Cypermethrin resistance and reproductive types in onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae)

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Cypermethrin resistance and reproductive types were examined for *T. tabaci* strains. Some arrhenotokous and thelytokous strains encoded the sodium channel mutation (T929I) involved in cypermethrin resistance. However, the resistance levels varied to some degree among the strains. A cytochrome P450 inhibitor, piperonyl butoxide, showed different synergistic effects on the strains examined. These results suggest that fundamental and additional levels of cypermethrin resistance in *T. tabaci* are conferred respectively by reduced sensitivity of the sodium channel and by cytochrome P450-mediated detoxification. © Pesticide Science Society of Japan

Keywords: COI, cytochrome P450, insecticide resistance, mutation, pyrethroid, sodium channel.

**Introduction**

Onion thrips, *Thrips tabaci* Lindeman, is a destructive agricultural pest worldwide.1) *T. tabaci* is also known as a vector of *Tomato spotted wilt virus* (TSWV),2) and *Iris yellow spot virus* (IYSV).3) The pyrethroid resistance of *T. tabaci* has been reported in some countries such as Japan, the USA, Canada, and Australia.4–8)

The mechanisms of pyrethroid resistance have been characterized in many insect pests including *T. tabaci*. Nerve insensitivity associated with amino acid mutations in the sodium channel is known as a major mechanism of pyrethroid resistance. Reportedly, *T. tabaci* strains that showed high levels of resistance had two amino acid mutations (M918T and L1014F) heterozygously.9) One moderately resistant strain was homozygous for amino acid mutation at the T929I site.9)

Cytochrome P450 (CYP450), an important degradation system for the metabolism of xenobiotics and endogenous compounds in insects,10) is another important mechanism of pyrethroid resistance. In thrips, the involvement of CYP450 in cypermethrin resistance was reported for *Thrips palmi* Karny.11)

Actually, *T. tabaci* has two distinct reproductive types: thelytoky and arrhenotoky. Thelytoky, an original reproductive type in Japan, shows complete parthenogenetic reproduction.12) Arrhenotoky is a form of parthenogenesis in which unfertilized eggs develop into haploid males.12) In Japan, arrhenotoky first appeared in Shimane Prefecture in 1990.13) The reproductive types of *T. tabaci* were examined previously for pyrethroid resistance.14) All insects with M918T and L1014F were judged as thelytokous. Most insects encoding T929I exhibited arrhenotoky. Takezawa14) determined reproductive types using nucleotide sequences encoding the mitochondrial cytochrome oxidase I (COI). However, Sogo et al.15) showed that some arrhenotokous insects were phylogenetically clustered incorrectly into the clade of thelytoky according to the method using COI sequences. Therefore, the relation between reproductive type and pyrethroid resistance must be re-examined. In this study, reproductive types of field-collected strains were determined based on progeny production of the adult females. Furthermore, nucleotide sequences corresponding to the three mutation sites in the sodium channel gene were examined for insects of different reproductive types.

**Materials and Methods**

1. **Insects**

In all, nine *T. tabaci* strains were collected in the field at three locations (Kochi, KOC; Tokushima, TOK; Kagawa, KAG) (Table 1). The TTU laboratory strain was obtained from T. Murai (Utsunomiya University, Japan). Insects were maintained with germinated fava bean (*Vicia faba* L.) at 23°C under a long photoperiod (16L:8D).

2. **Bioassay**

The leaf-dipping bioassay method17) was used for this study with a slight modification. Briefly, kidney bean leaves (3×3 cm) were dipped for 3 min in more than five concentrations of cypermethrin (Agrosrin 6.0% E.C.; Sumitomo Chemical Co., Ltd.) containing the spreading agent (0.02%, Agrura; Agro Kanesho Co., Ltd.). For the control test, kidney bean leaves were dipped in distilled water containing the spreading agent. The treated leaves were allowed to air-dry at room temperature and were inserted into plastic vials (10 mL) that had been coated in advance with the same concentrations of cypermethrin. Ten female adults...
were introduced into plastic vials and were kept at 23°C. Each bioassay was replicated three times. Mortality was recorded at 24 hr after treatment. Insects showing a lack of response to probioassay was replicated three times. Mortality was recorded at individually and were allowed to oviposit on fava beans for more

Females of the laboratory and field-collected strains were reared 6. Determination of reproductive types

\[ \text{′} - gcgaacgtttgctttgatcc-3 \]

(5  were sequenced directly using the primer, Tt-Na-direct-seq4 3 (5 ′ -gctgtcagtcttcatcct-3 )).\)

IIS6 of the sodium channel gene were amplified using PCR according to the manufacturer’s instructions.

