significantly different (*P*<0.05) among DBM larvae or pupae collected from either highland (CH) or lowland (SG) areas. Percent infection was significantly higher (*P*<0.05) for both larvae and pupae (71.3 and 66.8%) in the CH than in the SG (10.0 and 2.4%). It was also noted that the dead DBM larvae and pupae collected from the field had an abundance of *Nosema* spores especially DBM from the highlands. Percent mortality was significantly (*P*<0.05) higher in smaller instars (I and II) than larger instars (III and IV) even at lower spore concentration (4,260 spore/µl) and 24 h after treatment. There was also evidence that *Nosema* had developed resistance to the antibiotic (Fumidil-B) commonly used in making artificial diet for DBM. The recommended Fumidil-B seems to be ineffective in stopping disease development even at 400 ppm (220 ppm – current recommendation). Other antibiotics showed no better effect in controlling the disease infection than Fumidil-B. Temperature treatment was also unable to check the disease development even at 50°C. There was evidence that *Diadegma semiclausum* was involved in horizontal transmission of *Nosema* spores among DBM larvae. *Nosema* was observed to have a negative effect on the diurnal behaviour of DBM and *D. semiclausum*. Because the time spent by severely infected parasitoids was less than that of less or uninfected parasitoids, the prevalence of disease in the field might have a negative impact on the role of the parasitoid as an effective biological control agent of DBM.

Keywords

*Nosema bombycis*, diamondback moth, *Diadegma semiclausum*, antibiotic, temperature

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.), is a major insect pest of crucifer crops worldwide. In Malaysia, DBM was reported to cause serious damage on crucifers in 1925 (Ho 1965) and, since the 1940s, the main method of control has been the use of insecticides. The demand for these synthetic chemicals has been substantial and seems endless. Such over-dependence on insecticides has led to several pesticide-related problems such as resistance development to almost all insecticides including *Bacillus thuringiensis* (Bt) by DBM, hazards to non-target organisms, environmental pollution, poisoning and residues in the harvested produce (Ooi 1986, Loke *et al*. 1997).

An integrated DBM management (IPM) package stressing the use of DBM biological control agents in combination with other suitable methods such as insect growth regulators and the microbial insecticide (*Bacillus thuringiensis*, Bt), taking crop phenology into consideration and applying need-based treatments of pesticides has been used since the 1980s (Ooi 1986, Lim 1986, Ooi 1992, Loke *et al*. 1997). However, the problem of crop damage due to DBM seems to persist, since outbreaks of this pest occur at least every 2–3 years (Syed 1992). We noted that only a few farmers have used chemical and microbial insecticides alternately to control DBM.

DBM field populations in Malaysia have been naturally infected by several insect pathogens such as viruses (nuclear polyhedrosis virus, NPV and granulosis virus, GV), fungi (*Zoophthora radicans* and *Paecilomyces fumosoroseus*) and protozoa (*Nosema* spp. and *Vairimorpha* spp.) (Canning *et al*. 1999, Hussan 1992, Lim 1986, Ibrahim & Low 1993, Idris & Sajap 2001). However, their impact on the DBM population does not seem to reduce chemical insecticide use for controlling DBM.
As with most microsporidia infecting other insects (Steinhaus 1949), the impact of *Nosema bombycis* on DBM field populations has so far not been considered important. This is probably because of its slow killing action on the host. To date there are few scientific papers about microsporidia infecting DBM (Idris *et al.* 1997, Idris & Grafius 1999, Canning *et al.* 1999). However, the impact of *Nosema* on DBM and its parasitoids in laboratory culture is very severe (AK Hussan, AM Shelton and D Dough, personal communication). For example, laboratory cultures of DBM have to be renewed after two or three generations. *Nosema* infection of DBM reared in the laboratory may contribute to a compound effect if used for experiments. In the field, *Nosema*-infected DBM may have a negative impact on the parasitoid population of DBM, as was reported for the impact of *N. pyrasuta* on *Lydella thompsoni* (Diptera: Tachindae), a parasitoid of the European corn borer, *Ostrinia nubilalis* (Lewis 1982). This may be one of the factors that indirectly hamper the role of DBM parasitoids and IPM programs for controlling DBM, especially in Malaysia.

