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Diadegma semiclausum as a Possible Factor for the Horizontal Transmission of Microsporidial Disease of Diamondback Moth, *Plutella xylostella* L.

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Abstract: A study was conducted to test the hypothesis that *Diadegma semiclausum* is one of the factors involved in horizontal transmission of the microsporidial disease of the diamondback moth (DBM), *Plutella xylostella* L. Results showed that 41% of the larvae exposed to presumably microsporidia contaminated the adult *D. semiclausum* died before pupation, and all of the dead larvae were observed to have abundant microsporidia spores. None of the DBM larvae in the control treatment died before pupation. The microsporidia spores were detected on the body of the parasitoid adults (*D. semiclausum*) of both sexes, indicating that the parasitoid get contaminated with spores during eclosion or host finding bout. The spores were also detected within parasitoid's body (abdomen) of both sexes, and within the sex organ of the adult parasitoid female. This suggests that parasitoid is infected with the disease during immature stages. The presence of spores within female sex organ (plus ovipositor) could explain why many larvae died before pupation as the parasitoid oviposition (parasitism) behaviour indirectly transmit the disease spore to the host (DBM) larvae.

Key words: Microsporidia, *Diadegma semiclausum*, *Plutella xylostella*, horizontal transmission

Introduction

Diadegma semiclausum is a major larval parasitoid of diamondback moth (DBM), *Plutella xylostella* L. (Ooi, 1992). In the field, parasitism rate can be as high as 90% (Sastrosiswojo & Sastrodihardjo, 1986; Ooi, 1992). Higher parasitism rate by *D. insulare* on DBM was also reported in North America (Idris & Grafius, 1993; Biever *et al*., 1992; Harcourt, 1986). Study on the interaction between DBM and other factors influencing the population dynamics of both insects are crucial.

The microsporidium *Vairimorpha imperfecta (Nosema bombycis* Negali)(Canning *et al*., 1999) was reported to cause a major problem to laboratory rearing of DBM and its parasitoids (Idris *et al*., 1997). In the field the percent of microsporidia infection on DBM vary with altitudes (Idris & Sajap, 2001). Percent infection seemed to be higher in DBM of highland than that of lowland population. This may be due to the difference in temperatures and parasitoid species. The effect of temperatures on survival and infection of *Nosema apis* and *Nosema portugal* was reported by Schulz-Langer (1957, in Fuxa & Tanada, 1987), and Bauer, L., (1999, Michigan State University, USA – personal communication) respectively.

Studies on the impact of microsporidial diseases on the parasitoids were done by Geden *et al*. (1995), Zchori-Fein *et al*. (1992), Sajap and Lewis (1988), Orr *et al*. (1994a and 1994b), Cassentine & Lewis (1987). Their results seemed to suggest that the prevalence of microsporidial diseases in the host population has a negative impact on parasitoid population dynamics in the field. However, none of them mentioned that the parasitoid itself is indirectly involved in transmitting the diseases.

According to Maddox (1987, in Fuxa & Tanada, 1987) there are many associations in which parasitoids are involved in the epizootiology of microsporidial diseases. However, only a few of these associations have been examined, and they have revealed some fascinating interactions. Not only are the diseases often vectored in these associations, but the population dynamics and reproductive strategies of many parasitoids are also greatly influenced by the presence of these protozoan diseases. The role of parasitoid in host-pathogen system with parasitoid-dependent phoretic transmission is particularly important both at very low and high host densities (Fuxa & Tanada, 1987). This is because the parasitoid could help in sustaining the enzootics at very low host densities as well as create an epizootic.

Up to date there has been no study on the role of parasitoid in epidemiology of microsporidia disease of DBM. This study was conducted to test the hypothesis that *D. semiclausum* is a factor responsible for horizontal transmission of microsporidial disease in DBM.