The genomic DNA fragments corresponding to domains IIS4–IIS6 of the sodium channel gene from \( T. \ tabaci \) (GenBank/EMBL/DDBJ accession No. LC164017). The PCR conditions were 1 cycle of 3 min at 94°C, 40 cycles of 15 sec at 94°C, 30 sec at 50°C, and 1 min at 72°C; and final extension of 72°C for 7 min. Amplified DNA fragments were sequenced directly using the primer, Tt-COI-direct-5′ (5′-gtctgatcagtttattttaacagcc-3′).

7. Nucleotide sequencing
Nucleotide sequencing was conducted using a dye terminator cycle sequencing kit (Applied Biosystems) and a DNA sequencer (3130xl; Applied Biosystems).

Results

1. Reproductive type determination
Reproductive types of the examined strains are presented in Table 2. The TTU, KOC50, KOC2, TOK12, TOK6, KAG1, and KAG2-1 strains were judged as arrhenotokous according to progeny production of the adult females. The remaining three strains (KOC2442, KOC16, and TOK401) were regarded as arrhenotokous. Based on the COI sequences, KOC16 was classified as thelytokous.

2. Bioassay
The LC\textsubscript{50} values of 10 strains examined were 0.013 mg/L (KOC50)–393.06 mg/L (TOK401) (Table 2). The resistance level of the TOK401 strain was estimated as 30, 235-fold higher than that of the KOC50 strain (Table 2).

3. Nucleotide sequence analyses of the sodium channel gene
The DNA fragments corresponding to domains IIS4–IIS6 of the

<table>
<thead>
<tr>
<th>Strain</th>
<th>Location</th>
<th>Host plant</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTU</td>
<td>Utsunomiya City, Tochigi Pref.</td>
<td>Allium cepa</td>
<td>2005</td>
</tr>
<tr>
<td>KOC50</td>
<td>Hataeda, Nankoku City, Kochi Pref.</td>
<td>Allium fistulosum</td>
<td>2011</td>
</tr>
<tr>
<td>KOC2</td>
<td>Hondo, Shimanto-cho, Takaoka-gun, Kochi Pref.</td>
<td>Asparagus officinalis</td>
<td>2012</td>
</tr>
<tr>
<td>KOC2442</td>
<td>Hataeda, Nankoku City, Kochi Pref.</td>
<td>Allium fistulosum</td>
<td>2011</td>
</tr>
<tr>
<td>KOC16</td>
<td>Hataeda, Nankoku City, Kochi Pref.</td>
<td>Allium fistulosum</td>
<td>2011</td>
</tr>
<tr>
<td>TOK12</td>
<td>Hegoawa, Mugi-cho, Kaiifu-gun, Tokushima Pref.</td>
<td>Allium fistulosum</td>
<td>2012</td>
</tr>
<tr>
<td>TOK6</td>
<td>Hegoawa, Mugi-cho, Kaiifu-gun, Tokushima Pref.</td>
<td>Allium fistulosum</td>
<td>2012</td>
</tr>
<tr>
<td>TOK401</td>
<td>Mugi-cho, Kaiifu-gun, Tokushima Pref.</td>
<td>Allium fistulosum</td>
<td>2011</td>
</tr>
<tr>
<td>KAG1</td>
<td>Kita, Ayagawa-cho, Ayauta-gun, Kagawa Pref.</td>
<td>Allium cepa</td>
<td>2015</td>
</tr>
<tr>
<td>KAG2-1</td>
<td>Shichika, Manno-cho, Nakatado-gun, Kagawa Pref.</td>
<td>Asparagus officinalis</td>
<td>2013</td>
</tr>
</tbody>
</table>
Amplified DNA fragments were sequenced directly. According to the signal appearance at the T929I site on the sequence chromatograms, the KOC2, KOC2442, KOC16, TOK6, TOK401, and KAG2-1 strains were inferred as resistant homozygotes for the mutation site (Table 2). The remaining four strains (TTU, KOC50, TOK12, and KAG1) were regarded as susceptible homozygotes (Table 2). No strain with both M918T and L1014F was found in this study.

4. Synergistic analysis with PBO

The synergistic ratios of PBO for 10 strains were 0.81 (KOC50)–3.74 (TOK401) (Table 2).

Discussion

Reproductive type determination of T. tabaci based on progeny production of the adult females takes a considerable amount of time. A time-saving method for discrimination of the reproductive types using the COI sequences was reported by Takeuchi and Toda. However, some insects exhibiting arrhenotoky were classified incorrectly as showing thelytoky according to the COI-based method. In fact, in this study, the reproductive type of one strain (KOC16) was predicted incorrectly based on COI-based method (Table 2). Consequently, reproductive types determined by progeny production of the adult females were used in this study.

