Carboxylesterase isozymes responsible for organophosphate resistance in the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae)

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**Abstract**

Carboxylesterase isozymes responsible for organophosphate resistance in the cotton aphid, *Aphis gossypii* Glover, were evaluated by inhibitory experiments on isoelectric focused bands. Differences in the carboxylesterase band patterns were detected between organophosphate-susceptible and resistant clones, and the activity of all bands was equally inhibited by fenitrooxon in both clones. The activity was also inhibited by carbaryl, methomyl and K2 (2-phenoxy-4H-1,3,2-benzodioxaphosphorin 2-oxide) in a resistant clone. Fenitrooxon showed the highest inhibitory activity. On the other hand, in the peach-potato aphid, *Myzus persicae* (Sulz.), used as a comparative aphid species, no difference was detected in the carboxylesterase band patterns between organophosphate-susceptible and resistant clones. A single band, however, showed different activity between the clones. The band, whose activity was inhibited by fenitrooxon, may be the isozyme responsible for insecticide resistance in *M. persicae*. These results indicate that all carboxylesterase isozymes, but not the particular isozyme in *M. persicae*, are related to organophosphate resistance, and that overall enzyme activity determines the degree of resistance in *A. gossypii*.

**Key words:** *Aphis gossypii*, *Myzus persicae*, organophosphate resistance, carboxylesterase, isoelectric focusing

**INTRODUCTION**

The cotton aphid, *Aphis gossypii* Glover, one of the most important cosmopolitan pests of many crops, has developed a high resistance to organophosphates, carbamates, and pyrethrroids in Japan (Saito, 1989, 1990; Souda and Ohkubo, 1992; Hama et al., 1995; Saito and Ikeda, 1998). The degree of resistance to organophosphates correlates positively with the amount of carboxylesterase (Sun et al., 1987; Hama and Hosoda, 1988; Takada and Murakami, 1988; Saito, 1989) and is decreased by a carboxylesterase inhibitor, e.g., K2 (2-phenoxy-4H-1,3,2-benzodioxaphosphorin 2-oxide), in synergistic experiments (Saito et al., 1995).

Carboxylesterase shows different electrophoresis patterns for organophosphate-susceptible and resistant clones of *A. gossypii* (Takada and Murakami, 1988; O'Brien et al., 1992; Saito, 1993; Suzuki and Hama, 1998), but it is not known whether the cotton aphid has a particular isozyme such as E4 (Devonshire, 1977; Devonshire and Sawicki, 1979) responsible for insecticide resistance in the peach-potato aphid, *Myzus persicae* (Sulz.). Isozyme E4 of *M. persicae* provides both hydrolysis and sequestration activity to insecticides (Devonshire and Moores, 1982). The carboxylesterase of *A. gossypii*, however, acts only as a sequestering protein for organophosphates, lacking the role of a hydrolyzing protein (Suzuki et al., 1993), and then it loses its activity. In *A. gossypii*, therefore, each band of carboxylesterase electrophoresed may indicate a role in resistance to organophosphates by inhibiting their activity with chemicals. In this paper, we evaluated the carboxylesterase isozymes responsible for insecticide resistance in *A. gossypii* by isoelectric focusing, and compared it to the enzyme of *M. persicae*.

**MATERIALS AND METHODS**

**Aphid clones.** The susceptible *A. gossypii* clone (S) was provided by Dr. H. Takada of Kyoto Prefectural University. The organophosphate-resistant *A. gossypii* clone (M) was col-
lected in 1989 from a melon plant in Iwata, Shizuoka, Japan. Each clone was reared on excised leaves of eggplant and cucumber, respectively, at 23°C in the laboratory. The organophosphate-susceptible clone (PS) and resistant clone (PR) of *M. persicae* were cloned from populations supplied by Zen-No Cooperative (Hiratsuka, Kanagawa, Japan), and then reared on excised leaves of *Brassica rapa* L. in the laboratory under the conditions described above. The susceptibility of these aphids to insecticides is shown in Table 1.

**Chemicals.** Five chemicals, fenitroxon (>98% purity), carbaryl (>98%), methomyl (90.8%), K2 (2-phenoxy-4H-1,3,2-benzodioxaphosphorin 2-oxide) (>98%) and DEF (S,S,S-trIBUTYL PHOSPHOROTHIOATE) (74%) were used as potential carboxylesterase inhibitors.