This study was conducted to investigate (1) the prevalence of *N. bombycis* infection in field populations of DBM in Malaysia, (2) behaviour of *Nosema*-infected DBM and *D. semiclauseum*, (3) effect of antibiotics and temperature on *Nosema* infection, (4) percent mortality of DBM larvae infected with *N. bombycis* and (5) the possibility of *D. semiclauseum* being involved in horizontal transmission of *N. bombycis*.

**Materials and methods**

**Prevalence of *Nosema* infection in DBM field populations**

Two sites were selected, the Cameron Highlands (CH) in Pahang and Serdang-Gombak (SG) in Selangor, Malaysia, which are 400 km apart. The CH and SG sites represent highland and lowland cabbage growing areas, respectively. The altitudes from which samples were taken were 1700 - 1800 m (Kea Farm) and 3 - 10 m above sea level at CH and SG, respectively. The average daily temperature was 15 – 25°C for CH and 29 – 35°C for SG. Sampling was carried out over a period of two days at each site in the month of October 1998 (season I) and April 1999 (season II). DBM larvae and pupae were collected from 10% (∼90 plants) of the cabbage plants (selected randomly prior to sampling) per respective fields per site. At least 50 larvae or pupae were collected from each site per sampling per occasion.

The percentage of infection in the larvae or pupae was determined by examining impression smears of individuals using phase-contrast microscopy at 400x. The intensity of infection was determined from ten randomly selected individuals. These individuals were homogenized in 1 ml distilled water using a tissue-homogeniser. A drop of homogenate was pipetted onto a haemocytometer and *Nosema* spores were counted. Chi-squared tests were used to test for differences in percentage of infection and mean infection intensity among locations and seasons.

**Mortality of DBM larvae infected with *Nosema bombycis***

DBM eggs and artificial diet used were obtained from MARDI (Malaysian Agriculture Research and Development Institute). The *Nosema*-free DBM eggs were placed in 15 cm diameter Petri dishes for hatching. Spore solutions were prepared by crushing 50 infected DBM larvae in centrifuge tubes, adding 20 ml distilled water and centrifuging three times at 2,500 rpm for 10 minutes at 10°C. The spore pellet collected at the bottom of the tube was diluted to a 10⁻³ spore concentration (407150, 41420, 4260 and 420 spores/µl) following a serial dilution method. A slice of artificial diet (5 x 5 x 2 mm) was wet with 50 µl spore solution of the required spore concentration for 3 minutes and placed in a multi-well plate. Disease-free DBM larvae of known instars were placed in the wells (one larva per well) for one day under laboratory conditions. Larvae were transferred to another multi-well plate, fed on a spore free-diet and mortality was recorded at 24, 48 and 72 h after treatment. Dead larvae were crushed in a tissue grinder tube to which 1 ml of distilled water was added and then shaken. Ten µl of the larval solution was pipetted and placed onto a haemocytometer for spore counting. Data were analysed using two-way ANOVA and probit analysis. Distilled water was used to treat the diet in the control.

**Effect of antibiotics on disease-infected DBM**

The *Nosema*-infected DBM eggs and untreated artificial diet (provided by MARDI) were put in hatching cups placed in a growth chamber. Four concentrations of Fumidil-B (100, 200, 300 and 400 ppm) were prepared. A slice of artificial diet 4 cm² and 0.1 cm thick (without Fumidil-B) was soaked in a solution of Fumidil-B for 15 minutes (to ensure diet was impregnated by the antibiotic), air dried for 2 h and placed in a 15-cm diameter Petri dish. Five I instar DBM larvae (3 h after hatching) were randomly selected and placed
in Petri dishes with diet (25 larvae per treatment). Diet was changed daily. Mortality of larvae was recorded every other day, starting at two days after hatching and continuing until the tenth day when most surviving larvae had started to pupate. The untreated diet (treated with distilled water) was used as a control. Each treatment was replicated four times. In another experiment, Fumidil-B, Suprim, Albendazole and Tetracycline at 50, 100, 200, 300 and 400 ppm were tested as above. Percent mortality was calculated as the total number of larvae per replicate minus the accumulated dead larvae and divided by the total larvae x 100. Data were analysed by one-way ANOVA and the treatment means were separated by Fisher’s Protected LSD test.