The LC50 values of the KOC2, KOC2442, KOC16, TOK6, TOK401, and KAG2-1 strains were 70.982 mg/L (KAG2-1)–393.06 mg/L (TOK401) (Table 2). The agriculturally recommended concentration of cypermethrin for T. tabaci in onions is 30 mg/L in Japan. Direct sequencing showed that the six strains had T929I. One strain showing a moderate level of resistance to cypermethrin contained T929I. These results suggest that the six strains with T929I are cypermethrin resistant. However, the resistance level of the TOK401 strain was estimated as 5.5-fold higher than that of the KAG2-1 strain, suggesting the involvement of other resistance mechanisms.

The involvement of CYP450 in cypermethrin resistance was reported for T. palmi. To examine the involvement of CYP450 at various levels of resistance of the resistant T. tabaci strains, a synergism test using PBO was executed. PBO caused 3.09-fold and 3.74-fold decreases in the resistance ratio for the KOC16 and TOK401 strains, respectively. The synergistic effects of PBO in the resistance were limited for the KOC2, KOC2442, TOK6, and KAG2-1 strains. These results suggest that CYP450 is involved differentially in the cypermethrin resistance of T. tabaci strains. PBO is also known to block nonspecific esterases in some insect species. The involvement of nonspecific esterases in the cypermethrin resistance of T. tabaci must also be examined.

<p>| Table 2. Toxicity of cypermethrin and synergistic effect of PBO on Thrips tabaci |</p>
<table>
<thead>
<tr>
<th>strain</th>
<th>reproductive type*</th>
<th>phenotype</th>
<th>COI</th>
<th>T929I</th>
<th>treatment</th>
<th>n</th>
<th>LC50 (mg/L)</th>
<th>95% CL</th>
<th>RR&lt;sup&gt;4&lt;/sup&gt;</th>
<th>SR&lt;sup&gt;f&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>KOC2</td>
<td>TH</td>
<td>TH</td>
<td>SS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>cypermethrin</td>
<td>151</td>
<td>94.885</td>
<td>81.762–107.776</td>
<td>7,299</td>
<td>1.00</td>
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<tr>
<td>KOC2442</td>
<td>AR</td>
<td>AR</td>
<td>RR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>cypermethrin</td>
<td>244</td>
<td>112.905</td>
<td>87.978–137.379</td>
<td>8,685</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>KOC16</td>
<td>AR</td>
<td>TH</td>
<td>RR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>cypermethrin</td>
<td>195</td>
<td>148.968</td>
<td>134.568–164.627</td>
<td>11,459</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>TOK12</td>
<td>TH</td>
<td>TH</td>
<td>SS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>cypermethrin</td>
<td>166</td>
<td>0.033</td>
<td>0.029–0.041</td>
<td>3</td>
<td>1.00</td>
<td></td>
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<tr>
<td>TOK6</td>
<td>TH</td>
<td>TH</td>
<td>RR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>cypermethrin</td>
<td>205</td>
<td>146.781</td>
<td>124.486–158.835</td>
<td>11,291</td>
<td>1.00</td>
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<tr>
<td>TOK401</td>
<td>AR</td>
<td>AR</td>
<td>RR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>cypermethrin</td>
<td>221</td>
<td>393.060</td>
<td>336.079–482.295</td>
<td>30,235</td>
<td>1.00</td>
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<tr>
<td>KAG1</td>
<td>TH</td>
<td>TH</td>
<td>SS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>cypermethrin</td>
<td>196</td>
<td>0.054</td>
<td>0.009–0.077</td>
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<tr>
<td>KAG2-1</td>
<td>TH</td>
<td>TH</td>
<td>RR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>cypermethrin</td>
<td>236</td>
<td>70.982</td>
<td>57.813–81.006</td>
<td>5,460</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

*AR: arrhenotoky, TH: thelytoky. **RR: Resistant homozygote for the T929I site. **SS: Susceptible homozygote for the T929I site. **CL: confidence limit. **Resistance ratio (RR): cypermethrin LC<sub>50</sub> of each strain/cypermethrin LC<sub>50</sub> of KOC50 strain or cypermethrin+PBO LC<sub>50</sub> of each strain/ cypermethrin+PBO LC<sub>50</sub> of KOC50 strain. **Synergist ratio (SR): LC<sub>50</sub> of cypermethrin alone/LC<sub>50</sub> of cypermethrin+PBO. **PBO: piperonyl butoxide.
Takezawa\textsuperscript{14}) reported that most strains encoding T929I are arrhenotokous. In the present study, T929I was encoded not only in arrhenotoky but also in thelytoky, suggesting that both reproductive types have the potential to encode the mutation. To date, all insects encoding M918T and L1014F show thelytoky.\textsuperscript{9,14}) No insect encoding both mutations has been obtained in Shikoku Island to date (this study by Aizawa is unpublished). The genetic potential of arrhenotokous strains to encode both mutations heterozygously must be examined in future studies.

**Acknowledgements**

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**References**

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