INTRODUCTION

Aphids rank among the most serious insect pests attacking greenhouse crops due to their very rapid population increase, wide host range and high insecticide resistance (Yano, 2003). Four natural enemies and two microbial insecticides were available as biological control agents against aphids in Japan as of March 2009 (Food and Agricultural Materials Inspection Center, 2009). Natural enemies need to be introduced into greenhouses immediately after the appearance of pests on crops because late release will result in unsuccessful control (Yano, 2003); however, strict monitoring of pest abundance on vegetable crops is impractical because it is such a time-consuming process (Nagasaka and Oya, 2003).

The “banker-plant system” is a very efficient tool to resolve the problems described above. It is a method that involves the introduction of a whole reproduction unit including a natural enemy, and its prey insect with host plants in greenhouses (van Lenteren and Woets, 1988). A technique using an exotic natural enemy, Aphidius colemani Viereck (Hymenoptera: Braconidae), has been applied widely for controlling cotton aphids, Aphis gossypii Glover and green peach aphids, Myzus persicae (Sulzer) (Hemiptera: Aphididae). A. pisum, R. maidis and S. akebiae were accepted by A. gifuensis. S. akebiae showed the most successful parasitism by A. gifuensis among the six aphid species tested, with a mummification rate of 71.7% and emergence rate of 96.7%. No parasitism was observed on R. padi, an alternative host available in the banker-plant system with an exotic parasitic wasp, Aphidius colemani (Hymenoptera: Braconidae). A. gifuensis females reared on S. akebiae had the same developmental period and body size as those reared on M. persicae, with no significant differences. They also demonstrated successful parasitic performance in A. solani and M. persicae. These results suggest that S. akebiae should be a promising alternative host for use in a banker-plant system with A. gifuensis.

Use of *Sitobion akebiae* (Hemiptera: Aphididae) as an alternative host aphid for a banker-plant system using an indigenous parasitoid, *Aphidius gifuensis* (Hymenoptera: Braconidae)

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Abstract

Six species of legume or cereal-feeding aphids, *Acyrthosiphon pisum*, *Aphis craccivora*, *Megoura crassicauda*, *Rhopalosiphum maidis*, *Rhopalosiphum padi* and *Sitobion akebiae* (Hemiptera: Aphididae), were tested as candidates for alternative hosts of *Aphidius gifuensis* (Hymenoptera: Braconidae), an indigenous natural enemy attacking vegetable pest aphids *Aulacorthum solani* and *Myzus persicae* (Hemiptera: Aphididae). *A. pisum*, *R. maidis* and *S. akebiae* were accepted by *A. gifuensis*. *S. akebiae* showed the most successful parasitism by *A. gifuensis* among the six aphid species tested, with a mummification rate of 71.7% and emergence rate of 96.7%. No parasitism was observed on *R. padi*, an alternative host available in the banker-plant system with an exotic parasitic wasp, *Aphidius colemani* (Hymenoptera: Braconidae). *A. gifuensis* females reared on *S. akebiae* had the same developmental period and body size as those reared on *M. persicae*, with no significant differences. They also demonstrated successful parasitic performance in *A. solani* and *M. persicae*. These results suggest that *S. akebiae* should be a promising alternative host for use in a banker-plant system with *A. gifuensis*.

Key words: *Aphidius gifuensis*; *Sitobion akebiae*; alternative host; banker-plant system
Aphidius gifuensis Ashmead (Hymenoptera: Braconidae) is an aphid parasitoid distributed in East Asia, including Japan (Takada, 1992). This is expected to serve as an effective biological control agent against A. solani and M. persicae because of its high parasitizing potential for those aphids (Ohta and Ohtaishi, 2006); however, a banker-plant system for A. gifuensis has not yet been established. The substitute hosts used in the banker-plant system need high acceptance by the parasitoids, successful development of their progeny, and high parasitism of newly-emerged parasitoids against pest insects. In this study, we conducted three experiments to measure the parasitic performance of A. gifuensis for six candidate aphids and then determined a promising alternative host aphid to sustain reproduction of an A. gifuensis population but that would be harmless to greenhouse eggplants and green peppers.

MATERIALS AND METHODS

Insects. The A. gifuensis population originated from M. persicae mummies on eggplants in Nangoku City, Kochi Prefecture, Japan, in April 2004. Stock was cultured in an insectary controlled at 25±1°C with a 14L10D photoperiod (humidity not regulated). M. persicae on young leaves of Japanese radish were offered to the parasitoids as hosts in a plastic rearing cage (30 cm width, 25 cm depth, 35 cm height). Mummified aphids on the leaves were moved to another cage and kept collectively during the adult stage. Newly emerged parasitoids were fed 5% honey solution. Female wasps less than 48 h after emergence and with no experience of parasitism were provided in the following experiments. Collection data and condition of all aphids used in this work are summarized in Table 1.

