Estimation of Out-Crossing Rate in *Monochoria korsakowii* Using the Herbicide Resistance Trait as a Marker

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In order to discuss the rate of dispersion of the resistance gene of *Monochoria korsakowii*, we quantitatively estimated the out-crossing rates using the herbicide resistance trait as a marker on experimental populations. Two insect species, *Apis mellifera* and *Xylocopa circumvolans*, were observed on the flowers and the estimated out-crossing rates ranged from 30.4 to 67.8% with an average of 36.2%. The finding indicates that the trait can be transmitted via pollen by bees and expressed in hybrid offspring and further implies that the subsequent spread of resistance can occur through pollen migration. Thus, the evolution of resistance will be rapid.

**Keywords:** herbicide resistance, *Monochoria korsakowii*, out-crossing, pollen dispersal, sulfonyleurea herbicide.

**INTRODUCTION**

*Monochoria korsakowii* Regel et Maack (Pontederiaceae) is an annual, emergent aquatic weed in Hokkaido Prefecture, Japan. A biotype of weed resistant to the bensulfuron-methyl (BSM) [methyl α-(4,6-dimethoxy-pyrimidin-2-yl-carbamoylul-]famoyl) -o-toluate], the first sulfonyleurea (SU) herbicide used in Japan, has been discovered in Hokkaido rice-fields that were treated with BSM-based herbicides for five consecutive years. It was the first confirmed occurrence of herbicide resistance resulting from the use of an SU herbicide in Japan. The inheritance of resistance to SU herbicides has been studied, indicating that resistance was dominant and controlled by a single, nuclear gene. This research quantitatively estimated the out-crossing rate in *M. korsakowii* using herbicide resistance trait as a marker in experimental populations, and discussed the relationship between the rate of dispersion of the resistance gene and the mating system of this paddy weed.

**MATERIALS AND METHODS**

1. **Plant Materials**

   Seeds were collected from two populations code-named: SA1 and KAM. SA1 was collected from plants that had survived SU herbicide treatment in a rice field in Nagano Town, Hokkaido Prefecture and KAM was collected from plants that had never been treated with SU herbicide in a marsh near Kamioka Town, Akita Prefecture, Japan. SA1 was confirmed to be resistant (R) to BSM whereas KAM was susceptible (S) to the herbicide. Furthermore, resistance was dominant and was controlled by a single, nuclear gene.

2. **Out-Crossing Experiment**

   Three experimental populations were established in the field at the Department of Lowland Farming, Tohoku National Agricultural Experiment Station on May 22, 1996. The soil was a grey lowland soil (18.8% sand, 44.3% silt, and 26.9% clay). In each population, 24 SA1 pollen donor plants were hand-planted in a regular 10-cm square grid design and a single KAM pollen receptor plant (the target) was hand-planted at the center. We carefully observed the insects visiting the flowers and their behavior during the flowering periods of these plants. During ten days from Sept. 6 to 15, just one flower per individual plant was allowed to bloom each day; all other flowers were removed. In November, capsules on the target plant were harvested, and samples of seeds from each capsule were germinated after breaking their dormancy. Seedlings were planted in 15.8-cm diameter pots and at the one-leaf stage were treated with 75 g a.i. ha⁻¹ BSM as granules, which is equivalent to the recommended use rate, under submerged conditions with 2 to 4 cm water depth. Dead plants were considered susceptible, and surviving plants were considered resistant. Out-crossing rate, *t*, estimated using the expression \( t = H/p \), where *H* is the frequency of heterozygotes and *p* is the frequency of R homozygotes.

**Table 1. Out-crossing rates, *t* (calculated as \( H/p \)), in the experimental populations of *Monochoria korsakowii***

<table>
<thead>
<tr>
<th>Date of flowering</th>
<th>Proportion of heterozygotes</th>
<th>Estimated out-crossing rates, <em>t</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 6, 1996</td>
<td>42.1</td>
<td>43.8</td>
</tr>
<tr>
<td>7</td>
<td>10.0</td>
<td>10.4</td>
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<tr>
<td>8</td>
<td>37.0</td>
<td>38.5</td>
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<td>9</td>
<td>20.0</td>
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<tr>
<td>10</td>
<td>54.9</td>
<td>57.2</td>
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<tr>
<td>11</td>
<td>65.1</td>
<td>67.8</td>
</tr>
<tr>
<td>12</td>
<td>40.2</td>
<td>41.9</td>
</tr>
<tr>
<td>13</td>
<td>25.0</td>
<td>26.0</td>
</tr>
<tr>
<td>14</td>
<td>14.9</td>
<td>15.5</td>
</tr>
<tr>
<td>15</td>
<td>38.1</td>
<td>39.7</td>
</tr>
<tr>
<td>Average</td>
<td>34.7</td>
<td>36.2</td>
</tr>
</tbody>
</table>

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RESULTS AND DISCUSSION

Both R and S biotypes flowered synchronously in the experimental populations. An average of 140.9 seeds per fruit were harvested from the targets, KAM pollen receptor plants. Observed proportions of heterozygous seeds per fruit of the target plants ranged from 10.0 to 65.1% with an average of 34.7%. Estimated out-crossing rates ranged from 10.4 to 67.8% with an average of 36.2% (Table 1).

Two insect species, *Apis mellifera* and *Xylocopa circumvolans*, were observed on the flowers in the experimental populations. *Apis mellifera* foraged only pollen from this nectarless flower. When foraging pollen, the bee landed on the flower with its head upward, grasped the small yellow stamens positioning above the large stamen, then collected pollen from the anther with its fore- and mid-legs. During this, the bee abdomen is between the style and large stamen. Although the diameter of the abdomen is narrower than the distance between the stigma and the anther, the abdomen occasionally touched these structures as the bee moved (Fig. 1). The behavior was similar to that of *Apis cerana japonica* observed in Kyoto by Wang et al. *Xylocopa circumvolans* is a large bee that also foraged pollen from the flower. The bee approached the flower with its head upward but the flower was immediately bent downward by the body weight of the insect. The bee hung on the flower, curled its body over the small stamens, clasped the yellow anthers with the fore- and mid-legs, and emitted a sound characteristic of buzz-pollination. During this, the bee abdomen touched the stigma on one side, while simultaneously touching the large anther on the other side.

Gene flow is a potent evolutionary force that may serve to speed the rate of resistance evolution through introducing initial resistant individuals to new populations at much higher frequencies than natural mutation rates. Many factors will influence the rate of gene flow between populations including the reproductive biology of the species and the overall structure of the population. In addition, the mode of inheritance of the resistance trait influences the probability of occurrence and the subsequent survival of initial mutants, both before and after selection.

The weed produces two kinds of enantiomorphic flowers which develop alternatively on each branch of the inflorescence; the style curves sideways either to the left or right. The flower also has dimorphic stamens (heteranthery). Each flower has five small stamens with yellow anthers as well as one larger stamen with a purplish-blue anther. The large anther and the stigma are symmetrical with respect to the median plane of the flower and the small anthers are at the central upper position in the flower. The enantiotyly has been interpreted as a mechanism that promotes cross-pollination. Moreover, resistance was dominant and controlled by a single, nuclear gene. For these reasons, the estimated out-crossing rates were high, ranging from 10.4 to 67.8% with an average of 36.2%. This finding indicates that the trait can be transmitted via pollen and expressed in hybrid offspring and further implies that the subsequent spread of resistance can occur through pollen migration. Thus, the evolution of resistance will be rapid.

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