Stage-specific parasitism of *Diadegma semiclausem*: Consequence for the parasitoid and its host, *Plutella xylostella*

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**ABSTRACT**

The Ichneumon wasp, *Diadegma semiclausem*, is an important larval-prepupal endoparasitoid of the diamondback moth, *Plutella xylostella*. Parasitism of *Plutella* larvae of different instars and different developmental ages of the 4th instar of the pest and the consequences for both the parasitoid and the host were investigated at 24°C in laboratory. *Diadegma semiclausem* preferred parasitizing host larvae in 2nd and 3rd instars over 4th instars. Parasitism decreased with increasing host age of the 4th instars. Development time, final adult size, adult fecundity and longevity of the parasitoid varied with the host instar at initial parasitization. Parasitoids with initial parasitism in the 4th instar host had the shortest development time, and had the largest body size, followed by those in the 3rd instar, and then by those in the 2nd instar. Parasitoid females starting parasitism in 3rd instar host had the highest fecundity and the greatest longevity. However, the survival rate of the parasitoid during immature stages and the fecundity of the parasitoid adult female in the first day post-emergence did not vary with the host instar at initial parasitization. Parasitized host larvae exhibited longer development time compared with unparasitized ones, no matter when the larvae were parasitized initially. The host prepupae developed from parasitized larvae were not able to pupate, and the duration of the host staying in the prepupal stage increased with increase of host instar at parasitism. The parasitized larvae consumed similar amounts of cabbage leaf as the healthy larvae with the exception that parasitized larvae consumed significantly less cabbage leaf than healthy larvae during their 4th instar, when parasitism occurred at early 2nd instar.

**INTRODUCTION**

The diamondback moth (DBM), *Plutella xylostella* (L.), is a major notorious destructive pest of cruciferous vegetables throughout the world, especially in Southeast Asia (Talekar and Shelton 1993). The annual cost to keep its population in check is estimated to be approximately US$1 billion (Talekar and Shelton 1993).

*Diadegma semiclausem* Hellen is an important larval-prepupal endoparasitoid of DBM, and is considered to be one of the most effective parasitoids for keeping DBM under control among the more than 90 parasitoids of DBM (Talekar and Shelton 1993). It was considered to have originated in Europe, widely distributed in the Palaeartic region (Waterhouse and Norris 1987, Azidah et al. 2000). However, to date this parasitoid has been distributed widely in Indo-Malayan region since it was first introduced into New Zealand from England (Waterhouse and Norris 1987), then into Australia (Waterhouse and Sands 2001), Papua New Guinea (Saucke et al. 2000), Indonesia (Sastrosiswojo and Sastrodihardjo 1986), Malaysia
(Ooi 1992), Philippines (Amend and Basedow 1997), Japan (Noda et al. 2000), Korea (Kwon et al. 2003), Taiwan and Yunnan Provinces in China (Talekar et al. 1992, Chen et al. 2001).

There were many reports on the biological characteristics of *D. semiclausum* (Ooi 1980, Abbas 1988, Yang et al. 1993, 1994, Talekar and Yang 1993, Lee et al. 1995, Furlong and Pell 2000, Lavander et al. 2005), but several results relevant to host stage preference and host larval performance after parasitism are not consistent. First, on host stage preference, Talekar and Yang (1991) reported that this parasitoid preferred parasitizing 2* (L2) and 3* (L3) DBM larvae and never parasitized 4* (L4) instars, and proposed that its larger ovipositor was the reason why the wasp parasitized few 1* (L1) instar larvae. However, Vos (1953) reported an average of 47% parasitism of DBM larvae in L4 in Indonesia. Yang et al. (1993) documented that *D. semiclausum* oviposition in L1-L3 produced more parasite males than females, but oviposition in L4 produced significantly more females than males, implying that *D. semiclausum* could parasitize L4. Lee et al. (1995) reported the parasitism preference in order was L2 > L3 > L4 > L1.

Second, on host duration after parasitism, Yang et al. (1994) did not find significant differences between the parasitized and unparasitized (healthy) host larvae; neither did Furlong and Pell (2000). However, during culture maintenance of the parasitoid in our laboratory, it was observed that parasitized host larvae have an obviously prolonged larval duration. In addition, at times the prolonged prepupal duration after parasitism was much longer than that reported by Furlong and Pell (2000).