Parasitism of A. gifuensis on six candidate aphid species as alternative hosts and four vegetable pest aphid species. Parasitic performance of A. gifuensis on various aphid species was evaluated by mummification rates of aphids exposed to the parasitoids and emergence rates of new adult parasites from aphid mummies. Six candidate aphids for the substitute host of A. gifuensis, Acyrthosiphon pisum (Harris), Aphis craccivora Koch, Megoura crassicauda Mordvilko, Rhopalosiphum maidis (Fintch), R. padi and Sitobion akebiae (Shinji) (Hemiptera: Aphididae) were examined with four vegetable pest aphids, A.

Table 1. Collection data and condition of ten aphid species used in the experiments

<table>
<thead>
<tr>
<th>Aphidsa</th>
<th>Origin</th>
<th>Host plantsb used for the stock culturing and tests</th>
<th>Apterous nymphs offered in the tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Place and date</td>
<td>Plant</td>
<td>Age (day)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candidates for alternative host</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acyrthosiphon pisum</td>
<td>Morioka, Iwate in August 1998</td>
<td>Pea Broad bean (Unknown)</td>
<td>1–2</td>
</tr>
<tr>
<td>Aphis craccivora</td>
<td>Tsu, Mie in April 2004</td>
<td>Bush betch Broad bean (Unknown)</td>
<td>3–4</td>
</tr>
<tr>
<td>Megoura crassicauda</td>
<td>Tsu, Mie in April 2004</td>
<td>Bush betch Broad bean (Unknown)</td>
<td>1–2</td>
</tr>
<tr>
<td>Rhopalosiphum maidis</td>
<td>Ayabe, Kyoto in August 2002</td>
<td>Sweetcorn Barley (Kashima-mugi)</td>
<td>1–2</td>
</tr>
<tr>
<td>Rhopalosiphum padi</td>
<td>Zentsuji, Kagawa in March 1999</td>
<td>Unknown Barley (Kashima-mugi)</td>
<td>3–4</td>
</tr>
<tr>
<td>Sitobion akebiae</td>
<td>Kurashiki, Okayama in May 2005</td>
<td>Barley Barley (Kashima-mugi)</td>
<td>2–3</td>
</tr>
<tr>
<td>Vegetable pests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aphis gossypii</td>
<td>Tsu, Mie in May 2004</td>
<td>Cucumber Cucumber (Sharp-1)</td>
<td>3–4</td>
</tr>
<tr>
<td>Aulacorthum solani</td>
<td>Tsu, Mie in May 2004</td>
<td>Eggplant Soybean (Suzukari)</td>
<td>2–4</td>
</tr>
<tr>
<td>Macrosiphum euphorbiae</td>
<td>Tsu, Mie in May 2004</td>
<td>Eggplant Eggplant (Senryo-nigou)</td>
<td>2–3</td>
</tr>
<tr>
<td>Myzus persicae</td>
<td>Tsu, Mie in April 2004</td>
<td>Cabbage Japanese radish (Wase-40-nichi)</td>
<td>3–4</td>
</tr>
</tbody>
</table>

a All aphids were reared at 20±1°C with a 14L10D photoperiod on their own host plants.
b Cultivar names are indicated in parentheses.
c Mean±SD of length from frontal sinus on head to top of cauda measured in 20 aphids.
d Mean±SD of maximum width of abdomen measured in 20 aphids.
gossypii, A. solani, Macrosiphum euphorbiae (Thomas), Macrosiphum persicae (Hemiptera: Aphididae), and M. persicae. The parasitic response of wasps to aphids would be influenced by the body size of the hosts offered because larger aphids can be detected more easily by parasitoids. For example, Takada (1975) showed that the relative frequency of host-finding by A. gifuensis was higher for large nymphs of M. persicae in the fourth instar than small ones from the first to third instar; however, the body sizes of aphid nymphs vary according to species, even within the same developmental stage. Therefore, as shown in Table 1, we used apterous aphid nymphs with almost equivalent body sizes but different developmental stages for the present study. Approximately one hundred aphid nymphs were placed on their host crop seedling (see Table 1) planted in a plastic pot (7.5 cm dia., 6.5 cm height) and then introduced in a rearing cage (15 cm width, 25 cm depth, 30 cm height) with five female adults of A. gifuensis. The cages were kept in a climate chamber at 25 ± 1°C with fluorescent lights. Our previous study (Ohta and Ohtaishi, 2004) suggested that five parasitoids per replication should be sufficient to insure that all aphid nymphs are attacked during the test. Six hours later, parasitoids were removed from the cage and plants with aphids were left as they were. The numbers of mummified and intact aphids were recorded eight days after treatment. Adult parasitoids emerged from mummies were counted after a further five days. The tests were repeated five to eight times for each aphid species.

