Effect of low temperature and short day length exposure on the development of *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae)

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(Received 29 August 2005; Accepted 7 June 2006)

Abstract

Developmental responses of an indigenous aphid parasitoid, *Aphidius gifuensis* were compared when incubated at a low or high temperature and a short or long day length. Host aphid mummification and parasitoid emergence from mummies were observed with very high probabilities of over 80% and 90%, respectively, at all treatments. Sex ratios of emerged parasitoids remained constant at approximately 0.6. Developmental periods of parasitoid progenies reared with a short day length were approximately equal to those with a long day length for both sexes, although the duration from mummy to emergence at 15°C significantly differed between short and long day lengths. These results were summarized as *A. gifuensis* complete development under low temperature and short day length conditions of 15°C and 10L-14D instead of entering larval diapause as mummies. We thus conclude that *A. gifuensis* populations introduced into domestic greenhouses can increase and work effectively as biological control agents against pest aphids even during the hibernial season with low temperature and short day length conditions.

Key words: *Aphidius gifuensis*; day length; development; diapause; temperature

INTRODUCTION

*Aphidius gifuensis* Ashmead is a solitary aphid parasitoid distributed in East Asian countries including Japan, China, Korea and Taiwan (Takada, 1992). This wasp exhibits high parasitizing potential for the green peach aphid, *Myzus persicae* (Sulzer) and the foxglove aphid, *Aulacorthum solani* (Kaltenbach) (Takada, 1976; Yamamoto, 1997; Ohta et al., 2001; Ono et al., 2002; Ohta and Ohtaishi, 2004). Eggplants and green peppers have been infested with two common aphids, *M. persicae* and *Aphis gossypii* Glover in Japanese greenhouses, but additional damage by *A. solani* is also now increasing. This seems to be caused by limiting chemical pesticide application to conserve other introduced natural enemies and bumble bees. *Aphidius colemani* Viereck, a commercially produced aphid parasitoid, can attack *M. persicae* and *A. gossypii*, but not *A. solani* (Nagasaka and Oya, 2003). Therefore, *A. gifuensis* is expected to be a candidate for use as a biological control agent against *M. persicae* and *A. solani* feeding on eggplants and green peppers in domestic greenhouses.

Aphid parasitoids in the temperate zone usually enter diapause in response to photoperiod and temperature changes in autumn (Christiansen-Weniger and Hardie, 1997). Eggplants and green peppers are generally cultivated from September to the following June in domestic greenhouses. Day length shortens to approximately ten hours at the winter solstice in central Japan (National Astronomical Observatory, 2004). Nighttime temperatures during the coldest season are controlled to 10–12°C for eggplants and 18–20°C for green peppers, although daytime temperatures rise to 28–30°C (Ishibashi, 2004; Takahashi, 2004). Thus low temperatures and short day length in winter may constrain the successful application of *A. gifuensis* introduced against *M. persicae* and *A. solani* in greenhouses because of parasitoid diapause induction. The objective of this study is to investigate the biological response of *A. gifuensis* under low temperature and short day length conditions. Brodeur and McNeil

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DOI: 10.1303/aez.2006.555
(1989) and Christiansen-Weniger and Hardie (1997, 1999) demonstrated that *Aphidius nigripes* Ashmead and *Aphidius ervi* Haliday enter diapause in the third or last larval instar inside their host aphid mummies when exposed to photophases under critical day lengths. Also, they found that sensitivity to diapause-inducing conditions was greatest during embryonic and young larval stages in both parasitoids. Accordingly, this work focuses on development in the mummy stage of *A. gifuensis* exposed to low temperature and a short photoperiod during the embryonic and larval stages in the laboratory.

**MATERIALS AND METHODS**

The aphids used in this study originated from an apterous colony of *M. persicae* collected from cabbages in vegetable fields at the National Agricultural Research Center for Western Region (34.5°N, 133.4°E) in 1996. *A. gifuensis* stock culture was established from *M. persicae* mummies on potato leaves in the fields described above. Aphids and parasitoids were reared on radish leaves (*Raphanus sativus* L., c.v. ‘Tokinashi’) and *M. persicae* as their hosts respectively, in a climate room regulated at a constant temperature of 25°C, relative humidity of 60±10% and a photoperiod of 16L-8D.

