Effects through Successive Selection with Fenvalerate on Malathion-Resistant Strains of the Rice Brown Planthopper and the Small Brown Planthopper*

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Strains of the rice brown planthopper and small brown planthopper with 360- and 370-fold resistance to malathion were respectively and successively selected with fenvalerate to trace the changes of LD50 values of malathion and fenvalerate. The LD50 value of malathion markedly decreased during the first five to six generations of selection in both planthoppers, but little changed by further selection (equilibrated state). In both planthoppers, the LD50 value of malathion in the final (19th) generation of selection was about 1/4 that of the parent strains. In the fenvalerate-selected strain of the rice brown planthopper, the LD50 values of fenitrothion, diazinon and phenthoate decreased to 1/2 to 1/5 during the selection of the first seven generations. In the fenvalerate-selected strain of the small brown planthopper, on the other hand, decreases in the LD50 value of these insecticides were minimal. The LD50 value of fenvalerate increased in the fenvalerate-selected strains of the two planthoppers. This increase, however, did not proceed in proportion to the change in the level of resistance to malathion; the LD50 value did not markedly increase before the level of resistance to malathion reached an equilibrium. In the final (19th) generation of selection, the LD50 value of fenvalerate was about 11-times as high as those in the corresponding parent strains in the rice brown planthopper, and about 5-times in the small brown planthopper.

INTRODUCTION

The insecticidal activity of fenvalerate was found to be more prominent in the resistant strains than in the susceptible strains of the green rice leafhopper, *Nephotettix cincticeps* Uhler, the rice brown planthopper, *Nilaparvata lugens* Stål and the small brown planthopper, *Laodelphax striatellus* Fallén. In these insect pests, a negative correlation relationship was detected between the malathion resistance level of the insects and the insecticidal activity of fenvalerate.

Development of insecticide resistance in the green rice leafhopper has recently become an important problem in western Japan, and planthoppers have been showing a similar tendency. As one countermeasure for insecticide resistance, compounds which were effective against resistant strains, to which the insects may develop a negatively correlated cross-resistance must be developed. A valuable clue for the development of such compounds will be given through the examination of the insecticidal mechanism of fenvalerate, which is more effective against resistant strains than against susceptible strains.

In the present study using the rice brown planthopper and the small brown planthopper, the authors followed the relation between the malathion resistance level in insects and the insecticidal activity of fenvalerate in the course of successive selection of the malathion-
resistant strains with fenvalerate.

MATERIALS AND METHODS

Susceptible (LE) and malathion-resistant (Rm) strains of the rice brown planthopper and the small brown planthopper were used. The insecticides used in this experiment were malathion (purity, 95.5%), fenitrothion (97.3%), diazinon (95.0%), phenthoate (98.2%) and fenvalerate (94.8%).

The Rm strain of the rice brown planthopper and the small brown planthopper was selected with fenvalerate as described by Okuma and Ozaki. Fenvalerate was diluted with acetone to an appropriate concentration. A constant volume of this solution was dropped into a glass vial (1.1 cm in diameter and 10.4 cm in length) by a micrometer syringe, and another 50 μl of acetone was added to each vial in order to spread the insecticide uniformly on the inner surface of the vial. After acetone was evaporated completely, about 30 fourth- to fifth-instar larvae were placed into each vial, and were allowed to contact with the thin film of insecticide at 25±1°C. Mortality was checked at intervals. When it reached about 70%, the insects were transferred into a glass container in which rice seedlings were placed. Final mortality was 70 to 75%. The survivors were reared on the rice seedlings at 25±1°C under 16-hour illumination per day. Selection was conducted in each generation. During the selection, the final mortality of each generation was maintained at 70 to 75% by regulating both dose and duration of exposure to the insecticides. For both planthoppers, selection started with about 5000 individuals of the Rm strain. Successive selection was performed using about 2000 individuals per generation.