Development of A. gifuensis on four selected aphid species. We measured the developmental time and adult body size of A. gifuensis reared on two alternative host candidates, A. pisum and S. akebiae, and two vegetable pest aphids, A. solani and M. persicae, on which successful parasitism by A. gifuensis was observed in the first experiment. The aphids were exposed to A. gifuensis females and maintained using the same procedure as for the previous test. Treated aphids were checked daily to ascertain the developmental state until parasitoid emergence. Mummified aphids were individually confined to small glass tubes (2.1 cm dia., 4.5 cm depth) and kept under the initial conditions. The dates of adult wasp appearance from mummies were recorded with their sex to determine developmental periods from egg to adult emergence of A. gifuensis. Only female parasitoids were transferred to another glass tube with no water and food. The lengths of forewings and hind leg tibiae of dead females were also measured with a binocular microscope. Intact aphids and mummies without parasitoid emergence during three days after the last mummification or emergence were excluded from this experiment.

Parasitism of A. gifuensis developed in S. akebiae on vegetable pest aphids. Another stock culture of A. gifuensis reared on S. akebiae was established from the original population for this study. Parasitoid wasps maintained for three generations and more were offered to the tests. Parasitism efficiency of the parasitoids in two pest aphids, A. solani and M. persicae, was measured using the same methodology as in the first experiment. In the previous test, some aphids, particularly A. solani, were observed leaving host plants and staying inside the rearing cage; therefore, these aphids were returned to the leaves daily with a fine brush until the first observation to check for mummification.

Statistical analyses. Data on parasitic performance of A. gifuensis for different aphid species were combined for all repetitions and then analyzed statistically by a Tukey-type multiple comparison test (Zar, 1999) for the first experiment and a chi-square test for the third test. Parasitoid emergence rates from A. gossypii, M. euphorbiae and R. maidis mummies obtained in the initial test were excluded from a post-hoc comparison because their numbers were too small to analyze statistically. A Tukey-Kramer test was used for comparison of the developmental period and body size of the parasitoids fed on the four aphid species selected in the second experiment.

RESULTS

Parasitism of A. gifuensis on six candidate aphid species as alternative hosts and four vegetable pest aphid species

The results of the first experiment are summarized in Table 2. Aphids mummified by A. gifuensis were observed on A. pisum, R. maidis and S. akebiae among alternative host candidates, and all vegetable pest aphids tested, A. gossypii, A. solani, M. euphorbiae and M. persicae although R. maidis, A. gossypii and M. euphorbiae showed very few mummies in this study. No mummies were found on A. craccivora, M. crassicauda and R. padi. The
mummification rate of S. akebiae was 71.7%, with no significant difference from M. persicae (p > 0.05, Tukey-type multiple comparison test). The rates of mummification of A. pisum and A. solani exposed to A. gifuensis were significantly lower (34.7 and 48.1%, respectively) than those of M. persicae and S. akebiae (p < 0.05); however, emergence rates from A. pisum, S. akebiae, A. solani and M. persicae mummies were all 80% or more despite some significant differences (p < 0.05).

**Development of A. gifuensis on four selected aphid species**

The developmental period and body size of A. gifuensis females parasitizing A. pisum, S. akebiae, A. solani and M. persicae are given in Table 3. A. gifuensis emerged from A. pisum were characterized by a significantly longer developmental time, and larger body size than those from A. solani, M. persicae and S. akebiae (p < 0.05, Tukey-Kramer test). Parasitoids fed on A. solani completed their development in a shorter time than those on other aphids (p < 0.05). Their body size was slightly smaller than those on A. pisum but larger than those on S. akebiae and M. persicae. There were no significant differences in developmental period and body size between parasitoids reared on S. akebiae and M. persicae (p > 0.05).

**Parasitism of A. gifuensis developed in S. akebiae on pest aphids**

A. gifuensis reared on S. akebiae showed high parasitic efficiencies on A. solani and M. persicae.
of 93.8 and 85.5%, respectively, for the mummification rate, and 86.9 and 93.2%, respectively, for the emergence rate, with no significant differences (p > 0.05, chi-square test) (Table 4).