Third, Yang et al. (1994) found that parasitized larvae consumed less cabbage leaf than the healthy larvae did, and food consumption by parasitized larvae in comparison with healthy larvae was significantly lower in L4 when initial parasitism happened at L2. This was not always the case. We felt during the culture maintenance that the parasitized larvae sometimes consumed less while sometimes not, in comparison with unparasitized larvae.

The current study was set up to examine the parasitism of *P. xylostella* by *D. semiclausum* in relation to host age in more detail, and to investigate the influence of host age at initial parasitism on the development, survival and resultant adult ability to parasitize DBM larvae of *D. semiclausum*, and to determine the effect of parasitism on the host development and food consumption as well. It was hoped that the information obtained would help to clarify the different reports on host-instar dependent parasitism in this host-parasitoid system and to provide novel knowledge of the developmental interactions between the host and the parasitoid, and benefit the mass production of this parasitoid.

### MATERIALS AND METHODS

Parasitoid and Host Insect Cultures

DBM was originally collected from brassica vegetable crop fields in the eastern suburbs of Hangzhou (30°14′N), Zhejiang Province, China in September 2003. The stock culture of DBM, used as host material throughout this study, was reared on potted cabbage, *Brassica oleracea* var. *capitata*, cv. Jingfeng No. 1, and maintained at 24 ± 2°C, 60%-80% relative humidity (RH) and a 14L:10D photoperiod in a temperature-controlled room. The host plants were grown in an insecticide-free greenhouse prior to being transplanted in pots at their rosette stage.

The culture of *D. semiclausum* was established in November 2003 from 100 parasitoid cocoons obtained from a stock culture at the Institute of Plant Protection, Academy of Agricultural Science of Yunnan Province, China (Chen et al. 2001). Maintenance of the parasitoid culture was done by exposure of 2-3
day-old wasps to L2 or L3 on potted cabbage plant in a 50 cm × 50 cm × 50 cm ventilated cage.

DBM had been reared for 4-6 generations and the parasitoid for 2-4 generations in the laboratory prior to the experiments.

Stage-specific Parasitism

To obtain host larvae physiologically identical in instar or age, larvae in L1 were reared in an incubator at 24 ± 0.5°C and 75% RH with a 14L:10D photoperiod, and observed every 12 hours. The newly formed L2 were collected from the colony and used in the experiment. The newly formed L3 and L4 were obtained with the same method. The young L4 were cultured in the same incubator to obtain the larvae at different ages. Wasps used in this experiment were 1-2 day-old mated females, and had been fed with 20% honey solution. Two sets of experiments were conducted.

In the first set of experiments, 30 DBM larvae of the same instars and ages were introduced onto a leaf held in a plastic container (Shi et al. 2004), and allowed to settle and establish feeding sites for one hour, and then one D. semiclau summ female wasp was introduced to each container for oviposition for 6 hours.

In the second set of experiments, 30 DBM larvae consisting of 10 larvae each of newly molted L2, L3 and L4 were introduced onto a leaf in a container as mentioned above. One hour later one D. semiclau summ female wasp was introduced to each container for oviposition for 6 hours.

After a 6-hours exposure, each larva was dissected within one day under a Leica stereomicroscope, and the numbers of host containing egg(s) and the number of parasitoid eggs inside each larva were recorded. The experiment in the first set was replicated 10 times for each instar and each age in L4. The experiment in the second set was replicated 20 times.

Effect of Host Instar on the Parasitoid

To obtain larvae parasitized by D. semiclau summ, single DBM larvae that were obtained in the way described above were exposed to a female parasitoid in a test tube (18 mm in diameter, 80 mm in height), and replaced with another one after being stung once by the wasp. With this method, 40-60 parasitized larvae could be obtained per hour. Each female was allowed to sting only five larvae and then replaced with another female. Thus, more female parasitoids would be involved in the experiment in order to avoid the maternal effects.