Materials and Methods

Source of Insects and Artificial Diet: The disease-free DBM larvae of University Putra Malaysia (UPM) strain and artificial diet were provided by the Malaysian Agriculture Research and Development Institute (MARDI). Pupae of *D. semiclausum* were collected from cabbage fields in Cameron Highland, Pahang, Malaysia, and temporarily kept in refrigerator at 4ºC. Twenty *D. semicalausm* pupae of similar age (based on the external appearance (Bolter & Laing, 1983) were put in a clear 300 ml plastic container, 2.0 cm and 1.5 cm diameter lids on the top and sides, as the emergence cages, and placed 50 cm under white inflorescence light. The cotton wool wetted with diluted honey was placed on the bottom side of the cage as food source for the newly enclosed parasitoid adults. The presumably disease-infected parasitoid adults were than allowed to mate for 5 days before used in experiments. At least 70% of *D. semicalausm* collected in the Cameron Highland was infected by microsporidia (Idris & Sajap, 1999 and 2000 – unpublished data).

Effect of *Vairimorpha*-infected parasitoid on percent mortality host larva, number of host and parasitoid pupae formed and adults emerged, and percent of dead larvae containing *Vairimorpha* spores: Thirty DBM second instar larvae were placed in a modified clear plastic container as a parasitism arena, with four slices (0.2 x 2.0 x 2.0 cm^3) of artificial diet and left for 24 h. A five-days mated female *D. semiclausum* were randomly selected from the emergence cages using aspirator and released into the parasitism arena through the hole on top lid. A tissue paper wetted with diluted honey was inserted through the side hole of the cage as food source for the parasitoid adult. The food was replaced daily. Each parasitoid was allowed to parasitize DBM larvae for 4 hours, took out and kept in freezer for use in next study. Presumably parasitized DBM larvae were reared individually in 14.5 cm diameter petri dish, fed artificial diet as above and kept at laboratory environment until pupation. Experiment was replicated eight times (eight parasitism arenas). For a control treatment, similar set (number) of DBM second instar larvae were used but without being exposed to parasitoid adult. Number of larvae died before pupation, pupae formed, adult parasitoid and DBM emerged were recorded. The presence of spores within the dead larvae was also observed under microscope at 400 X magnification.

Presence of spores within parasitoid pupae: Eighty DBM second instar larvae were exposed for parasitism (four replicates, 20 larvae per replicate per parasitoid female) for four hours and reared as above until pupation. The one-day parasitoid pupae were taken out of the cocoon and placed in a test tube half filled with 70% alcohol. The test tubes were shaken on electric shaker for one minute to dislodge any possible micsrosporidium spores from the larvae body after which parasitoid larvae were placed into different test tubes and shaken again. This process was repeated four times. Pupae were then placed on glass slides, added with a drop of distilled water and crushed using a cover slip. The presence of spores was observed under a compound microscope at 400 X magnification.

Presence of spores on and within adult parasitoid females: Ten and 20 adult females collected from above study and from the field were killed immediately after emergence or placed in a test tube (one parasitoid per test tube) under hot sun in the field for 15 minutes. The dead parasitoid adults were then kept temporarily in freezer. Each individual female was centrifuged in a tube filled with 50 ml of 70% alcohol, shaken for 10 min after which the parasitoid was taken out and kept for use in next experiment. The supernatant was centrifuged for 10 min at 13,000 rpm and 10 °C and was removed, leaving the pellet at the bottom of the tube. Five ml distilled water was added into this and shaken. One ml spore suspension was pipetted out, placed on a glass slide and covered with 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 their sex organ and within the abdomen. The individual female sex organ was pulled out using fine forceps, placed on the glass slides and added a drop of distilled water on to it before crushing it using cover slip and observed for spore presence as before. The abdomen of similar females was dissected and the internal body parts were examined for the presence of spores as before.

Presence of spores on and within adult parasitoid males: A total of 20 males (10 from the above study and another 10 from the field) were treated as females to examine the presence of spores on the body and the internal organs within the abdomen.

Data analysis: Number of DBM larvae died before pupation, percent parasitism and adult emergence (parasitoid and DBM) were analyzed using paired-t test (MINITAB version 13).