Effect of temperature on disease-infected DBM

One-day-old *Nosema*-infected DBM eggs were dipped into a hot water bath at various temperatures (20, 25, 30, 35, 40, 45, 50 or 55°C) for 2, 3 or 6 h. In another study, the eggs were subjected to different temperatures (by putting them into a growth chamber) as above for 1 or 2 h. Treated eggs were placed in 15 cm diameter Petri dishes (100 eggs per dish) with artificial diet for 3 days (when all eggs had hatched). Larvae were transferred to other Petri dishes (10 larvae per dish), fed the same diet (changed every two days) until pupation. Number of IV instar larvae surviving was recorded. Data were analysed using two-way analysis of variance (ANOVA).

Involvement of *Diadegma semiclausum* in horizontal transmission of disease

Pupae of *D. semiclausum* were collected from cabbage fields in the Cameron Highlands, Pahang, Malaysia and temporarily kept in a refrigerator at 4°C. Twenty *D. semiclausum* pupae of a similar age were selected and placed in emergence cages consisting of clear 300 ml plastic containers with 2.0 cm and 1.5 cm diameter lids on the top and sides. Cages were placed 50 cm below a white fluorescent light. Cotton wool with diluted honey was placed on the bottom of the cage as food for the newly eclosed parasitoid adults. The parasitoid adults were allowed to mate for five days before being used in the experiment.

Thirty DBM II instar larvae were placed in a modified clear plastic container (see above) as a parasitism arena with four slices (0.2 x 2.0 x 2.0 cm) of artificial diet for 24 h. A 5 day old mated female *D. semiclausum* was randomly selected from the container using an aspirator and was released into the parasitism arena via the hole in the top lid. A paper tissue moistened with diluted honey was inserted through the side hole to provide food for the parasitoid adult. The food was replaced every day. Each parasitoid was allowed to parasitise DBM larvae for 4 hours and was then taken out and kept in the freezer for use in the next study. The presumed parasitised DBM larvae were reared individually in 14.5 cm diameter Petri dishes, fed artificial diet as above and kept under laboratory conditions until pupation. The experiment was replicated eight times. For a control, DBM larvae were not exposed to the parasitoid adult. Numbers of larvae that died before pupation, pupae formed, adult parasitoids and DBM emerging were recorded.

Eighty DBM II instar larvae were exposed for parasitism (four replicates, 20 larvae per replicate per parasitoid female) and then reared as above until pupation. The one-day-old parasitoid pupae were taken out of their cocoons using forceps and placed in a test tube filled with 70% alcohol. The test tubes were shaken on an electric shaker for one minute to dislodge any possible microsporidian spores adhering to the body of the parasitoid pupae, after which the pupae were placed into different test tubes and shaken. This process was repeated four times. Pupae were then placed onto a glass slide with a drop of distilled water and crushed using a cover slip.

Ten adult females from the laboratory study were killed immediately after emergence, while 20 adult females collected from the field were killed by placing the test tube containing each parasitoid in sunlight in the field for 15 minutes. The dead parasitoid adults were kept temporarily in the freezer. Each individual female was put into a centrifuge tube filled with 50 ml of 70% alcohol, shaken as above for 10 min after which the parasitoid was taken out and kept for use in the next experiment. The supernatant was centrifuged for 10 min at 13,000 rpm at 10°C. The supernatant was poured out, leaving the pellet at the bottom of the tube. Five ml of distilled water was added to the tube and shaken as before. One ml of spore suspension was pipetted onto a glass slide and covered with a cover slip after which the presence of spores was observed as above. Similar female adults were again subjected to inspection for the presence of spores in the sexual organs and within the abdomen. The abdomen of similar females was dissected using dissecting scissors and knives to take out just the internal body parts which were examined for the presence of spores. A total of 20
males (10 from the above study and another 10 collected from the field) were also treated as for the females, to examine the presence of spores on the body and in the internal organs within the abdomen.

The presence of spores was observed under a compound microscope at 400x magnification. The number of DBM larvae that died before pupation, percent parasitism and adult emergence (parasitoid and DBM) were analysed using paired t-tests.

