BIOLOGICAL CONTROL

Biology of *Diadegma* sp. (Hymenoptera: Ichneumonidae), a Parasitoid of *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), from Reunion Island

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Neotropical Entomology 31(2): 271-274 (2002)

Biologia de *Diadegma* sp. (Hymenoptera: Ichneumonidae), um Parasitóide de *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), da Ilha Reunião

RESUMO - O gênero *Diadegma* compreende espécies capazes de parasitar larvas de *Plutella xylostella* (L.) (DBM). Foram realizados estudos de laboratório com uma população de *Diadegma* sp. oriunda da Ilha da Reunião, para determinar sua capacidade de parasitismo e a influência da temperatura na sua fecundidade, longevidade, oviposição e o consumo foliar de larvas parasitadas de DBM. A taxa de parasitismo foi de 70% de larvas de segundo e terceiro estádios e 53% de quarto estádio. A 15°C, não houve parasitismo. A 20°C e 25°C, a porcentagem de parasitismo foi próxima a 70%. A 29°C, a porcentagem de parasitismo foi similar (70%), mas houve maior produção de machos. Larvas parasitadas de DBM consomem 35% menos superfície foliar do que larvas não parasitadas. Essa espécie de *Diadegma* é um possível candidato para ser introduzido em áreas tropicais, onde nenhuma espécie adaptada é conhecida, e onde pode ser usada como complemento às aplicações de *Bacillus thuringiensis.*

PALAVRAS-CHAVE: Inimigos naturais, repolho, temperatura, parasitismo, traça-das-crucíferas.

ABSTRACT - The genus *Diadegma* have some species able to parasite *Plutella xylostella* (L.) (DBM). Laboratory studies were carried out on a *Diadegma* sp. from Reunion Island to elucidate its ability to parasite DBM larvae, and to determine the influence of the temperature on its fecundity, longevity, oviposition and the consumption of cabbage leaves by parasitized DBM larvae. Parasitism rates were close to 70% for the L2 and L3 instar, and 53% for the L4. At 15°C, there were no progeny. At 20°C and 25°C, the percentage of parasitism was near 70%. At 29°C, the percentage parasitism was similar but the number of males produced was higher. Parasitized DBM larvae consumed 35% less leaf surface than non parasitized larvae. This *Diadegma* sp. is a possible candidate for introduction into tropical areas, where no tropically adapted *Diadegma* species are known and could complement *Bacillus thuringiensis* treated systems.

KEY WORDS: Natural enemies, cabbage, temperature, parasitism, diamondback moth.

The diamondback moth (DBM), *Plutella xylostella* (L.), is considered the major pest of Brassicacea in the world (Talekar & Shelton 1993). DBM populations resistant to chemical pesticides have been recorded for many years (Johnson 1953, Tabashnik *et al*. 1987, Shelton & Perez 1993) and more recently it was also proved resistant to *Bacillus thuringiensis* Berliner toxins (Kirsch & Schmutterer 1988, Hama *et al*. 1992, Tabashnik *et al*. 1992, Shelton & Perez 1993). Other control methods have been tried to control these resistant populations which have the options is the use of natural enemies.

More than 90 species of natural enemies are noted from different DBM stages (Goodwin 1979). The *Diadegma* genus is currently under revision (Noyes 1994) and the identification (by K. Horsmann, taxonomist from the Zoologisches Institut III der Universitat Würzburg Lehrstuhl für Tierökologie from Austria) of *Diadegma* sp., performed by and reported on here, has not yet been confirmed in the species level. Because of their efficacy in parasitizing different larval instar of DBM, about ten species of *Diadegma* have been introduced world-wide for biological control (Lim 1986, Talekar & Yang 1993). However, no *Diadegma* spp. have been successfully introduced into a tropical habitat. The endemic Reunion *Diadegma* sp. is active as a *Plutella* parasitoid from sea level

to the limits of cabbage cultivation (C1500m). The aims of this work were: to elucidate the percent parasitism of L2, L3 and L4 larval instar of *Plutella*; to test the influence of temperature (15-29°C) on fecundity, longevity, oviposition rate and percent parasitism and to compare the consumption of cabbage leaves by parasitized and non-parasitized larvae.

Material and Methods

Parasitism of DBM Larval Stages. Three containers of transparent plastic 45 cm square (a) and one of 45 x 80 x 90 cm (b), were used for this test. The top of each container was covered with nylon mesh. An open plastic box, 20 x 15 x 10 cm (c), containing DBM larvae on a cabbage leaf was placed in each container (a and b) to be offered to *Diadegma* females. DBM larvae were introduced into the containers through a 15 cm diameter hole in one side of each container. Five pairs of *Diadegma* were introduced into each one of the three containers (a) , the first of which had 50 DBM L2 larvae, the second 50 L3 larvae and the third 50 L4 larvae. Fifteen *Diadegma* pairs were introduced into the large container (b), which had 150 DBM larvae (50 larvae of each instar, L2, L3 and L4). A streak of honey and a saturated cotton wick were placed inside each container to provide nutrition for the parasitoids. Twenty four hours later, the DBM larvae in the boxes (c) were removed to complete their life cycle. The larvae from the large container (b) were sorted into other boxes by instar (L2, L3, L4) and left to develop. All the boxes contained DBM larvae were placed on an insectary to complete their life cycle.