Results

Number of larvae surviving, pupae formed, adults emerged and percent of dead larvae containing microsporidia (*Vairimorpha*) spores: The percent mortality of DBM larvae was significantly ($t = 5.73$, $df = 7$, $P < 0.05$) higher for the treated (parasitized) larvae than that of control (Table 1). There was no significant ($t = 0.83$, $df = 7$, $P > 0.05$) difference in the number of *D. semiclausum* pupae and DBM formed in this experiment. Interestingly, the number of DBP pupae formed was significantly ($t = 10.02$, $df = 7$, $P < 0.05$) lower in treated than in control. The number of *D. semiclausum* adults emerged was significantly ($t = 7.34$, $df = 7$, $P < 0.05$) higher than that of DBM adults. As for the pupae the number of adult DBM emerged was also significantly ($t = 9.12$, $df = 7$, $P < 0.05$) lower in the treated than in the control treatment. All the dead larvae in treatment had microsporidia spores. As expected there was no parasitoid pupae formed and none of the dead DBM larvae contain microsporidia spores as compared with that of the control treatment.

Presence of spore within parasitoid pupae, on and within adult parasitoid female or males: Results about the presence of microsporidia spores within parasitoid pupae, on and within adult parasitoid female or males are shown in Table 2. There was over 85% of the parasitoid pupae containing microsporidia spores. The microsporidia spores were observed on and within the body of both sexes of the parasitoid irrespective of whether the samples were field-collected or laboratory-reared. On the body, the females collected from the fields seemed to have higher number of spores (85%) than the males (50%). Within the body of both sexes of the parasitoid collected from the fields, however, there seemed to be not much difference in the percentage of individual parasitoid adults having spores. The laboratory-reared individual parasitoids were observed to have relatively less microsporidia spores on their body than those of field collected individuals. However, the laboratory-reared parasitoid adults had a relatively more spores within their body as compared to those field-collected parasitoid adults. Most (80 – 90%) female sex organs (genitalia + ovipositors) of both field-collected and laboratory-reared parasitoid adults had microsporidia spores.

Discussion

Results of present study indicated that the microsporidia disease caused by *V. imperfecta* was transmitted by *D. semiclausum* as 100% of them had microsporidia spores (Table 1). Although we did not count the number of spores per gm larva, the abundance of spores per larvae observed was enough to support our argument that larval mortality (41.3%) was mainly because of *Vairimorpha* infection transmitted by *D. semiclausum*. The microsporidial disease transmitted by parasitoid in other host-pathogen system was reported by Brown (1987, in Fuxa & Tanada, 1987), Geden *et al*. (1995) and Sajap and Lewis (1988).

The mean numbers of parasitoid pupae (7.3 ± 4.3) and adult emergence (5.2 ± 3.2) was very low (Table 1), less than 30% of the total 30 larvae tested. This indicates that

Table 1: Percent mortality of parasitized diamondback moth (DBM) larvae, mean number of pupae formed and adult DBM and parasitoids emerge and percent of larvae containing microsporidia spores

Treatment	% Mortality before pupation	No. of pupae		No. of adults emerged		% of dead larvae containing spores
		D. semiclausum	BDM	*D. semiclausum*	BDM	
Treated	41.3 ± 4.6A	7.3 ± 4.3a	8.7 ± 3.5aA	5.2 ± 3.2a	7.4 ± 4.2bA	100
Control (N = 480)	0.1 ± 0.22B	NR	28.6 ± 3.7B	NR	28.5 ± 3.6B	0

NR, Not related - as the DBM larvae was not exposed for parasitism
Means in row with similar lowercase letter are not significantly different (t test, $P < 0.05$)
Means in column with similar uppercase letter are not significantly different (t-test, $P < 0.05$)

Table 2: Microsporidia spores detected within parasitoid pupae, on and within parasitoid females or males body.