The stung larvae in groups (25-30 larvae each) were reared on cabbage leaves in plastic containers until all larvae formed DBM cocoons. Newly formed DBM cocoons were collected and reared in groups in test tubes until D. semiclau summ formed its own cocoon inside the DBM cocoon. Newly formed parasitoid cocoons were collected and reared singly until adults emerged. All rearing was carried out in incubators at 24 ± 0.5°C, 75% RH and a 14L:10D photoperiod. The insects were observed every 12 hours during the course of the rearing. The time of cocoon formation for both DBM and parasitoid and the time of parasitoid emergence were recorded. Any dead DBM larva and prepupa were dissected under a Leica stereomicroscope to determine whether they had been parasitized.

After adult emergence, two samples were taken to determine their fecundity, longevity and body size. Female wasps in the first sample were maintained in the same incubators as mentioned above, and used to determine their fecundity and longevity. They were first kept with males for 24 hours to ensure mating, then individually exposed to 20 L3 reared on a cabbage leaf in a plastic container for 6 hours each day until they died. The females were provided with 20% honey solution ad libitum at each exposure. The exposed larvae were dissected within one day after exposure under a Leica stereomicroscope, and the number of parasitoid eggs inside the larvae was recorded to determine parasitoid fecundity. The times of wasp death were recorded
to estimate their longevity. Female wasps in the second sample were killed in 75% ethanol and the length of their body, forewing and ovipositor were measured to the nearest 0.01 mm through a Leica stereomicroscope equipped with a calibrated ocular micrometer.

Effect of Parasitism on Host Development and Food Consumption

The method to obtain physiologically consistent host larvae and parasitized larvae was the same as that described above. Single DBM larvae initially parasitized at newly formed L2, L3 or L4 were reared on cabbage leaf discs (4.3 cm in diameter) in a Petri dish in an incubator at 24 ± 0.5°C, 75% RH and a 14L: 10D photoperiod until DBM pupated or the parasitoid formed cocoons. Healthy DBM larvae of identical age were reared under the same conditions and served as the control. Host larvae were checked once every 12 hours to record development. The leaf discs were replaced every 24 hours, and area fed by the larvae was measured to the nearest 0.1 mm to estimate larval food consumption.

Data Analysis

DBM individuals which died during rearing without parasitoids in their body at dissection and still formed DBM pupa after being stung by D. semicleausum were excluded from the calculation of the percentages of parasitoid cocoon formation and adult emergence.

There are many indices used to assess parasitism preference (Cook 1978). In this study, the following formula was used to calculate the relative parasitism index where L2, L3 and L4 were provided to D. semicleausum simultaneously:

\[ P_i = \frac{N_i}{\sum_i N_i} \]

Where, \( P_i \) is the relative parasitism index of age class i, \( N_i \) is the number of host larvae parasitized in age class i, and \( k \) is the total number of age classes included in the preference experiment.

All proportional data were transformed by arcsine square root before analysis of variance, and were back-transformed to proportions for presentation. Mean values were separated using Tukey’s honestly significantly different (HSD) test when significant differences among several mean values were detected by analysis of variance (ANOVA). Comparison between the two mean values, such as the data of developmental time and food consumption by parasitized and unparasitized, was done by Student’s t-test. All statistical analyses were performed using the statistical software package, STATISTICA (StatSoft, Inc. 2003).

 RESULTS

Stage-specific Parasitism

When L2, L3 and L4 were provided separately to parasitoid females, the number of parasitized host larvae and the number of eggs oviposited by the wasp in L2 or L3 were significant higher than those in L4, and the number of parasitized host larvae decreased with increase of age in L4 (Table 1). Female adults could oviposit into larvae that had been oviposited previously, leading to occurrence of superparasitism (two eggs in the same host, but only one could complete its life cycle). The percentage of those females ranged from 30% - 70%, and the percentage of superparasitized host larvae reached 14.75% in total as females were exposed to newly formed L4. However, no female adult produced superparasitized host larvae when they were exposed to larvae in the 3rd day postmolting to L4 at the initial exposure.
Table 1 Parasitism (means ± SE) of Plutella xylostella larvae at various ages by Diadegma semiclauus