**Isoelectric focusing and inhibition of carboxylesterase activity.** Isoelectric focusing of carboxylesterase was conducted as detailed elsewhere (Saito, 1993). Adult apterous viviparae were homogenized in 200 μl of 1/15 M phosphate buffer (pH 7.2). In *A. gossypii*, the number of individuals per sample (1 to 20 aphids) was adjusted based on the degree of carboxylesterase activity. In *M. persicae*, 1 aphid per sample was used. Then, 20 μl of homogenate was applied to a filter paper on a precast amphotoline polyacrylamide gel plate (pH 3.5–9.5; Pharmacia LKB, Sweden). Gels were electrofocused at a constant power of 5 W for 30 min, and then at 10 W for 3 h after removing the filter paper. The gel was cooled during the run by circulating cold water (10°C).

Focused gel plates were cut into strips for each lane and incubated in a series of 3 to 5 concentrations of each chemical at 30°C for 10 min with gentle shaking, as described by Kono and Sato (1987). The appropriate solution of chemicals was prepared as follows: each chemical was dissolved in acetone, then 1 ml of the acetone solution was added to 30 ml of 1/15 M phosphate buffer (pH 7.2).

Strips were stained for activity with 0.1% Fast Blue RR in 1/10 M phosphate buffer (pH 6.2) containing 0.03% *a*-naphthyl acetate. The absorbance of each band was measured at 600 nm using a densitometer (Model CS-910; Shimadzu, Japan). The isoelectric point (pI) of each band was estimated from pI marker proteins (pI range 4.7–10.6; LKB, Sweden) run in parallel with samples on the gel.

**RESULTS**

In *A. gossypii*, susceptible aphids (S) had 3 major active bands, at pI 5.25, 5.3 and 5.4 (Fig. 1), and resistant aphids (M) generally had 4 major active bands, at pI 5.3, 5.4, 5.5 and 5.8 (Figs. 2 to 6). These aphids thus showed different electrofocusing patterns of carboxyl

![Fig. 1. Zymogram of carboxylesterase treated with fenitroxon in susceptible *A. gossypii* (clone S). Concentrations of fenitroxon and the isoelectric point (pI) of major bands are shown.](image-url)

### Table 1. Susceptibility to insecticides of clonal aphids of *A. gossypii* and *M. persicae*

<table>
<thead>
<tr>
<th>Species</th>
<th>Clone</th>
<th>Malathion</th>
<th>Fenitrothion</th>
<th>Carbaryl</th>
<th>Methomyl</th>
<th>Permethrin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. gossypii</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>S</td>
<td>8.69</td>
<td>4.15</td>
<td>2.06</td>
<td>1.95</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>239</td>
<td>69.0</td>
<td>66.4</td>
<td>13.0</td>
<td>0.181</td>
</tr>
<tr>
<td><em>M. persicae</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>PS</td>
<td>&lt;500</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>&gt;500</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are expressed as LD<sub>50</sub> (μg/g aphid) (Saito et al., 1995).
<sup>b</sup>Values are expressed as LC<sub>50</sub> (ppm).
Carboxylesterase of *Aphis gossypii*

Fig. 2. Zymogram of carboxylesterase treated with fenitroxon in resistant *A. gossypii* (clone M). Concentrations of fenitroxon and the isoelectric point (pI) of major bands are shown.

Fig. 5. Zymogram of carboxylesterase treated with methomyl in resistant *A. gossypii* (clone M). Concentrations of methomyl and the isoelectric point (pI) of major bands are shown.

Fig. 3. Zymogram of carboxylesterase treated with carbaryl in resistant *A. gossypii* (clone M). Concentrations of carbaryl and the isoelectric point (pI) of major bands are shown.

Fig. 6. Zymogram of carboxylesterase treated with DEF in resistant *A. gossypii* (clone M). Concentrations of DEF and the isoelectric point (pI) of major bands are shown.

Fig. 4. Zymogram of carboxylesterase treated with K2 in resistant *A. gossypii* (clone M). Concentrations of K2 and the isoelectric point (pI) of major bands are shown.

Esterase. S aphids, in particular, lacked 2 bands at pI 5.5 and pI 5.8, which were major active bands in M aphids. On the focused gel-strips treated with fenitroxon, the carboxylesterase activity of all bands was inhibited equally in both S (Fig. 1) and M aphids (Fig. 2), and the enzyme activity was also inhibited by carbaryl (Fig. 3), K2 (Fig. 4), and methomyl (Fig. 5) in M aphids. Fenitroxon indicated the highest inhibitory activity, causing the esterase activity to disappear completely at a concentration of 10^{-5} M. DEF showed little inhibition even at a high concentration of 10^{-2} M (Fig. 6).