Diurnal behaviour of *Nosema*-infected DBM and *D. semiclausum*

Pupae of DBM and *D. semiclausum* were collected from a cabbage field near Kea Farm, Cameron Highlands. The pupae were individually kept in a transparent plastic container (11 x 12 x 10 cm) that was placed in a growth chamber environment (20°C, 12 h light: 12 h dark, installed 20 cm above the container, and 50-70% relative humidity) until adult emergence. Adults were maintained in the growth chamber and fed honey water on cotton wool placed on the floor of the container. The flying, feeding, moving, grooming and resting behaviour of DBM or *D. semiclausum* was observed for 2 h (0900 – 1100 h) from outside the chamber on the 5th day after emergence. After the behaviours were observed, each insect was crushed in a centrifuge tube filled with 50 ml distilled water and centrifuged three times (10 minutes each) at 2,500 rpm at 10°C. Ten ml of distilled water was added to the spore pellet which was then shaken and diluted to 10^-2 spore solution. One ml of this spore solution was pipetted onto a haemocytometer for counting of spores.

For parasitism behaviour (approaching and attacking the host larvae exposed to individual *D. semiclausum* female in parasitism arena – similar clear plastic container as above), eleven 5 day old parasitoid females were tested in a similar environment as before. Data were analysed using either chi-square or regression analysis.

**Results and discussion**

**Prevalence of *N. bombycis* in DBM field populations**

There was a significant difference in the percentage of *N. bombycis* infection (*P*<0.05) and mean infection intensity (*P*<0.05) among DBM larvae or pupae collected from different sites (CH and SG) and seasons. The percentage of infection of DBM larvae and pupae in CH was much higher (71.3 and 66.8%) than in SG (10.0 and 2.4%). The mean intensities of *Nosema* infection (number of spores per larva) of DBM larvae were 259.3 x 10^5 and 150.2 x 10^5 in CH while in SG the mean intensity was 14.0 x 10^5 and 2.0 x 10^5. However, the range of infection intensity on DBM larvae in CH (0.02 x 10^7 - 8.0 x 10^7 and 0.01 x 10^7 - 9.7 x 10^7) was wider than that of SG (0.03 x 10^7 – 0.27 x 10^7 and 0.01 x 10^7 – 0.02 x 10^7). The mean intensity of infection and range of infection intensity per pupa showed similar trends as for DBM larvae. The mean infection intensity of DBM pupae in CH was much higher (67.2 x 10^5 and 22.3 x 10^5) than that of SG (26.5 x 10^5 and 7.0 x 10^5).

The microsporidian infection was comparatively more severe in DBM collected from the highlands than in the lowland areas. Temperature may have influenced the severity of infection. Low temperatures in the highlands (CH) would have prolonged larval developmental periods (Ooi 1986). This may have increased the success of infection as DBM larvae were exposed for a longer time to *Nosema* spores. High temperature in the lowland area may cause the insect growth rate to outpace disease development in DBM leading to less infection compared with the highland DBM population. Low sunlight intensity in the CH probably inactivated fewer *Nosema* spores that contaminate plant leaf surfaces (Sikorowski & Lashomb 1977). Consequently, DBM larvae in this area may have ingested more spores while feeding on cabbage leaves than DBM larvae at SG. Unlike DBM larvae, the difference in percentage of *Nosema* infection on DBM pupae between the populations from CH and SG in both seasons was smaller (13.3% and 12.51 for CH and 8.1 and 7.0% for SG respectively). This was probably due to the fact that many infected DBM larvae in CH failed to develop to the pupal stage.

**Mortality of DBM larvae infected with *Nosema bombycis***

Percent mortality was significantly (*P*<0.05) higher in smaller instars (I and II) than larger instars (III and IV) even at low spore concentration (4,260 spore/µl) and 24 h after treatments. The mortality reached 100% when smaller instars were infected with a higher spore concentration (407,150 spore/µl) whereas mortality of larger larvae reached 40% at 72 h after treatment. No mortality was observed in the control. Similar results were also reported for *Bombyx mori* larvae infected by *N. bombycis* (Lian 1991). The LC50 (number of spores per µl) of smaller larvae was also significantly lower than the LC50 value of larger larvae at 48 and
72 h after treatment. At 24 h after treatment, LC50 values for instars I and II was 15,955 and there was no mortality of instars III and IV. However, at 72 h after treatment, the LC50 values were 3206 for instars I and II, and 7,955 and 11,516 for instars III and IV. This indicates that more spores are needed to kill larger larvae. The sudden increase in numbers of spores in smaller larvae may have damaged the host cell membrane to a greater degree than in larger larvae (Jurand et al. 1967).