**DISCUSSION**

Three legume- and three cereal-feeding aphids were evaluated as potential alternative hosts of *A. gifuensis* in the present study (Table 1), since none of these cause damage to eggplants and green peppers (The Japanese Society of Applied Entomology and Zoology, 2006). In the first experiment, three aphid species, *A. pisum*, *R. maidis* and *S. akebiae*, among the alternative host candidates were accepted by *A. gifuensis*. *S. akebiae* particularly indicated high parasitic efficiency by *A. gifuensis* with a mummification rate of 71.7% and an emergence rate of 96.7% (Table 2). There was no significant difference between *S. akebiae* and *M. persicae* in the mummification rate. Also, the second experiment showed that the developmental time and adult body size of *A. gifuensis* females parasitizing *S. akebiae* were not significantly different from those on *M. persicae*, although some differences from *A. pisum* and *A. solani* were observed (Table 3). This means that *A. gifuensis* progeny can complete development on *S. akebiae* as well as on *M. persicae*.

Apterous nymphal aphids with almost equivalent body sizes but different developmental stages were used for the second experiment (see Table 1), in order to prevent the encounter probability of *A. gifuensis* with the aphids in an experimental cage from being influenced by the aphid’s body size. On the other hand, Sequeira and Mackauer (1992, 1994) reported that developmental rates and fecundity of *Aphidius ervi* Haliday (Hymenoptera: Braconidae) parasitoids reared on pea aphids, *A. pisum*, varied with the host stage at parasitization. Thus, the results obtained in the first and second experiments could also be affected by differences in the developmental stage of aphid nymphs offered in the tests.

Host preference in aphid parasitoid wasps can be influenced by the host species on which the parasitoids are reared (Storeck et al., 2000). *A. ervi* parasitoids were commonly recorded from *A. pisum* on lucerne and *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) on nettles in the UK (Cameron et al., 1984); however, their laboratory tests showed that the *A. ervi* population obtained from *A. pisum* did not parasitize *M. dirhodum* despite high mummy production on *A. pisum*. This implies that the value of an alternative host will depend on the characteristics by which parasitoids will switch between it and the original host (regarded as pest aphids in this study) (Powell and Wright, 1988). In the third experiment, *A. gifuensis* wasps reared on *S. akebiae* demonstrated successful parasitism against pest aphids, *A. solani* and *M. persicae*, as well as the original population fed on *M. persicae* (Table 4). From experimental results of three laboratory tests, we can conclude that *S. akebiae* should be a promising alternative host aphid for use in the banker-plant system for *A. gifuensis*.

*A. gifuensis* exhibited successful parasitism on *A. solani* and *M. persicae*, but not on *A. gossypii* and *M. euphorbiae*, among the four vegetable pest aphids tested in the first experiment (Table 2). This result agrees with the host range of indigenous aphid parasitoids in Japan reviewed by Takada (2002); however, the mummification rate of *A. solani* was significantly lower than that of *M. persicae* (Table 2). Some dead aphids of *A. solani* were observed on the bottom of the rearing cage and in plant pot tubs with water. *A. solani* is likely to be easily disturbed by the parasitic behavior of *A. gifuensis*, i.e. escaping or dropping from the initial

<table>
<thead>
<tr>
<th>Pest aphids offered to the parasitoids</th>
<th>No. mummies + intact aphids</th>
<th>Mummification rate (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Emergence rate (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aulacorthum solani</em></td>
<td>580</td>
<td>93.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Myzus persicae</em></td>
<td>744</td>
<td>85.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
|<sup>a</sup> No. mummies/(No. mummies + intact aphids)×100.  
<sup>b</sup> No. emerged adult parasitoids/No. mummies×100.  
<sup>c</sup> No significant differences between *A. solani* and *M. persicae* (chi-square test, p > 0.05). |
position on leaves after encountering parasitoids (unpublished data). Additional treatment of returning dropped aphids to the leaves probably led to a substantial increase in the mummification rate of *A. solani*, 93.8% in the third experiment (Table 4); therefore, the mummification rate of *A. solani* shown in Table 2 could be underestimated due to the loss of some parasitized and disturbed aphids.

* A. colemani, a commercially produced exotic parasitoid, can parasitize *R. padi* but not *S. akebiae* (unpublished data). This means that the banker-plant system for *A. gifuensis* containing *S. akebiae* would be unavailable to *A. colemani* and vice versa. Thus, simultaneous introduction of two different banker-plant systems for *A. gifuensis* and *A. colemani* may be useful to control *A. solani*, *A. gossypii* and *M. persicae* on eggplants and green peppers in a greenhouse, although further research is required to establish an appropriate technique.

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The authors express special thanks Professor H. Tsumuki (Okayama University) and Dr. H. Shimoyakawa (Kochi Agricultural Research Center) for supplying us with *S. akebiae* and *A. gifuensis* populations, respectively. We are also grateful to Dr. M. Miyazaki for identifying *S. akebiae*. This research was supported financially by the research project, “Development of new biorational techniques for sustainable agriculture” by the Agriculture, Forestry and Fisheries Research Council of Japan.

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