*M. persicae*, however, showed no difference in electrofocusing patterns of carboxylesterase between susceptible (PS) and resistant (PR)
aphids: both had 4 bands, at $pI$ 4.9, 5.4, 5.5 and 5.6, although PR aphids showed significantly higher activity in the band at $pI$ 4.9 than PS aphids (Fig. 7). Fenitroxon inhibited only the activity of this single band (Fig. 8).

**DISCUSSION**

Carboxylesterase appears to be a significant factor in resistance to organophosphates in *A. gossypii* (Sun et al., 1987; Hama and Hosoda, 1988; Takada and Murakami, 1988; Saito, 1989; Saito et al., 1995). O’Brien et al. (1992) found that isoelectric focusing of carboxylesterase showed both quantitative and qualitative differences in patterns between the aphids susceptible and resistant to insecticides. Saito (1993) has already shown that total carboxylesterase activity in organophosphate-resistant aphids was significantly higher than that in susceptible aphids, and the latter lacked a band at $pI$ 5.8 in isoelectric focusing, a major band in resistant aphids. Suzuki and Hama (1998) has also shown that bands at $pI$ 5.57 and $pI$ 5.83 detected as a single protein of 52 kDa may play a prominent role in the expression of resistance to organophosphates in the aphids. These findings support our data showing marked activity in bands at $pI$ 5.5 and $pI$ 5.8 in resistant aphids. Accordingly, these bands may indicate increased carboxylesterase activity in organophosphate-resistant cotton aphids.

It has been shown that carboxylesterase of *A. gossypii* detoxifies fenitroxon by sequestration, without hydrolytic action (Suzuki et al., 1993). As the carboxylesterase activity of all bands was inhibited by fenitroxon in our study, these results strongly suggest that all isozymes of the enzyme are related to organophosphate resistance and overall activity of the enzyme correlates positively with the degree of resistance.

On the other hand, it has been shown that carboxylesterase plays roles as both a sequestering and hydrolysing protein to detoxify insecticides in *M. persicae* (Devonshire and Moores, 1982) used as a comparative aphid species. Possible carboxylesterase action in *M. persicae* thus cannot be evaluated through inhibition experiments such as our study using only detectable sequestration action. Electrophoresis of carboxylesterase is useful in detecting isozyme E4, because insecticide resistance in *M. persicae* is caused by increased carboxylesterase activity in the single isozyme (Devonshire, 1977; Devonshire and Sawicki, 1979; Devonshire and Moores, 1982). Based on a significantly higher activity at $pI$ 4.9 in resistant aphids, the band was estimated to be isozyme E4 in the former studies. In *A. gossypii*, however, band $pI$ 4.9 was not detected in any carboxylesterase patterns in either susceptible or resistant aphids, suggesting that the cotton aphid has no isozyme E4. Thus, all isozymes of carboxylesterase, but not the particular isozyme such as E4 in *M. persicae*, may be responsible for organophosphate resistance in *A. gossypii*.

Suzuki et al. (1993) determined the inhibitory activity ($I_{50}$) of fenitroxon ($1.4 \times 10^{-8} \text{M}$), K2 ($4.9 \times 10^{-7} \text{M}$), carbaryl ($7.5 \times 10^{-7} \text{M}$), methomyl ($1.1 \times 10^{-7} \text{M}$) and DEF ($>10^{-3} \text{M}$)
for carboxylesterase of *A. gossypii* in vitro. Their results agree practically with our data using electrofocusing, because overall carboxylesterase acts as a sequestering protein toward these chemicals.

O’Brien et al. (1992) suggested that gene amplification may be associated with insecticide resistance in both *A. gossypii* and *M. persicae*. Suzuki and Hama (1998) have suggested that other mechanisms, e.g., mutation in the structural gene, changes in transcription regulation and/or posttranslational modification may be related to the overproduction of carboxylesterase in resistant *A. gossypii*. In our study, a dramatic difference in carboxylesterase patterns between the susceptible and resistant cotton aphids cannot be accounted for by a genetic phenomenon.

Japanese *A. gossypii* is roughly divided into 2 groups based on their carboxylesterase activity, and their qualitative variations are apparently associated with quantitative variations in carboxylesterase patterns (Saito, 1993). These variations also appear to be closely related to biotypes with different preference for host plants (Saito, 1989, 1990, 1991, 1993; Hosoda et al., 1992). Studies of the basic biology may aid in clarifying more detailed insecticide resistance in *A. gossypii*.

ACKNOWLEDGEMENTS

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REFERENCES