Parameters		Percent of sample observed with spores
Within parasitoid pupae (n = 80)	On the body of adult	85.53 ± 10.31 (mean ± S.E.)
	Females (n = 30)	85 (field) and 50 (laboratory - 1st generation, L-G1)
	Males (n = 20)	50 (field) and 40 *L-G1)
Within the body (abdomen)		
	Females (n = 30)	65 (field) and 75 (L-G1)
	Males (n = 20)	58 (field) and 70 (L-G1)
Sex Organs (females, n = 30)		90 (field) and 80 (L-G1)

microsporidia infection had a negative impact on parasitoid population in the fields or in the laboratory rearing works. However, our field observation (unpublished data) indicated that this parasitoid was really abundant despite the high disease (microsporidia) incidence and pesticide usage. Percent parasitism cannot be estimated in this study due to the dead of parasitized DBM larvae. The parasitism rate of DBM larvae by *D. semiclausum* and it's closely related species, *D. insulare*, in the field was ranging from 20 to over 80% (Ooi, 1992; Idris & Grafius, 1993) despite the prevalence of microsporidia infection in the field population of DBM. Probably, the parasitoid is capable of avoiding the negative impact of the microsporidia disease. If this is the case then both *Vairimorpha* and *D. semiclausum* could be synergists to each other in controlling DBM in the field. However, Siegel *et al*. (1987) reported that the level of *N. pyrausta* infection in the European corn borer (ECB) corresponds to level of infection in its parasitoid, *Macrocentrus grandii* (Hymenoptera: Braconidae), and that infection had reduced the number of parasitoid exiting the host (ECB) as well as the emergence of the parasitoid adults. 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, living less long and producing only 12 – 50% offsprings of the uninfected ones.

The *Vairimorpha* spores were found within the parasitoid pupae as well as on and within the body of the parasitoid adults of both sexes (Table 2). The presence of spores within the parasitoid pupae indicates that the parasitoid larvae were not able to sequester the spores they accidentally consumed while feeding on its host tissues, and instead they get infected with the disease. This result may also explain, why many emerged parasitoid adults had deformed wing and smaller than its normal size. In *Macrocentrus ancylivorus* (Hymenoptera: Braconidae) cultures, infected individuals can be recognized by conspicuous white patches on the metasomal underside, and in extreme cases the infection results in severely deformed abdomens (Allen & Brunson, 1945).

The microsporidia spores were detected on the body of parasitoid adults of both sexes irrespective of field-collected or laboratory-reared individuals (Table 2). The adult parasitoid might have contaminated with microsporidia spores during the eclosion process, host finding or parasitism behaviour. The possibility of getting contaminated by the spores seemed to be a relatively low in the laboratory (low percent of samples observed had spores) than in the field conditions. This may be due to the fact that hosts, exposed for parasitism were free of disease infection as compared to those host larvae in the field. Percent of samples (parasitoid adults) having spores within their body (abdomen) were somewhat lower for field-collected than those of laboratory-reared irrespective of sexes (Table 2). This indicates that severity of infection was relatively higher for laboratory-reared parasitoid. Surprisingly, both field-collected and laboratory-reared parasitoid's sex organs (genitalia + ovipositor) contaminated or infected by the microsporidia spores. This is the primary organ of parasitoid that is in contact with its host during oviposition. Although we did not specifically observe the parasitoid egg for spore presence, result of this preliminary observation indicated that *D. semiclausum* was one of the possible factors involved in horizontal transmission of microsporidia disease of DBM. Other possible factors were the host's fecal, meconium, silk threat, regurgitates and exuviae (shaded skin) (Idris & Grafius, 1999). The microsporidian was shown to be transmitted maternally, within the egg (i.e., transovarian transmission, with 100% efficiency by *M. raptor* on the filth-breeding flies (Geden *et al*., 1995), but with less efficiency by *Pediobius foveolatus* (Hymenoptera: Eulophidae) on the Mexican bean beetle (Chapman & Hooker, 1992).

Although the transmission is most likely through oviposition (parasitism) activities as the spores were detected within the sexual organs of the parasitoid, the spores are also potentially distributed on the host plants during the host finding bout – from which the host larvae may accidentally pick up the spores while feeding or wondering around activity. Because microsporidia disease infecting DBM may have a negative impact on *D. semiclausum* as well, further research is required to determine the degree of impact of disease on parasitoid population dynamic. This is important, as the preliminary survey has indicated that the disease prevalence in the field was as high as 70% but the parasitoid was observed to be highly abundant all year round despite the heavy pesticide usage to control DBM. There may be some kind of synergistic interaction between the microsporidia and the parasitoid that can be manipulated for better management of DBM using

these two biological control agents. However, further research is needed to be done to investigate this possibility.

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