Selection for imidacloprid resistance in *Nilaparvata lugens*: cross-resistance patterns and possible mechanisms

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Abstract: A field population of brown planthoppers (*Nilaparvata lugens* Stål) was collected and selected for imidacloprid resistance in the laboratory. The resistance increased by 11.35 times in 25 generations and the resistance ratio reached 72.83 compared with a laboratory susceptible strain. The selected resistant strain showed obvious cross-resistance to all the acetylcholine receptor targeting insecticides tested (monosultap 1.44-fold, acetamiprid 1.61-fold, imidacloprid homologues JS599 2.46-fold and JS598 3.17-fold), but not to others. Further study demonstrated that TPP and DEM had no synergism on imidacloprid. However, PBO displayed significant synergism in some different strains, and the synergism increased with resistance (S strain 1.20, field population 1.43 and R strain 2.93). PBO synergism to cross-resistant insecticides was also found in the resistant strain (monosultap 1.25, acetamiprid 1.39, JS598 1.94 and JS599 2.02). We concluded that esterase and glutathione S-transferase play little role in imidacloprid detoxification. The increase of the P450-monooxygenases detoxification is an important mechanism for imidacloprid resistance and target resistance may also exist in this species.

Keywords: *Nilaparvata lugens*; resistance mechanism; cross-resistance; synergism; P450-monooxygenases; acetylcholine receptors

1 INTRODUCTION

The brown planthopper, *Nilaparvata lugens* Stål, is a major rice pest in many parts of Asia. Insecticides have been extensively used for its control and resistance to various insecticides has been reported in different countries and areas.1–3 In China, organophosphates and carbamates have commonly been used in the past, and in the 1980s an insect growth regulator, buprofezin, was thought likely to be a useful insecticide for controlling this pest.4,5 However, it is slow acting and has little effect on the adults and eggs.6 As an advance on these, imidacloprid was introduced in the early 1990s and, having high activity and long-lasting effect, it has become the primary insecticide for controlling *N lugens* in China.7

Imidacloprid, like other systemic insecticides, displays prolonged persistence which is likely to generate high selection pressure for resistance.8 Soil application and seed treatment lead to pests at all stages receiving prolonged exposure to this kind of insecticide.9 Resistance to imidacloprid has been reported in a range of species including silverleaf whitefly, western flower thrips, Colorado potato beetle, German cockroach and house fly,10–16 but, because of its characteristics, including a novel mode of action,17 imidacloprid resistance in field populations appears to develop slowly and the mechanism is not well understood.

In some species, the significant synergism of piperonyl butoxide (PBO) indicated that P450-detoxification could be an important biochemical mechanism for imidacloprid resistance.16,18 However, target-site resistance has not yet been found, even with *Myzus ssp*19 or *Bemisia tabaci* Genn,20 in which resistance ratios of greater than 100 have been reported.

In this paper an imidacloprid resistant strain of *N lugens* was selected in the laboratory. In order to reveal the resistance mechanism, cross-resistance to some other insecticides and the effects of three synergists were studied.

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2 MATERIALS AND METHODS

2.1 Insects

The susceptible strain (S) of *N. lugens* was a laboratory strain, obtained from Jiangsu Academy of Agricultural Science in April 2000, which had been collected before imidacloprid was introduced and had been reared in greenhouse for more than 10 years. The field population (F) was collected from hybrid paddy rice in Jiangsu, China in August 2002. The imidacloprid-resistant strain (R) was a laboratory strain selected from a field population (P) originally collected 2 years before from the same field as F. Insects were kept in field web cages in the growing season and indoors at 25 (±1) °C, humidity 70 ~ 80% and 16:8 h light:dark in winter (from October to April).

2.2 Insecticides and synergists

Imidacloprid (97%) was purchased from Red Sun Group Corporation (Nanjing, China). Malathion (99.9%), fenobucarb (98.4%) and fenvalerate (94.0%) were gifts from Professor Toru Nagata of Ibaraki University in Japan. Monosultap (92.4%) and acetamiprid (90%) were provided by the Jiangsu Academy of Agricultural Science. JS598 (94.6%) and JS599 (86.5%), two analogues of imidacloprid, were supplied by the Jiangsu Pesticide Research Institute. Triphenyl phosphate (TPP, reagent grade,) and diethyl meteate (DEM, reagent grade) were from Sigma.

