Individual Variation of Acetylcholinesterase Sensitivity to Propoxur in the Green Rice Leafhopper, *Nephotettix cincticeps* Uhler (Hemiptera : Deltocephalidae)\(^1\)

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It has been shown that carbamate resistance in the green rice leafhopper is mainly due to much reduced sensitivity of acetylcholinesterase (AChE) to inhibition by carbamates and it is controlled by a single gene (Hama and Iwata, 1971, 1978; Iwata and Hama, 1972). It was also proved that much reduced AChE sensitivity was attributed to the appearance of a mutant enzyme, i.e., a modified AChE, which could be distinguished from a wild enzyme, i.e., a normal AChE, by DEAE-cellulose column chromatography (Hama, 1976). Recently, the enzyme of a multiple-resistant Nakagawara strain (N) used as a parent resistant strain in genetic study (Hama and Iwata, 1978) was found to consist of two different enzymes, i.e., normal and modified AChE's, with an activity ratio of about 1:1 (Hama et al., 1980).

In other words, the N strain seemed homozygous for carbamate resistance but not homozygous for reduced sensitivity of AChE. Then, sensitivity of AChE to an inhibitor was measured individually to get further information and two different patterns were found in individual variation of AChE sensitivity in this insect.

Strains of the green rice leafhopper used are listed in Table 1. All strains except for the N strain have been reared without insecticidal selection pressure. The N strain has been continuously selected in several generations with Bassa (2-n-sec-butylphenyl methylcarbamate) and more recently with propoxur (2-isopropoxyphenyl methylcarbamate). Three- to eight-days-old adults were used.

Sensitivity of AChE to an inhibitor was determined by the same technique as in Oppenorth et al. (1977), which was based on Ellman’s method (Ellman et al., 1961), with slight modifications. A head of an adult was homogenized in 0.22 ml of distilled water on a ground glass and the homogenate was used as the enzyme preparation. Enzyme preparation (0.15 ml), 1.24 ml of 0.1 M phosphate buffer, pH 7.4, and 0.05 ml of 0.01 M 5,5'-dithiobis(2-nitrobenzoic acid) were taken into a cuvette (10×5 mm), while all reagents except for enzyme preparation, which was replaced by distilled water, were taken in a reference cuvette. Both cuvettes were kept at 30°C in a spectrophotometer (Hitachi 556) connected with a recorder (Hitachi 056). A few minutes later 0.05 ml of 0.015 M acetylthiocholine iodide (a final concentration of 5×10^{-4} M) was added to both cuvettes and stirred quickly and absorbance change at 412 nm was recorded at a chart speed of 10 mm/min. A few minutes later 0.01 ml of 1×10^{-4} M propoxur in acetone (a final concentration of 6.7×10^{-4} M) was injected into a sample cuvette and absorbance change was continuously recorded. The amount of acetone added resulted in a few percent increase in AChE activity.

Although absorbance change increased linearly with time prior to the addition of an inhibitor, it later varied from samples tested. Absorbance change was completely suppressed, moderately suppressed or hardly suppressed. In the cases

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Table 1. Strains of Green Rice Leafhopper Used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Location collected</th>
<th>Year collected</th>
<th>Characteristics of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>Sendai, Miyagi</td>
<td>1969</td>
<td>Susceptible</td>
</tr>
<tr>
<td>N</td>
<td>Nakagawara, Ehime</td>
<td>1970</td>
<td>Organophosphorus and carbamate</td>
</tr>
<tr>
<td>Nakagawara '75 (N '75)</td>
<td>do.</td>
<td>1975</td>
<td>do.</td>
</tr>
<tr>
<td>Nakagawara '79 (N '79)</td>
<td>do.</td>
<td>1979</td>
<td>do.</td>
</tr>
<tr>
<td>Ōzu '79</td>
<td>Ōzu, Ehime</td>
<td>1979</td>
<td>do.</td>
</tr>
<tr>
<td>Kumamoto '74</td>
<td>Kumamoto, Kumamoto</td>
<td>1974</td>
<td>do.</td>
</tr>
<tr>
<td>Saga '75</td>
<td>Kawasoe, Saga</td>
<td>1975</td>
<td>do.</td>
</tr>
</tbody>
</table>

* All strains except for the N strain are insects reared without insecticidal selection.

Fig. 1. Variations of AChE sensitivity in head homogenates of individual green rice leafhoppers. Ratio \((v_1/v_0)\): \(v_0\) = Absorbance change per min prior to the addition of propoxur (final concentration \(6.7\times10^{-4}\) M), \(v_1\) = Absorbance change per min 3 min after the addition of propoxur. Details are given in the text. In the S strain ratios of AChE sensitivity were 0 in all individuals tested (n = 100).
where absorbance change was moderately suppressed, absorbance increased with a very gentle curve. Then, the ratio of absorbance change per min after 3 min of adding propoxur ($n_1$) to that prior to adding propoxur ($n_0$) was given as an indicator of AChE sensitivity.

Individual variations of AChE sensitivity for several strains are shown in Fig. 1. Although females were used in most strains, both sexes were used in the Nakagawa and Ōzu strains. Since there were no differences in individual variation of AChE sensitivity between females and males in both strains, data on females and males were pooled.

In the S strain, AChE sensitivity ratios were 0 in all individuals tested (n = 100). In the N strain, AChE sensitivity ratios centered on 0.5, ranging from 0.4 to 0.7. The ratios in the Nakagawa and Ōzu strains seemed to be divided into three groups: 0–0.05, 0.2–0.35 and 0.4–0.7. No individuals with much less sensitive AChE, whose ratio was more than 0.7, were detected in the Nakagawa and Ōzu strains collected in 1970 and 1975.

In the Nakagawa and Ōzu strains collected in 1979, however, individuals with much less sensitive AChE, whose ratio was more than 0.7, were detected with high frequency and there were no individuals with highly sensitive AChE, whose ratio was less than 0.4.

The changes in pattern of AChE sensitivity in the Nakagawa insects indicate that a decrease in AChE sensitivity had occurred stepwise, i.e., a moderate decrease occurred, followed by a steeper decrease.

In contrast to the Nakagawa insects, the Kumamoto and Saga strains were clearly divided into three groups, i.e., 37.7 and 52% of the individuals of the respective strains had highly sensitive AChE (ratio = 0), 52.2 and 42% had moderately less sensitive AChE (ratio of 0.35 to 0.75) and 10.1 and 6% had much less sensitive AChE (ratio of 0.8 to 0.95).

If these three groups correspond to homozygotes for a wild AChE, heterozygotes and homozygotes for a mutant AChE, respectively, the expected ratios of heterozygotes and homozygotes for a mutant AChE could be calculated from the observed frequency of homozygotes for a wild AChE by the Hardy-Weinberg equation. The expected frequencies of the two phenotypes were calculated to be 47.4 and 14.9% in the Kumamoto strain and 40.2 and 7.8% in the Saga strain, whereas observed frequencies were 52.2 and 10.1% in the Kumamoto strain and 42 and 6% in the Saga strain. No significant deviations were observed at a 5% level of probability of the observed from the expected ratios in either strain.

Study on the inheritance of reduced AChE sensitivity in this insect is underway.

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REFERENCES