In the fenvalerate-selected strains, the lethal doses of fenvalerate and malathion were determined with appropriate generations. At the 7th generation, the lethal doses of fenitrothion, diazinon and phenthoate were also determined. Insecticides were diluted to appropriate concentrations with acetone (for rice brown planthopper) or with methanol (for small brown planthopper whose body size is much smaller), acetone or methanol solution was applied to the abdomen of female adults 3 to 5 days after emergence. The volume applied was 0.25 μl for the rice brown planthopper and 0.036 μl for the small brown planthopper. Treated insects were given rice seedlings and placed in a room maintained at 25±1°C. After 24 hr, the mortality was recorded.

RESULTS AND DISCUSSION

LD₅₀ values of malathion and fenvalerate in the susceptible (LE) and Rm strains of the rice brown planthopper and the small brown planthopper were shown in Table 1.

The Rm strains of the rice brown planthopper and the small brown planthopper used in this selection experiment showed about 360- and 370-fold resistance to malathion, respectively, while their susceptibility to fenvalerate was 5.7 and 8.7 times, as large as those of the corresponding LE strains.

**Table 1 LD₅₀ values of malathion and fenvalerate against the susceptible (LE) and malathion-resistant (Rm) strains of the rice brown planthopper and the small brown planthopper.**

<table>
<thead>
<tr>
<th>Insect</th>
<th>Strain</th>
<th>Malathion (μg/g)</th>
<th>Fenvalerate (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice brown planthopper</td>
<td>LE</td>
<td>4.4</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Rm</td>
<td>1588</td>
<td>3.5</td>
</tr>
<tr>
<td>Small brown planthopper</td>
<td>LE</td>
<td>1.7</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Rm</td>
<td>635</td>
<td>0.14</td>
</tr>
</tbody>
</table>

**Fig. 1** Change in susceptibility to malathion (solid circle) and fenvalerate (hollow circle) in the malathion-resistant (Rm) strain of the rice brown planthopper during selection with fenvalerate.

P: parent strain.
Figures 1 and 2 show changes in malathion and fenvalerate susceptibilities of the fenvalerate-selected strains of both planthoppers. The LD$_{50}$ value of malathion in the fenvalerate-selected strain of the rice brown planthopper decreased to 1/2 in the 5th generation and to 1/3 in the 6th generation compared with that in the parent strain. This value scarcely changed from the 5th to 11th generations during selection, but began to decrease further in the 15th generation, and reached about 1/4 in the final (19th) generation of selection.

In the fenvalerate-selected strain of the small brown planthopper, the LD$_{50}$ value of malathion decreased in the 5th generation to 17 of that in the parent strain, followed by a slight increase in the 6th to 7th generations. There was little change in value after that. The degree of decrease of the LD$_{50}$ values of malathion attained in the 19th (final) generation of selection was comparable to that attained in the fenvalerate-selection of the rice brown planthopper.

Other experiments confirmed that the level of resistance changed little for five or more generations when Rm strains of the rice brown planthopper and the small brown planthopper having the same malathion resistance level were successively reared under no insecticidal pressure. The reason for the decrease in the resistance level in the present study should thus be sought in the selection with fenvalerate.

The Rm and fenitrothion-resistant (Rf) strains of the rice brown planthopper and the small brown planthopper show cross-resistance to various organophosphates. In both planthoppers, the effect of successive selection with fenvalerate on the susceptibility to fenitrothion, diazinon and phenthoate was studied, and results are shown in Table 2.

According to the result, the LD$_{50}$ values of fenitrothion, diazinon and phenthoate in the rice brown planthopper decreased to 1/2 to 1/5 of those in the parent strain after selection for seven generations. In contrast, in the small brown planthopper, the decrease of LD$_{50}$ value in the course of fenvalerate selection was much slower. The reason for this difference is unknown, but it may have some correlation with the relatively low degree of cross-resistance to these insecticides in the Rm strain of the small brown planthopper. These results may suggest the following hypothesis: Some of the resistant factors present in the Rm strains of the two planthoppers are involved in their elevated susceptibility to fenvalerate. These factors are considered to be non-specific to the mechanism for producing the resistance to malathion but common to the resistance-producing mechanism for all organophosphorus insecticides.

