**INTRODUCTION**

The wild mulberry silkmoth, *Bombyx mandarina* (Moore) (Lepidoptera: Bombycidae) inhabits a wide range in Japan, from Hokkaido to Kyushu, except the Amami Islands and Okinawa Prefecture. Studies of *B. mandarina* have been focused mainly on the relationship with the domesticated silkworm, *B. mori* (L.), to understand the domestication process. On the other hand, the ecology of *B. mandarina* has been poorly studied because this insect is not recognized as a serious pest to damage mulberry fields (Ômura, 1943, 1950; Saito and Agata, 1955). Recently, the genetic structure of Japanese populations of *B. mandarina* has been investigated (Yukuhiro et al., 2011, 2012a, b), and we plan to investigate the seasonal occurrence, distribution, and flight distance of *B. mandarina*. Pheromone traps are a useful tool to collect samples for these studies or for behavioral tracking in the wild. They are also used to collect wild silkmoths in order to monitor introgression of transgenes accompanied by test rearing of transgenic silkworms.

The sex pheromone of *B. mandarina* has been identified as (E)-10, (Z)-12-hexadecadien-1-ol (bombykol), as in *B. mori* (Butenandt et al., 1959; Kuwahara et al., 1984; Daimon et al., 2012). Traps baited with 1 mg of bombykol attracted more *B. mandarina* male moths in the field than those containing a virgin female of *B. mandarina* or *B. mori* (Kuwahara et al., 1984). Traps with 0.1 mg of bombykol have been also used to attract male moths (Daimon et al., 2012). In this report, we examined the pheromone trap setting conditions of pheromone dose and trap set height to collect *B. mandarina* male moths efficiently.

**MATERIALS AND METHODS**

**Pheromone traps**

We used SE traps (Fig. 1a, Sankei Chemical Co., Ltd., Kagoshima, Japan) for the pheromone dose tests. Each trap was equipped with a sticky plate (sticky plate for SE traps; Sankei Chemical Co., Ltd.). A rubber septum (sleeve stopper septum; Sigma-Aldrich, St. Louis, MO, USA) loaded with bombykol (Shin-Etsu Chemicals Co., Ltd., Tokyo, Japan) dissolved in hexane or a virgin female moth of *B. mori* (Daizo, p50) was placed in a plastic cup at the center of a sticky plate.

The traps used in the trap set height tests were made by folding cardboard into triangular prisms with both ends open (Fig. 1b). Each trap was equipped with half of a sticky plate. A rubber septum loaded with 1 mg of bombykol dissolved in hexane was placed at the center of the sticky plate.

**Pheromone dose tests**

Five traps with different lures were prepared as one set to compare the effect of pheromone dose on attracting moths, including three rubber septa with different amounts of bombykol (0.1, 1, and 10 mg), one without bombykol, and a *B. mori* female moth. The test fields are listed in Table 1. The traps were hung at 10-m intervals, and trap position was changed daily in rotation. Three or four sets of traps were installed at different locations of each field in triplicate or quadruplicate. The sticky plates were examined daily and changed if they were covered with insects. Female moths were replaced every day.

**Trap height tests**

Three traps were hung at heights of 0.3, 1.3, and 2.3 m as one set to compare the effect of trap height on attracting moths. Each trap was baited with a rubber septum loaded with 1 mg of bombykol. The test fields are listed in Table 1. The traps were hung at 10- and 5-m intervals...
in Tsukuba and Maebashi, respectively. Trap position was changed daily in rotation. Three sets of traps were installed in different locations in triplicate at Tsukuba. The sticky plates were not changed during each period because they were never fully covered with insects.

**RESULTS AND DISCUSSION**

**Pheromone dose**

In total, we collected 16-64 male moths of *B. mandarina* in traps baited with 10 mg of bombykol during each test period at Fuchu (Tokyo), Tsukuba (Ibaraki), and Hokuto (Yamanashi). Fewer than three moths were caught in each trap in the field in Morioka (Iwate). Hence, the results obtained for the first three fields were analyzed here. No moths were trapped if the rubber septa were not loaded with bombykol, confirming that the traps or sticky plates themselves did not attract *B. mandarina* male moths. The number of moths captured increased proportionally to the logarithm of the pheromone dose in all the three fields (Fig. 2).