<table>
<thead>
<tr>
<th>Age at initial parasitism</th>
<th>No. of larvae parasitized</th>
<th>No. of eggs laid per female</th>
<th>% larvae superparasitized</th>
<th>No. of Replicates produced superparasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 instar (12)</td>
<td>22.6 ± 2.1 a</td>
<td>24.9 ± 2.8 a</td>
<td>7.96</td>
<td></td>
</tr>
<tr>
<td>3 instar (12)</td>
<td>20.2 ± 1.9 a</td>
<td>23.4 ± 2.1 a</td>
<td>9.40</td>
<td>7</td>
</tr>
<tr>
<td>4 instar (12)</td>
<td>12.2 ± 0.6 b</td>
<td>14.8 ± 1.1 b</td>
<td>14.75</td>
<td>4</td>
</tr>
<tr>
<td>4 instar (36)</td>
<td>9.8 ± 0.8 b</td>
<td>10.1 ± 0.7 b c</td>
<td>3.06</td>
<td>3</td>
</tr>
<tr>
<td>4 instar (50)</td>
<td>5.3 ± 0.6 c</td>
<td>5.3 ± 0.6 c</td>
<td>0.00</td>
<td>0</td>
</tr>
</tbody>
</table>

a The numerals in parentheses are time in hours postmolting to the instar.
b Means within the same column followed by different letters are significantly different (P < 0.05, by Tukey’s HSD test).
c The superparasitism rate was calculated by dividing the total number of larvae with more than one parasitoid egg by the total number of larvae with parasitoid egg(s) in all ten replicates.

When L2, L3 and L4 were provided to D. semiclausum simultaneously, percentages of larvae parasitized in L2 and L3 were again significantly higher than those in L4, although the number of eggs oviposited by the female wasps in L3 was significantly higher than those in L2 or in L4. This demonstrated that the female wasp preferred parasitizing L3 and L2 to L4, as also indicated by the different relative parasitism indices between the three instars (Table 2). Seven of twenty females tested produced superparasitized larvae, and there were 1, 7 and 4 superparasitized larvae in L2, L3 and L4, respectively.

Table 2 Differential parasitism (means ± SE) of Plutella xylostella larvae between three instars by Diadegma semiclausum

<table>
<thead>
<tr>
<th>Age at initial parasitism</th>
<th>No. of larvae parasitized</th>
<th>No. of eggs laid in different instar</th>
<th>Relative parasitism index</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 instar (12)</td>
<td>6.0 ± 0.4 b</td>
<td>6.0 ± 0.4 b</td>
<td>0.3749 ± 0.0150 b</td>
</tr>
<tr>
<td>3 instar (12)</td>
<td>7.3 ± 0.3 a</td>
<td>7.8 ± 0.5 a</td>
<td>0.4374 ± 0.0177 a</td>
</tr>
<tr>
<td>4 instar (12)</td>
<td>3.4 ± 0.4 c</td>
<td>3.6 ± 0.5 c</td>
<td>0.1875 ± 0.0186 c</td>
</tr>
</tbody>
</table>

a The numerals in parentheses are time in hours postmolting to the instar.
b Means within the same column followed by different letters are significantly different (P < 0.05, by Tukey’s HSD test).
c An index of conditional proportion of parasitism for each of the instars when the total parasitism of the three instars is taken as unity. See text for mathematical definition.

Effect of Host Instar on the Parasitoid

Parasitoids starting parasitism in L4 had a significantly shorter development time from egg to cocoon formation than those starting parasitism in L2 and L3, and had a significant longer duration from cocoon formation to adult emergence than those starting parasitism in L2. However, the survival rates of both from egg to cocoon formation and from cocoon to adult emergence were not different among the ages at which the initial parasitism started (Table 3).