Effect of antibiotics on disease-infected DBM
The highest accumulated percent larval mortality (92.5% at day 10) was observed when larvae were fed untreated diet (Table 1). Mortality was significantly lower when larvae were fed diet treated with 200 ppm Fumidil-B solutions on day 8 and 10 than those treated with 100 ppm Fumidil-B. However, mortality was significantly lower when larvae were fed diet treated with 300 ppm of Fumidil-B compared with those fed with diet treated with 200 ppm. This indicates that the recommended rate of 220 ppm Fumidil-B used in preparation of DBM artificial diet is unable to contain development of the disease. Further increase in Fumidil-B concentration to 400 ppm significantly increased the percent mortality, indicating that there was a deleterious effect of Fumidil-B on DBM larvae because there were no Nosema spores observed from the dead larvae. Results of this study showed that the Nosema-infected colony of DBM reared at MARDI might have developed resistance to Fumidil-B.

Table 1. Mean percent mortality (accumulative) of diamondback moth larvae fed artificial diet treated with various concentrations of Fumidil-B

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Untreated</td>
<td>45.4 ± 5.6 a</td>
</tr>
<tr>
<td>50</td>
<td>20.5 ± 10.5 b</td>
</tr>
<tr>
<td>100</td>
<td>23.3 ± 11.3 b</td>
</tr>
<tr>
<td>200</td>
<td>15.5 ± 10.2 c</td>
</tr>
<tr>
<td>300</td>
<td>1.5 ± 2.4 d</td>
</tr>
<tr>
<td>400</td>
<td>0d</td>
</tr>
</tbody>
</table>

Means in column with same letters are not significantly different (Fisher’s Protected LSD, P>0.05)

Percent mortality of DBM larvae was significantly lower when fed on diet treated with Fumidil-B at all concentrations than when fed on diets treated with other antibiotics or untreated (Table 2). This indicates that Fumidil-B is comparatively a better antibiotic for treating a N. bombycis-infected DBM culture or preventing disease development. Since mortality of DBM was still 25.4% at 400 ppm of Fumidil-B, it is suggested that its concentration could be increased for total control of disease development.

Table 2. Percent mortality (mean) of disease-infected DBM larvae fed artificial diet treated with antibiotics of different concentrations

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suprim</td>
<td>75.3 ± 7.4 b</td>
<td>76.2 ± 10.2 b</td>
<td>72.1 ± 11.3 b</td>
<td>56.5 ± 8.4 c</td>
</tr>
<tr>
<td>Albendazole</td>
<td>85.3 ± 6.2 a</td>
<td>80.4 ± 11.3 b</td>
<td>75.6 ± 10.2 b</td>
<td>70.4 ± 8.5 b</td>
</tr>
<tr>
<td>Fumidil-B</td>
<td>55.5 ± 7.4 c</td>
<td>45.3 ± 6.7 c</td>
<td>40.2 ± 6.5 c</td>
<td>25.4 ± 4.5 d</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>85.7 ± 10.8 a</td>
<td>85.5 ± 9.5 a</td>
<td>85.2 ± 8.9 a</td>
<td>80.4 ± 9.3 b</td>
</tr>
<tr>
<td>Untreated</td>
<td>100a</td>
<td>100a</td>
<td>100a</td>
<td>100a</td>
</tr>
</tbody>
</table>

Means in column with same letters are not significantly different (Fisher’s Protected LSD, P>0.05).

Effect of temperature on disease-infected DBM
The percentage of DBM larvae surviving to IV instar (when Nosema-infected eggs were treated at 45°C for 3 h) was significantly higher (75.3%)(P<0.05) than when eggs were treated with other temperatures (Figure 1). The rate of survival was lower than 20% when eggs were dipped in hot water at temperatures below 35°C or higher than 50°C (6 h exposure). Eggs treated with various temperatures in the growth chamber
showed similar trends. This indicates that disease development is high at low temperatures, while high temperatures of hot water (>50°C) might have deleterious effects on development of larvae within eggs which resulted in a low hatching rate. Schulz-Langner (1957) reported that temperatures of 37°C and higher, suppressed *Nosema apis* in the honeybee, whose body temperature may reach 44°C. The number of hours the bees spent at 37°C in the hive proportionately retarded the development of the *Nosema* infection.