In the fenvalerate-selected strain of the rice brown planthopper, the LD$_{50}$ value of fenvalerate increased with repetition of the selection. The slope of the increase was relatively gentle.
up to the 9th generation, and became steeper after that. This value was 8-times higher than the parent strain in the 11th generation of selection, and about 11-times higher in the final (19th) generation. The LD$_{50}$ value of fenvalerate in the fenvalerate-selected small brown planthopper increased only slightly up to the 5th generation, followed by a rapid increase to about 6-times in the 10th generation as compared to that in the parent strain. After the 10th generation, no increase occurred. The pattern of these changes accompanied by the selection differed between the two planthoppers. The cause may reside in the difference in the resistance mechanism between the Rm strains involved.

In the fenvalerate-selected strains of both planthoppers, the LD$_{50}$ value of fenvalerate increased with generations. This increase, however, did not proceed in inverse proportion to the decrease in the level of malathion resistance. In the rice brown planthopper, the LD$_{50}$ value started to increase markedly after the malathion resistance level decreased to its lowest level, and in the small brown planthopper the decrease was remarkable after selection for five generations when malathion resistance reached equilibrium. This finding is not compatible with the previous finding in that there was a negative correlation between the level of malathion resistance and the LD$_{50}$ value of fenvalerate in these two species of planthoppers.$^{33}$

Figure 3 illustrates the relationship between LD$_{50}$ values of malathion and fenvalerate determined in certain generations of the fenvalerate-selected strain of the rice brown planthopper. In the same panel, LD$_{50}$ values of fenvalerate determined in populations with different malathion resistance levels (from Ozaki and Kassai$^{23}$) are plotted for comparison. In the fenvalerate-selected strain of the rice brown planthopper, it can be said that a marked increase in the LD$_{50}$ value of fenvalerate occurred in individuals corresponding to those which, by nature, had a high level of malathion resistance and thus relatively small variation in the fenvalerate LD$_{50}$ value was shown. As mentioned above, the fact was also found that the LD$_{50}$ value of fenvalerate did not show a sharp increase before the level of malathion resistance reached equilibrium.

These findings seem to suggest that the LD$_{50}$ value of fenvalerate in the Rm strain of the rice brown planthopper increased in the course of successive selection with this insecticide, independently from the change in the level of malathion resistance. In light of the result illustrated in Fig. 2, a similar phenomenon is suspected to have occurred in the fenvalerate-selected strain of the small brown planthopper.

When the Rm strains of the planthoppers were selected successively with fenvalerate, the level of resistance to malathion was thus decreased to some extent, but complete recovery of sensitivity to malathion was not attained. Initially, the authors expected that sensitivity of insect pests to an insecticide to which they had been resistant would easily recover when using a substitute compound to which the strains showed negatively correlated cross-resistance. The results of the present selection experiments, however, did not support the expectation. In the laboratory experiment, the selection factor (corresponding to fenvalerate in the present experiments) may act in a simpler condition than in the field experiment, accelerating the development of resistance. Such an accelerated development
of fenvalerate resistance seems to be a cause of the above mentioned incomplete recovery of sensitivity in the resistant strains. Additional laboratory experiments are needed by using other insecticides to which a resistant strain shows a negatively correlated cross-resistance to see if results are similar to those obtained here. The outcome of the present study suggests that a certain complicated mechanism is involved in the development of insecticide resistance in insect pests, and that the resistance is unexpectedly difficult to be encountered. This complexity in the relation between development of the resistance and the use of insecticide must be kept in mind in developing and using an insecticide which is expected to be effective on resistant strains of insect pests.

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REFERENCES