The experiment was carried out in an insectary under controlled conditions of temperature 26 ±1°C, relative humidity 75 ± 5% and 16h L, 8h D.

The numbers of parasitized larvae, dead larvae and the proportion of males and females of the F1 parasitoids were noted. The experiment was repeated ten times, 500 larvae from each external instar were exposed to parasitism and the data analysed by ANOVA (Newman-Keuls test at 5%).

Influence of Temperature on Fecundity, Longevity, Oviposition Rate, and % Parasitism. Four square containers (a), as used in the test above, were put in each of four walk-in chambers at different temperatures: 15°, 20°, 25° and 29°C and 16h D/8h N. Five *Diadegma* male-female pairs, which emerged the same day, were put in each container to facilitate mating and temperature acclimatisation (*Diadegma* was currently reared at 26±1°C). These parasitoids were fed on water and honey. The dead adults were counted each day to obtain the percentage mortality of both sexes.

The day after the *Diadegma* pairs were caged, 50 DBM L3 larvae on a cabbage leaf were introduced into each container. The number of DBM larvae exposed was proportional to the number of live females (10 larvae/1 female). The DBM larvae were taken out after 24h exposure to allow the parasitized DBM larvae to develop. Every day until all the *Diadegma* females were dead, this protocol was repeated, the DBM were offered to the parasitoids. When the parasitoid cocoons appeared, they were counted and placed individually into small petri dishes (3 mm diameter). On emergence, the *Diadegma* adults were counted and sexed.

This experiment was repeated three times and *Diadegma* female longevity and productivity, as well as the proportion of male and female from the F1 generation were calculated. The data obtained were analysed by ANOVA (Newman-Keuls test 5%).

Consumption of Cabbage Leaves by Parasitized and Non-Parasitized Larvae. The surface areas of 20 cabbage leaves were measured by image analysis software using a scanner. Ten parasitized DBM L2 larvae were placed on 10 leaves, and 10 non parasitized L2 were put on the other ten leaves. These larvae were observed each day and for each dead parasitized larva, one non parasitized larva was removed, so that the number of larvae on the different cabbage leaves was always the same. When all non-parasitized larvae had pupated, the pupae were removed and the remaining surface of each of the 20 cabbage leaves was measured. The data obtained for 100 parasitized larvae and 100 non parasitized larvae were analysed by ANOVA (Newman-Keuls test at 5%).

Results and Discussion

Parasitism of the L2, L3, L4 Larval Instars of DBM. When the DBM larvae were offered separated by instar (L2, L3, L4) to *Diadegma* females inside the containers (a), there was no significant difference in the percentage parasitism between each instar. On the other hand, when a mixed group of instars was presented, the L4 instar was less attractive (Table 1). DBM

Table 1. Influence of the larval instar on the mortality of host caterpillars, the productivity and % of parasitism by *Diadegma* females.

P. xylostella	L2		L3		L4	
	NC	C	NC	C	NC	C
Adults obtained	360 a ± 14,9	334 a ± 15,7	321 a ± 15,8	321 a ± 14,4	300 a ± 16,8	265 b ± 29,2
Parasitism (%)	72,0	66,8	64,2	64,2	60,0	53,0
Dead larvae	30 a ± 11,3	68 ab ± 20,7	45 a ± 16,3	107 b ± 15,6	87 b ± 15,5	138 b ± 27
Mortality (%)	6,0	13,6	9,0	21,4	17,4	27,6
Proportion male/female	1,0 a ± 0,15	1,0 a ± 0,11	1,5 a ± 0,3	2,1 a ± 0,38	1,6 a ± 0,36	2,3 a ± 1,14

L2: 2nd larval instar; L3: 3rd larval instar;L4: 4th larval instar; NC (no choice): larval instar separated (L2, L3, L4) during parasitism period; C (choice): larval instar mixed (L2, L3, L4) during parasitism period Means followed by the same letter in the lines are not significantly different by Newman-Keuls test (5% level).

L4 instar larvae moved slowly and did not react to the presence of *Diadegma* females, unlike the L2 and L3 stages which vigorously coil and jump. The former behaviour may seem to resemble the behaviour of diseased larvae which do not stimulate *Diadegma* females to oviposit. The slow moving or quiescent L4 instar larvae may possibly be taken for diseased larvae. This phenomenon was not seen when only L4 instar larvae were presented, when there was no choice. The percentage parasitism obtained with L2 and L3 instar was similar to that recorded for *D. semiclausum* (Hellén) where 66% of DBM larvae presented to females were parasitized (Abbas 1988).