Table 3 Development time and survival (means ± SE) of Diadegma semiclausum reared in Plutella xylostella larvae of various instars

<table>
<thead>
<tr>
<th>Age at initial parasitism</th>
<th>Development time in days</th>
<th>Egg to cocoon formation</th>
<th>Cocoon to adult emergence</th>
<th>Survive to cocoon formation</th>
<th>% coconns that produce wasps</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 instar (12)</td>
<td>8.0 ± 0.1 a (27)</td>
<td>7.4 ± 0.1 b (27)</td>
<td>90.4 ± 2.2 a (15)</td>
<td>93.6 ± 1.5 a (15) [316]</td>
<td></td>
</tr>
<tr>
<td>3 instar (12)</td>
<td>8.0 ± 0.2 a (41)</td>
<td>7.6 ± 0.1 ab (41)</td>
<td>89.2 ± 1.8 a (15)</td>
<td>91.8 ± 2.2 a (15) [300]</td>
<td></td>
</tr>
<tr>
<td>4 instar (12)</td>
<td>6.7 ± 0.2 b (30)</td>
<td>7.8 ± 0.1 a (30)</td>
<td>91.0 ± 1.5 a (17) [223]</td>
<td>88.4 ± 2.2 a (17) [297]</td>
<td></td>
</tr>
</tbody>
</table>

a The numerals in parentheses are time in hours postmolting to the instar.
b Means within the same column followed by different letters are significantly different (P < 0.05, by Tukey’s HSD test). The numerals in parentheses are numbers of replicates.
c The numerals in square brackets are total numbers of individuals included in all replicates.
Compared with those associated with L2 or L4 as the initial hosts, the resultant females associated with L3 as the initial host laid significantly more eggs in their lifetimes, and had significantly longer longevity; however, they did not lay more eggs in the first day post-emergence (Table 4). The adult females resulting from L4 as the initial host had the largest body size, followed by those from L3, and then by those from L2 (Table 4).

Table 4 The effect of Plutella xylostella larval instars at initial parasitization on the resultant female adult wasps of Diagema semicalausum (means ± SE)

<table>
<thead>
<tr>
<th>Larval age at initial parasitism²</th>
<th>No. of eggs laid per female³</th>
<th>Longevity in days¹</th>
<th>Body length</th>
<th>Fore wing length</th>
<th>Ovipositor length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In first day</td>
<td>In life time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 instar(12)</td>
<td>15.9 ± 0.7 a (26)</td>
<td>124.7 ± 7.7 a (10)</td>
<td>8.8 ± 1.0b (8)</td>
<td>4.7 ± 0.1 a (10)</td>
<td>1.2 ± 0.0 a (10)</td>
</tr>
<tr>
<td>3 instar(12)</td>
<td>15.6 ± 0.8 a (15)</td>
<td>184.6 ± 14.0 b (11)</td>
<td>13.1 ± 0.9a (11)</td>
<td>4.7 ± 0.1a (9)</td>
<td>3.5 ± 0.1 a (9)</td>
</tr>
<tr>
<td>4 instar(12)</td>
<td>14.8 ± 0.7 a (20)</td>
<td>110.7 ± 7.0, 9 (12)</td>
<td>6.7 ± 0.9b (11)</td>
<td>5.1 ± 0.1b (11)</td>
<td>1.4 ± 0.0 b (11)</td>
</tr>
</tbody>
</table>

¹ The numerals in parentheses are in hours postmolting to the instar.
² Means within the same column followed by different letters are significantly different (P < 0.05, by Tukey’s HSD test). The numerals in parentheses are numbers of replicates (females observed).

Effect of Parasitism on Host Development and Food Consumption

Compared with healthy DBM larvae, parasitized larvae had an obviously prolonged duration of L3, L4 and prepupal stages whenever the larvae were parasitized initially. But the duration of the parasitized larvae in L2 did not differ from that of healthy ones when the parasitism occurred at early L2 (12 hours postmolting to L2) (Table 5).

When parasitism occurred at early L2, the parasitized larvae consumed significantly less cabbage leaf than the healthy larvae during their L4, and consequently they consumed significantly less cabbage leaf in their lifetime. However, the food consumption of the parasitized larvae was not reduced when parasitism occurred at early L3 or L4 (12 hours postmolting to L3 or L4) (Table 6).