![Graph showing percent of DBM larvae surviving to IV instar after eggs were dipped in hot water of various temperatures for 1, 3 or 6 hours.](image)

**Figure 1.** Percent of DBM larvae surviving to IV instar after eggs were dipped in hot water of various temperatures for 1, 3 or 6 hours.

Involvement of *D. semiclausum* in horizontal transmission of the disease

The number of dead larvae was significantly (*P*<0.05) higher for the treated larvae than for the control (Table 3). The number of *D. semiclausum* adults that emerged was significantly (*P*<0.05) higher than that of DBM adults. However, there was no significant (*P*>0.05) difference between the number of *D. semiclausum* and DBM pupae formed in this experiment. All the dead larvae in the treatment had microsporidian spores, indicating that *D. semiclausum* is involved in horizontal transmission of spores to its host. Microsporidian diseases transmitted by parasitoids in other host-pathogen systems were reported by Brown (1987), Geden *et al.* (1995) and Sajap and Lewis (1988).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% mortality before pupation</th>
<th>% <em>D. semiclausum</em> adults emerged</th>
<th>% of dead larvae with spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>41.3 ± 4.6 a</td>
<td>22 ± 4.3 a</td>
<td>100 a</td>
</tr>
<tr>
<td>Unexposed</td>
<td>0.1 ± 0.2 b</td>
<td>0 b</td>
<td>0 b</td>
</tr>
</tbody>
</table>

Eight *D. semiclausum* individuals were used. Each had access to 30 DBM larvae for 3 hours. Means in column with same letters are not significantly different (Fisher’s Protected LSD, *P* > 0.05).

85.5% of parasitoid pupae contained *Nosema* spores (Table 4). The microsporidian spores were observed on and within the body of both sexes of the parasitoid. On the body, the females had more spores than the males. Within the body of both sexes of a parasitoid, however, there was no difference in the percentage of individual parasitoid adults having spores. Most (90.4%) parasitoid female sex organs had spores.
Table 4. Microsporidian spores detected within parasitoid pupae, on and within the body of parasitoid females or males

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Percentage of sample with spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within parasitoid pupae</td>
<td>85.5 ± 10.31</td>
</tr>
<tr>
<td>On the body of adult</td>
<td></td>
</tr>
<tr>
<td>Females (n=30)</td>
<td>85.7 ± 12.9</td>
</tr>
<tr>
<td>Males (n=20)</td>
<td>50.3 ± 7.8</td>
</tr>
<tr>
<td>Within the body (abdomen)</td>
<td></td>
</tr>
<tr>
<td>Females (n=30)</td>
<td>65.4 ± 9.6</td>
</tr>
<tr>
<td>Males (n=20)</td>
<td>58.8 ± 5.4</td>
</tr>
<tr>
<td>Sex organs (females, n=30)</td>
<td>90.4 ± 10.3</td>
</tr>
</tbody>
</table>

Results of this study also indicate that *Nosema* infection had a negative impact on the parasitoid populations in the field. However, our field observations (unrecorded data) indicated that this parasitoid was abundant despite high disease incidence and pesticide usage. Percent parasitism cannot be estimated in this study due to the death of parasitised and nonparasitised DBM larvae. The parasitism rate of DBM larvae by *D. semiclausum* and the closely related species, *D. insulare*, ranged from 20 to over 80% in the field (Ooi 1992, Idris & Grafius 1993). It is hypothesized that the parasitoid is capable of avoiding the negative impact of the microsporidian disease. Both the disease and the parasitoid could be synergists to each other in using DBM as a host. However, further research needs to be done to test these hypotheses. Nevertheless, Siegel et al. (1986) reported that the level of *N. pyrausta* infection in the European corn borer (ECB) corresponded to the level of infection in its parasitoid, *Macrocentrus grandii* (Hymenoptera: Braconidae) and that infection had reduced the number of parasitoids exiting the host (ECB) as well as the emergence of the parasitoid adults. Results of another study conducted by Geden et al. (1995) found that the *Muscidifurax raptor* (Hymenoptera: Pteromalidae) parasitoid of filth-breeding flies (Diptera: Muscidae), infected by *Nosema* disease had serious loss of fitness, with infected females taking longer to develop, having shorter lives and producing only 12–50% offspring of the uninfected ones. Although we did not specifically monitor the parasitoid egg for spores, the results of this preliminary observation indicated that *D. semiclausum* is one of the possible factors involved in horizontal transmission of microsporidian disease of DBM.