We compared the abilities of *B. mori* female moths and bombykol-loaded rubber septa to attract *B. mandarina* male moths. The numbers of moths captured in traps baited with a female moth were intermediate between those baited with septa containing 0.1 or 1 mg of bombykol at all three fields (Fig. 2). According to regression formulae estimated from the moth capture in bombykol-baited traps, a *B. mori* female was comparable to 0.13-0.21 mg of bombykol.

Trap maintenance is also a factor that should be considered depending on the frequency of trap exchange when collecting moths consecutively to investigate seasonal changes or when monitoring hybrids. Synthetic bombykol is more useful than female moths because the activity of bombykol-baited lures persists for 1 month outdoors (Kuwahara et al., 1984), but the short and variable longevity of moths requires frequent replacement of dead females. Catching an adequate number of moths also affects trap efficiency. If traps are filled with moths within a few days, it is necessary to check traps repeatedly to avoid biased sampling or underestimating the number of moths. As the practical capacity of a sticky plate is about 100 *B. mandarina* moths based on our experience, traps with 10 mg of bombykol, which attracted up to 64 moths in five days in this study, should be changed weekly. We have previously tested the efficiency of traps baited with 1 mg of bombykol and confirmed that they attract *B. mandarina* male moths effectively for 2 weeks (data not shown). Cost of the lures should also be considered. The cost to catch one moth is minimized when the lure is loaded with 1 mg of bombykol if both 1 mg of bombykol and a rubber septum are purchased at the same price.

**Trap height**

It was assumed that traps located at a higher position could disperse the sex pheromone over a wider area than those at a lower position to attract more male moths. However, we detected no difference in the number of moths

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**Table 1. Test fields**

<table>
<thead>
<tr>
<th>Fields</th>
<th>Dates</th>
<th>Sets</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pheromone dose test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fuchu, Tokyo</td>
<td>Nov. 12-17, 2014</td>
<td>3</td>
<td>Edge of mulberry fields, Tokyo University of Agriculture and Technology</td>
</tr>
<tr>
<td>Tsukuba, Ibaraki</td>
<td>Nov. 20-25, 2014</td>
<td>3</td>
<td>Border of rice paddies and woods at the foot of Mt. Tsukuba</td>
</tr>
<tr>
<td>Hokuto, Yamanashi</td>
<td>Nov. 3-8, 2014</td>
<td>3</td>
<td>Edge of mulberry fields, Genetic Resource Center, NARO</td>
</tr>
<tr>
<td>Morioka, Iwate</td>
<td>Oct. 31-Nov. 5, 2014</td>
<td>4</td>
<td>Tohoku Agricultural Research Center, NARO</td>
</tr>
<tr>
<td><strong>Trap height test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsukuba, Ibaraki</td>
<td>Nov. 4-13 and 17-26, 2015</td>
<td>3</td>
<td>Owashi Campus, Institute of Agrobiological Sciences, NARO</td>
</tr>
<tr>
<td>Maebashi, Gunma</td>
<td>Aug. 20-29, 2016</td>
<td>1</td>
<td>Edge of a mulberry field of a sericulture farm</td>
</tr>
</tbody>
</table>
Pheromone trap for Bombyx mandarina

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caught in traps set at the three heights of 0.3, 1.3, and 2.3 m, in both trap height tests at Tsukuba and Maebashi (Table 2). This finding suggests that Bombyx mandarina male moths fly around at random heights and are attracted only when they come close to the lures. We conclude that pheromone traps to collect Bombyx mandarina can be hung at any height practically on trees, fences, or poles. Traps should be kept off the ground to avoid entry of other insects.

Table 2. Numbers of moths captured in traps set at various heights

<table>
<thead>
<tr>
<th>Fields</th>
<th>Trap height (m)</th>
<th></th>
<th>χ² test*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3</td>
<td>1.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Tsukuba, Ibaraki</td>
<td>23</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>Maebashi, Gunma</td>
<td>15</td>
<td>18</td>
<td>13</td>
</tr>
</tbody>
</table>

* Null hypothesis is that there is no difference in the number of moths captured among the three trap heights.

Fig. 2. Relationship between pheromone dose and number of moth captured. The bombykol dose loaded on rubber septum is plotted on a logarithmic scale. Black circles, mean number of Bombyx mandarina male moths caught in a trap per day with standard deviation; dotted lines, regression lines; white triangles, number of moths caught in traps baited with a Bombyx mori female moth plotted on the regression lines.

ACKNOWLEDGMENTS

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