Table 5 Development time in days (means ± SE) of unparasitized healthy and parasitized Plutella xylostella larvae by Diagema semicalausum

<table>
<thead>
<tr>
<th>Age at initial stage²,³</th>
<th>Larva status</th>
<th>2nd stadium²</th>
<th>3rd stadium²</th>
<th>4th stadium²</th>
<th>prepupal observation³</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 instar(12)</td>
<td>Parasitized</td>
<td>2.2 ± 0.1 a (21)</td>
<td>2.1 ± 0.1 a(21)</td>
<td>2.5 ± 0.1 a(21)</td>
<td>1.1 ± 0.1 a(9)</td>
</tr>
<tr>
<td></td>
<td>Unparasitized</td>
<td>2.2 ± 0.1 a(27)</td>
<td>1.7 ± 0.1 b(27)</td>
<td>2.0 ± 0.1 b(27)</td>
<td>0.7 ± 0.1 b(27)</td>
</tr>
<tr>
<td>3 instar(12)</td>
<td>Parasitized</td>
<td>2.4 ± 0.1 a(36)</td>
<td>3.7 ± 0.2 a(36)</td>
<td>2.0 ± 0.2 a(27)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unparasitized</td>
<td>1.9 ± 0.1 b(33)</td>
<td>2.6 ± 0.1 b(33)</td>
<td>0.7 ± 0.1 b(15)</td>
<td></td>
</tr>
<tr>
<td>4 instar(12)</td>
<td>Parasitized</td>
<td>2.7 ± 0.1 a(30)</td>
<td>4.7 ± 0.1 a(21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unparasitized</td>
<td>2.2 ± 0.1 b(21)</td>
<td>0.7 ± 0.0 b(19)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

² The numerals in parentheses are time in hours postmolting to the instar.
³ Within the same age at initial observation, means at the same column followed by different letters are significantly different (P < 0.05, by Student’s t-test). The numerals in parentheses are numbers of replicates.
⁴ For parasitized host, the duration is the interval between host cocoon formation and the time when parasitoid forms its own cocoon inside the host cocoon, while for the unparasitized host, the duration is the interval between host cocoon formation and the time when host becomes pupa.
Table 6  Cabbage leaf consumption in square millimeters (means ± SE) by unparasitized healthy
and parasitized Plutella xylostella larvae by Diadegma semiclausum

<table>
<thead>
<tr>
<th>Age at initial observation</th>
<th>Larva status</th>
<th>n</th>
<th>2nd instar larvae</th>
<th>3rd instar larvae</th>
<th>4th instar larvae</th>
<th>In total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 instar (12)</td>
<td>Parasitized</td>
<td>21</td>
<td>6.8 ± 0.5 a</td>
<td>24.1 ± 0.9 a</td>
<td>147.0 ± 6.6 a</td>
<td>177.9 ± 7.0 a</td>
</tr>
<tr>
<td></td>
<td>Unparasitized</td>
<td>27</td>
<td>7.4 ± 0.6 a</td>
<td>25.9 ± 1.0 a</td>
<td>179.0 ± 9.2 b</td>
<td>212.3 ± 9.6 b</td>
</tr>
<tr>
<td>3 instar (12)</td>
<td>Parasitized</td>
<td>36</td>
<td>23.6 ± 1.4 a</td>
<td>169.9 ± 6.6 a</td>
<td>193.5 ± 7.3 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unparasitized</td>
<td>33</td>
<td>20.2 ± 1.1 a</td>
<td>166.8 ± 6.8 a</td>
<td>187.0 ± 7.2 a</td>
<td></td>
</tr>
<tr>
<td>4 instar (12)</td>
<td>Parasitized</td>
<td>30</td>
<td>207.1 ± 5.6 a</td>
<td>207.1 ± 5.6 a</td>
<td>207.1 ± 5.6 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unparasitized</td>
<td>21</td>
<td>225.3 ± 12.2 a</td>
<td>225.3 ± 12.2 a</td>
<td>225.3 ± 12.2 a</td>
<td></td>
</tr>
</tbody>
</table>

* The numerals in parentheses are in hours postmolting to the instar.

* Within the same age at initial observation, means at the same column followed by different letters are significantly different (P < 0.05, by Student's t-test).

n represents the number of host larvae which survived to cocoon formation (replicates).