This experiment was designed to compare developmental responses in immature stages of *A. gifuensis* incubated under four different abiotic conditions. Four cut radish leaves infested with 400 apterous nymphs of *M. persicae* aged 3 days old (approximately third instar) were prepared for the tests (100 aphids/leaf). The leaves were held individually in Erlenmeyer glass flasks (cont. 100 ml) with water. Approximately 50 female adult parasitoids (within 24 h after emergence) obtained randomly from the stock culture and four radish leaves with aphids were introduced into an acrylic rearing cage (30 cm in length and height, 25 cm in width) in a fluorescent-lighted cabinet of 25±1°C and 60±10% r.h. Six hours later parasitoids were removed and then radish leaves with exposed aphids were separately transferred to four growth cabinets regulated at a constant temperature of 15±1°C or 25±1°C and a photoperiod of 14L-10D or 10L-14D with 60±10% r.h. Escaped aphids, leaving the radish leaves and walking on the flasks or the inside walls of the rearing cage, were excluded from the experiments. Incubated aphids were checked daily for mummification and adult parasitoid emergence from mummies. Newly formed mummies were individually confined into small glass tubes (2.1 cm dia., 4.5 cm depth) and kept under the initial conditions. The sex of emerged parasitoids was recorded. Intact aphids, which had not become mummies within three days after the last mummification, were excluded from observation. Mummies from which no adult parasitoid appeared within three days after the last emergence were dissected so that their survival (dead/alive) and developmental stage (larvae/pupae/adult) could be assessed. The data obtained in these experiments revealed the mummification rates of *M. persicae* exposed to *A. gifuensis* (=No. mummies/(No. mummies+No. intact aphids)), emergence rates of adult parasitoids from formed mummies (=No. mummies from which adult parasitoids emerged/Total no. mummies), sex ratios of emerged parasitoids (=No. females/No. females and males) and developmental periods of *A. gifuensis* under four experimental conditions of low/high temperature and short/long day length. The disappearance and death of aphids during incubation were excluded from the calculations because the incidence was very low. This procedure was replicated five times.

Mummification rates, emergence rates and sex ratios were arcsine-transformed and statistically analyzed with two-way ANOVA. Developmental periods between short and long photoperiods were compared using identical temperatures and sexes because the differences between 15°C and 25°C and females and males have already been shown by Ohta et al. (2001).

**RESULTS AND DISCUSSION**

Mummification rates of *M. persicae* exposed to *A. gifuensis*, the emergence rates of new adult parasitoids from aphid mummies and their sex ratios are given in Table 1. Mummification rates and emergence rates were estimated at very high values of over 80% and 90% for all treatments. All mummies with no parasitoid emergence contained dead pupae or adults (no diapausing larvae observed). Mean sex ratios remained constant at 0.60–0.64. Two-way ANOVA showed no significant effects of temperature and photoperiod on the rates of aphid mummification, parasitoid emergence and sex
ratio, and no interactions between these two factors \((p>0.05:\) adjusted by the Bonferroni method to limit overall experimental error). Developmental periods for the juvenile stages of \(A. \ gifuensis\) are summarized in Table 2. Statistical differences between short and long day lengths were found only for durations from mummification to adult emergence of parasitoids reared at 15°C \((t\text{-test, } p<0.001: \) adjusted by the Bonferroni method to limit overall experimental error).

Christiansen-Weniger and Hardie (1999) indicated that diapausing parasitoids of \(A. \ ervi\) not only took a much longer time from the mummy stage to 

### Table 1. Mummification rates of \(M. \ persicae\) exposed to \(A. \ gifuensis\), emergence rates of adult parasitoids from mummies and their sex ratios

<table>
<thead>
<tr>
<th>Temperature (^\circ)C</th>
<th>Photoperiod</th>
<th>No. aphids tested(^a)</th>
<th>Mummification rate (^b)</th>
<th>Emergence rate (^c)</th>
<th>Sex ratio (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>14L-10D</td>
<td>442</td>
<td>87.7 (82.8–94.6)(^c)</td>
<td>98.1 (96.0–98.9)(^c)</td>
<td>0.60 (0.58–0.61)(^c)</td>
</tr>
<tr>
<td></td>
<td>10L-14D</td>
<td>425</td>
<td>89.8 (85.1–92.5)</td>
<td>97.3 (94.6–100)</td>
<td>0.64 (0.58–0.69)</td>
</tr>
<tr>
<td>25</td>
<td>14L-10D</td>
<td>477</td>
<td>92.0 (87.1–94.9)</td>
<td>96.0 (87.1–98.8)</td>
<td>0.64 (0.59–0.69)</td>
</tr>
<tr>
<td></td>
<td>10L-14D</td>
<td>467</td>
<td>87.7 (82.6–92.6)</td>
<td>97.8 (97.3–98.7)</td>
<td>0.61 (0.55–0.64)</td>
</tr>
</tbody>
</table>

\(^a\)Total number of aphids in five replications.
\(^b\)No. mummies/(No. mummies + No. intact aphids) \times 100.
\(^c\)No. mummies from which adult parasitoids emerged/No. mummies \times 100.
\(^d\)No. female parasitoids/No. emerged parasitoids.
\(^e\)Mean (Minimum–Maximum) in five replications.
\(^f\)Two-way ANOVA with data arcsine-transformed and the significance level adjusted by the Bonferroni method to limit overall experimental error.