The number of dead DBM larvae increased with the development of the larval stage in the mixed or isolated larval groups (Table 1). An ANOVA test carried out on the mortality of different instars showed significant differences. It is possible that the higher mortality occurring in L4 instar larvae is due to a virus (PolyDNAvirus) injected by the female *Diadegma* when ovipositing which affects the immune system of the attacked DBM larva, (Quicke 1997). This virus disturbs the metabolism and brings about the death of some of the larvae presented to the females.

The sex-ratio of the F1 progeny from the Reunion *Diadegma* species does not seem to be dependent on the larval instar presented to the ovipositing female, whether presented as mixed or single stage larvae (Table 1). With *D. insularis* (Cresson) and *D. semiclausum* (Fox *et al.* 1996), the proportion of males from young DBM larvae is greater, the larvae parasitized at the L4 instar stage giving more females (Yang *et al*. 1993). This data reinforces our conviction that the Reunion *Diadegma* species is neither *D. insularis* nor *D. semiclausum*.

Influence of Temperature on Fecundity, Longevity, Oviposition Rate and % Parasitism. Males had a longer life cycle than females at 25°C. At other temperatures, the life cycle was similar for both sexes. At 15°C and 29°C, the life cycle did not exceed one week (Table 2). The low temperatures affect the percentage parasitism of *Diadegma*. At 15°C there was no oviposition. At 20°C, 25°C and 29°C the percentage of parasitism was similar (Table 2). The sex ratio from *Diadegma* F1s was also influenced by temperature. At 20 and 25°C, the ratio was similar, but at 29°C there were three times more males than females (Table 2). At these temperatures the longevity of *Diadegma* species varied widely. At 25°C the longevity observed for this species was 15.2 and 6.7 days for males and females respectively. This compares with 16 to 22.3 days for *D. semiclausum* males and with 11.2 to14.6 days for females (Abbas 1988). For *D. insulare*, life cycles of 10.4 days for the males and 16.9 days for females were obtained (Mitchell *et al.* 1997).

At 20°C and 25°C, the percentage parasitism of *Diadegma* Reunion sp. was similar to that recorded for *D. semiclausum* (Talekar & Yang 1993). By contrast, at 15°C and 29°C, the percentage parasitism obtained with the Reunion species, 0% and 70% respectively, was very different to those observed with the two other species: more than 80% parasitism at 15°C and less than 50% at 29°C (Talekar & Yang 1991, Talekar & Yang 1993). These data suggest a tropical origin for the Reunion *Diadegma* species. *D. semiclausum* was apparently adapted to lower temperatures, typical of temperate areas or tropical highlands.

At 20°C the proportion of males in relation to females obtained during this study was similar to that for *D. semiclausum* (Abbas 1988), and *D. insulare* (Mitchell *et al*. 1997). However, at a continuous temperature of about 29°C, mating between the Reunion *Diadegma* species was disturbed resulting in three times more males than females. At 25°C, percentage parasitism was similar.

Consumption of Cabbage Leaves by Parasitized and non Parasitized DBM Larvae. L2 and L3 instar DBM larvae parasitized by *Diadegma* consumed about 33% less leaf surface compared with non parasitized larvae (Table 3). This phenomenon is important because as they consumed less, the parasitized larvae do not absorb a lethal dose of toxins from *B. thuringiensis* formulations and this allows *Diadegma* to develop and emerge (Monnerat & Bordat 1998).

This *Diadegma* sp. is a possible candidate for introduction into tropical areas, where no tropically adapted *Diadegma* species are known and could complement *B. thuringiensis* treated systems.

Table 3: Surface of cabbage leaves consumed by *P. xylostella* larvae.

	PL	NPL
Average surface area consumed (cm^2)	17,2 a	26,9 b
Surface area consumed (cm^2) (range)	7,23 – 27,6	18,2 – 36,8

PL: parasited larvae; NPL: non parasited larvae
Means followed by the same letter in the lines are not significantly different by Newman-Keuls test (5% level).

Table 2. The influence of different temperatures on adults of *Diadegma* sp.

Temperature	15°C	20°C	25°C	29°C
Number of larvae	1.120	1.880	1.010	1.030
Longevity of males(days)	9,8 b ± 1,16	13,0 a ± 0,78	15,2 a ± 1,10	7,8 b ± 0,76
Longevity of females (days)	7,4 b ± 0,68	12,5 a ± 0,78	6,5 b ± 0,77	6,8 b ± 0,60
Parasitism (%)	-	70	68	70
Proportion male/female (G1)	-	1,5 a ± 0,06	1,0 a ± 0,03	2,9 b ± 0,4

G1: First generation
Means followed by the same letter in the lines are not significantly different by Newman-Keuls test (5% level).

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Received 20/11/00. Accepted 12/04/2002.