= DISCUSSION =

We measured dorsal width of 10 first instar DBM larvae and ovipositor width of 10 D. semiclausum. The DBM L1 has a dorsal width of 566.0 ± 7.0 μm, and the wasp ovipositor has a width of 57.0 ± 2.4 μm. Theoretically, it is possible for the wasps to deposit their eggs inside L1. This has been verified by several authors (Talekar and Yang 1991, Yang et al. 1993, Lee et al. 1995). However, the DBM L1 is a leaf miner and feeds inside the host plant leaf until the end of the first stadium or the early beginning of the second stadium. Thus, the DBM L1 has very little opportunity to be exposed to the wasp. Therefore, the first instar P. xylostella larvae were not included in our experiments in this paper. Wasps deposited more eggs and parasitized more larvae when exposed to L2 or L3 than they did when exposed to L4, suggesting they preferred to parasitize L2 and L3 to L4. These results are consistent with Lee et al. (1995), but differ from Talekar and Yang (1991), who reported that this parasitoid parasitized only the first 3 instars and almost always failed to parasitize L4. Talekar and Yang (1991) did not carry out the non-choice experiment and did not indicate the age of L4 used in their experiment. Under non-choice conditions, the wasps were forced to parasitize L4, while under choice conditions, the wasps might shift parasitism from older to younger larvae, thus leading to almost never parasitizing L4. In addition, larger host larvae usually display stronger physical defenses to ovipositing females than smaller ones (Brodeur et al. 1996). Based on our results from the non-choice experiment, the proportion of parasitized larvae decreases with increase of age in L4 (Table 1). We supposed that the difference between the Talekar and Yang results and ours may be due to the fact that L4 used in their experiments were older than those used in ours.

Diadegma semiclausum is a solitary, koinobiotic larval-prepupal endoparasitoid of DBM. The parasitized DBM larvae can form cocoons, but are not able to pupate regardless of the host instar at oviposition, i.e. development of host prepupal stage was arrested (Ooi 1980, Shi et al. 2003, 2004). As the host instar at oviposition increases, the number of host instars that the parasitoid has to go through for completing egg-larval development decreases. Not surprisingly, developmental interactions between the host and the parasitoid become more complicated. Our data on consequences for D. semiclausum showed that as the host instar at oviposition increased from L2 to L4, the development time for the parasitoid decreased, while the size of resultant wasps increased; the resultant female wasps from L3 had the longest longevity and deposited the highest number of eggs in their lifetime, although they did not deposit more eggs in the first day post-emergence when compared to those wasps resulting from L2 or L4. However, survival rates of the parasitoid
both from egg to cocoon and from cocoon to adult emergence were not different among the host instars at oviposition (Table 3 and Table 4). Our data on consequences for host larvae indicated that parasitism resulted in increase of development duration but did not cause the increase of food consumption by host larvae, with an exception of an increasing food consumption when host insects were in the second instar at oviposition (Table 5 and Table 6).

Mackauer and Sequeira (1993) proposed two models to characterize two broadly different developmental strategies for koinobiotic parasitoids such as *D. semicalausum*, which face host quality constraints characterized by an “open”, dynamic resource environment. In the first model, parasitoids attacking low quality (i.e. small) hosts are predicted to exhibit a lag phase in development that allows the hosts to increase in size, whereas parasitoids attacking high quality (i.e. large) hosts are predicted to develop at a constant rate. Parasitoid development time thus varies with host quality at oviposition to maximize wasp biomass per unit of host resources. In the second model, parasitoid growth and development are assumed resource- and time-limited. Under such conditions, parasitoids again are predicted to initially delay development in low quality hosts but are also predicted to exhibit a compensatory increase in growth rate during late stages to balance fitness gains from increased size against losses from longer development times. In both models, parasitoid fitness is size-dependent. Our limited data on the performance of *D. semicalausum* associated with host instars indicate that wasp size may not be a reliable fitness parameter for this parasitoid and that the parasitoid shows complicated trade-offs between various life-history characteristics with regard to host exploitation.