**Diurnal behaviour of *Nosema*-infected DBM and *D. semiclausum***

It was observed that DBM adults spent significantly more time (75%) resting than moving (walking) (15%), grooming (5%), feeding (3%) or flying (2%). Although the observation time occurred at the time they are actively flying in the field (personal observation), the space (cage) and lack of external cues (host plant volatiles and pheromones) may have caused DBM to be less active. There was no significant relationship (*P*>0.05) between the total time spent resting and the spore concentration (number of spores/µl) per individual DBM adult. However, moving (*r*=0.67, *F*=27.7, df=1 & 18, *P*=0.001) and grooming (*r*=0.54, *F*=9.8, df=1 & 18, *P*=0.006) behaviours positively correlated with the spore concentration per insect. This indicates that DBM movement rate increased with the severity of disease infection. An increase in movement rate of disease-infected DBM helps the epizootics of the disease (Maddox 1987).

*D. semiclausum* adult females were observed spending significantly (*P*<0.05) more time moving (65%) than resting (25%), flying (5%), feeding (4%) or grooming (1%). It was not certain whether the space factor influenced the parasitoid diurnal behaviour in this study. However, in the field, the parasitoid was observed actively flying between 0800 and 1100 h on a clear or partly sunny day. Idris and Grafius (1998) also reported that diurnal flight behaviour of *D. insulare* is optimal around 1000 h under favourable weather conditions (15-23°C, at least partly sunny day, calm wind). Moving (*r*=0.90, *F*=15.2, df=1 & 13, *P*<0.05), flying (*r*=0.45, *F*=9.54, df=1 & 13, *P*<0.05) and grooming (*r*=0.75, *F*=34.4, df=1 & 13, *P*<0.05) behaviours were positively correlated with the spore concentration in the parasitoid body. This indicates that infected individual parasitoids spend more time on activities that are not related to parasitism. This was also shown by the negative relationship between the amount of parasitism behaviour in 2 h observation time and spore concentration per insect (*r*=-0.78, *F*=10.3, df=1 & 13, *P*=0.001) (Figure 2).
The management of diamondback moth and other crucifer pests

Figure 2. Relationship between the number of parasitism behaviours in 2 h of Diadegma semiclausum and Nosema bombycis spore concentration per insect.

Conclusion

The prevalence of N. bombycis infection in field populations of DBM in the Cameron Highlands of Malaysia and high mortality rate of DBM larvae indicates that this microsporidian disease is one of the important mortality factors of DBM. Could it be a potential candidate for a biopesticide of DBM? Interestingly, N. bombycis also infects other insects including Spodoptera litura, S. exigua, Delia radicum, Pieris rapae, Chrysodeixis eriosoma and Vallaga nigricornis (Idris et al., unpublished data). Nosema bombycis causes serious problems in laboratory cultures of DBM. Our results showed that hot water or high temperature treatment could not prevent disease development in a DBM culture. Antibiotic treatment (especially Fumidil-B) may be able to control the disease development at high concentrations (>400 ppm). However, further study of the antibiotic's deleterious effect on DBM at high concentrations is required. The influence of N. bombycis on the behaviour of DBM seems to have a negative impact on the spread of the disease. Nevertheless, the heavily disease-infected D. semiclausum spent less time on parasitism activities than did those less or uninfected individuals. This would have a negative impact on its role as a major mortality factor of DBM and its impact in integrated DBM management. The evidence of D. semiclausum being involved in the horizontal transmission of this disease among its host could increase disease prevalence. Whether or not this would eventually lead to its population becoming extinct is an area for further study.

References