### Table 2. Developmental periods of \(A. \ gifuensis\) on \(M. \ persicae\)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Temperature (^\circ)C</th>
<th>Photoperiod</th>
<th>No. parasitoids tested</th>
<th>Egg to mummification (^{t\text{-test}})</th>
<th>Mummification to emergence (^{t\text{-test}})</th>
<th>Total (^{t\text{-test}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>♀</td>
<td>15</td>
<td>14L-10D</td>
<td>228</td>
<td>12.7±0.05 ns</td>
<td>8.9±0.05 (p&lt;0.001)</td>
<td>21.6±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10L-14D</td>
<td>239</td>
<td>12.8±0.05 ns</td>
<td>9.3±0.07</td>
<td>22.1±0.07</td>
</tr>
<tr>
<td>♂</td>
<td>15</td>
<td>14L-10D</td>
<td>152</td>
<td>12.9±0.06 ns</td>
<td>7.9±0.06 (p&lt;0.001)</td>
<td>20.8±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10L-14D</td>
<td>132</td>
<td>12.8±0.07 ns</td>
<td>8.5±0.07</td>
<td>21.2±0.10</td>
</tr>
<tr>
<td>♀</td>
<td>25</td>
<td>14L-10D</td>
<td>275</td>
<td>6.6±0.03 ns</td>
<td>3.7±0.03 ns</td>
<td>10.3±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10L-14D</td>
<td>245</td>
<td>6.5±0.03 ns</td>
<td>3.6±0.03</td>
<td>10.1±0.02</td>
</tr>
<tr>
<td>♂</td>
<td>25</td>
<td>14L-10D</td>
<td>156</td>
<td>6.6±0.05 ns</td>
<td>3.4±0.04 ns</td>
<td>10.0±0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10L-14D</td>
<td>156</td>
<td>6.5±0.05 ns</td>
<td>3.3±0.04</td>
<td>9.8±0.05</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SE.
\(^b\)Significance level in each comparison was adjusted by the Bonferroni method to limit the overall experimental error.

Adult emergence than non-diapausuig parasitoids, but also had mummies with dark brown shells, while non-diapausuig mummies had light brown shells. Moreover, the sex ratio of diapausuig populations was male biased. In our study, neither abnormally colored mummies nor male-biased sex ratios were observed for any treatments. Female and male parasitoid offspring incubated at 15°C and 10L-14D required longer periods from mummification to emergence than those at 15°C and 14L-10D, but the differences were slight, within one day. Thus these observations and experimental results indicate that \(A. \ gifuensis\) progeny parasitiz-
ing *M. persicae* can complete their development under low temperature and short day length conditions of 15°C and 10L-14D instead of entering diapause or quiescence.

For long-day insects (i.e., insects that develop without interruption under long days and enter diapause in response to short days), low temperatures tend to promote diapause, whereas high temperatures prevent it (Taubert et al., 1986). Brodeur and McNeil (1989) found a similar pattern for *A. nigripes*: all parasitoid progeny entered diapause at 12L-12D and 15°C, but ca. 50% entered diapause under the same photoregime at 20°C, and only ca. 25% did so at 25°C. Furthermore, temperatures affect the host searching and oviposition behavior of adult parasitoids. Langer et al. (2004) investigated oviposition, flight and walking capacity of four aphid parasitoids at various low temperatures and concluded that temperatures above 10°C were needed for their activity. Greenhouse temperatures during the hibernal season fluctuate daily between 10–30°C for eggplants and 18–30°C for green peppers, but they average out at higher than 15°C (defined as the low temperature condition in our tests) (Ishibashi, 2004; Takahashi, 2004). Therefore, if *A. gifuensis* shows the same temperature response as the long-day insects mentioned above, this suggests that no diapause can be induced in *A. gifuensis* in domestic greenhouses cultivated with eggplants and green peppers even during winter. Also, foraging and oviposition activities of *A. gifuensis* adults in greenhouses are unlikely to be interrupted by low temperatures. This means that the *A. gifuensis* population released in greenhouses can increase and maintain its effectiveness as a biological control agent for pest aphids.

Geographically intraspecific variations in photoperiodic diapause response have been demonstrated in many insects: low latitudinal populations tend to have short critical photoperiods or in extreme cases nondiapause life cycles, whereas high latitudinal populations have long critical photoperiods (Taubert et al., 1986). *A. gifuensis* are distributed widely from Hokkaido (ca. 43°N) to Okinawa (ca. 26°N) in Japan (Takada, 2002). Parasitoids showing no diapause under low temperature and short day length in this study were derived from Fukuyama populations (34.5°N). The influence of geographical variations on diapause should thus be considered for the introduction of other local populations of *A. gifuensis* in greenhouses.

**ACKNOWLEDGEMENTS**

We thank Dr. A. Kawai (National Agricultural Research Center for Tohoku Region) for valuable comments on an earlier version of the manuscript.

**REFERENCES**


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