Irrespective of its timing relative to host development, parasitism by *D. semicalausum* inevitably increased the duration of subsequent host development, especially the prepupal stage, and this increase for the prepupal stage became greater with increase of the host age at oviposition (Table 5). Previous reports did not record significant difference in development duration between parasitized and unparasitized host larvae (Yang et al. 1994, Furlong and Pell 2000). However, Furlong and Pell (2000) reported a significant difference between parasitized and unparasitized prepupal stages, that is, the parasitized ones had longer duration than the unparasitized ones, and data provided by Yang et al. (1994) also showed a trend of increasing larval development duration for the parasitized (Yang et al. 1994, their Table 1). Our data on difference of host development prior to prepupa differ substantially from the results mentioned above. The difference may be credited to external (host plant, observation interval and others) or internal factors (genetic variation among population of DBM, maybe the parasitoid too) (Setamou et al. 2005). The host developmental changes may be brought about by the parasitoid’s influences on the nutritional status of the host (Lawrence and Lanzrein 1993). Although the mechanisms operating in this specific interaction are largely unknown, polydnaviruses and venoms injected into the host at oviposition by other ichneumon species have been shown to affect the host endocrine system and arrest host development (Dover et al. 1988, Dover and Vinson 1990, Beck et al. 2000, Cusson et al. 2000). The extension of duration in prepupal stage mediated by *D. semicalausum* was apparently beneficial to the development of the parasitoid, because it weakened the host constraints in time for the wasp development (Vinson and Iwantsch 1980).

The food consumption by parasitized larvae in L4 decreased significantly in comparison with that by unparasitized ones when oviposition happened at host L2. This is consistent with the report by Yang et al. (1994). However, when parasitism started with L3 or L4, the food consumption did not vary. These results are similar to those reported by Jiang et al. (2004) that when *Chilo partellus* (Lep., Pyralidae) was oviposited in L3 by *Cotesia flavipes* (Hym., Braconidae), parasitized *C. partellus* larvae consumed significantly less food than unparasitized, while when *C. partellus* was parasitized at L4, the food consumption by parasitized larvae was comparable to that by unparasitized. Parasitism by other ichneumon wasps was also observed to reduce food consumption (Rohlf and Mack 1983, Kumar and Ballal 1992, Khan
However, food consumption by host larvae may vary with temperature (Jiang et al. 2004).

The results of the present study have several important practical implications. First, as *D. semiclaustrum* eggs laid in L2-L4 have to survive host development until the hosts form cocoons (prepupal stage) to realize successful parasitism, and the parasitoid deposits very few eggs into late L4, percent parasitism of DBM by this parasitoid in the field may be estimated by sampling only all L4. Although such a simplified sampling method may result in a little overestimation of parasitism rate because of the prolonged duration in some of the parasitized larvae, or underestimation because of any further parasitization of young L4, it probably can produce results just as reliable as those obtained by more arduous procedures (Liu et al. 2000). Second, as the parasitized hosts by *D. semiclaustrum* never molt to pupae, when a new laboratory population of this parasitoid needs to be established, only DBM cocoons in prepupal stage need to be collected from the field, while the cocoons that have already survived to pupal stage can be discarded. Third, as L2 and L3 attracted higher parasitism, and female wasps resulting from L3 at parasitization had the highest fecundity (total number of eggs laid), L3 larvae should be chosen in culture maintenance or mass rearing of *D. semiclaustrum* to increase both productivity and quality of the parasitoid produced. Yang et al. (1993) found that oviposition in L4 resulted in production of more females than males (estimated to be 55% and 45% respectively based on their Figure 2), whereas oviposition in L3 resulted in the production of fewer females than males (estimated to be 35% and 65% respectively based on their Figure 2). However, Liu et al. (2004) have recorded a more than 75% female percentage for *D. semiclaustrum* collected from Beijing when using L2 and L3 host as oviposition substratum. Yang et al. (1993) also found that the sex ratio of the parasitoid progeny varied with female wasp age. Meanwhile, other factors will significantly affect the sex ratio of parasitoid progeny (Yang et al. 1994, Eliopoulos et al. 2003). Thus, based on the resultant female’s fecundity, using L3 as oviposition substratum might benefit more for culture maintenance and mass rearing of this parasitoid.

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