2.3 Resistance selection

About 50 seedling shoots (30 days-old, 30 (±2) cm) were placed in a plastic box (20 × 15 × 6 cm), with all roots immersed in an imidacloprid solution (0.05~3.00 mg litre⁻¹, depending on the resistance level, and emulsified with Triton-X 100). About 100 2nd-instar larvae were inoculated and caged (50 × 38 × 38 cm nylon web) at 16:8 h light:dark and 28 (±1) °C for 4 days. The surviving insects were transferred to another caged rearing box with fresh seedlings free from insecticides. In each generation, about 600 insects survived from 2000 treated larvae and were usually obtained and bred in the next generation, from which some insects were used for resistance selection and some for bioassay to check changes in resistance.

2.4 Bioassay

The bioassay followed the micro-topical application technique reported by Nagata. Three- to five-day-old macropterous adult females were used as test animals in this study. Under carbon dioxide anaesthesia, a droplet (0.04 µl) of acetone solution of insecticide was applied topically to the prothorax notum with a hand microapplicator (Burkard Manufacturing Co Ltd, Rickmansworth, UK). Thirty insects were treated at each concentration and every treatment was repeated three times. Controls used acetone alone. The treated insects were reared on the seedlings cultured soil-less in the rearing box at 25 (±1) °C and 16:8 h light:dark. The results were checked after 24 h. LD₅₀ values were determined on the basis of standard probit analysis²¹,²² as adapted to a personal computer.²³ In the synergism analysis, 2 µg of synergist (TPP, PBO or DEM) in 0.04 µl of acetone was delivered on to the prothorax notum of each female adult 1 h before the insecticide application, as described by Wen and Scott.¹⁶ The synergistic ratio (SR) was calculated as

\[
SR = \frac{\text{LD}_{50} \text{ value of insecticide alone}}{\text{LD}_{50} \text{ value of insecticide after synergist}}
\]

3 RESULTS

3.1 Imidacloprid resistance selection

*Nilaparvata lugens* of a field population (P) were collected in 2000 and continuously selected with imidacloprid for 25 generations in the laboratory. The change in LD₅₀ for imidacloprid during selection is shown in Fig 1. This shows that, in the first seven generations, selection with imidacloprid led to little

![Figure 1: The dynamics of imidacloprid LD₅₀ against *Nilaparvata lugens* during resistance selection. P: parental population; Fₙ: generation selected.](image-url)
increase in LD\textsubscript{50} (from 0.82 to 1.20 ng per pest). The LD\textsubscript{50} then increased steadily until F\textsubscript{22} gave a value of 8.76 ng per pest, and subsequently stayed at this level. Thus the 25-generation selection was associated with an increase in LD\textsubscript{50} of 11.35 times, giving a resistant strain with a resistance ratio (RR) of 72.83 compared with S strain (LD\textsubscript{50}: 0.12 ng per pest).

### 3.2 Cross-resistance evaluation

With both the laboratory susceptible strain and field population as controls, the resistant strain was tested for cross-resistance to different insecticides, and the results are shown in Table 1. These indicated that the R strain showed resistance to all the insecticides tested when an S strain was used as standard. Because the R strain was selected from a field population, the original field population could have a multiple resistance background. Otherwise, the laboratory strain, as usual, could also lose its tolerance to insecticides. Therefore, it was not acceptable to use these data as proof of cross-resistance. When the field population collected at the same site and with similar resistance background was used as a control, no obvious resistance was found with the non-acetylcholine receptor targeting insecticides malathion, fenobucarb and fenvalerate. However, the high RR ratio of monosultap (1.4-fold), acetamiprid (1.6-fold), JS599 (2.5-fold) and JS598 (3.2-fold) indicated that, during the selection, the R strain had developed some resistance to all the acetylcholine receptor targeting insecticides tested. The cross-resistance was in the order JS598 > JS599 > acetamiprid > monosultap. It seemed that the more similar the chemical structure was to imidacloprid, the higher the synergism.

### 3.3 Synergistic effects evaluation

The synergistic effects of PBO, TPP and DEM on imidacloprid were tested with the S and R strains and the field population of *N. lugens*. The results are shown in Table 2.

The results in Table 2 indicated no significant synergistic effect in any of the treatments with TPP and DEM, but moderate synergism was found with PBO. In the S strain and the field population, PBO had only a slight synergistic effect, but it significantly synergized imidacloprid against the R strain.

The synergistic effect of PBO on some other chemicals targeting the acetylcholine receptor was also tested with the R strain and the field population. The results were shown in Table 3. All treatments showed some synergism, apart from monosultap against the F population. With all the chemicals tested, synergism was higher with the R strain than with the F population, and was in the order JS599 ≈ JS598 > acetamiprid > monosultap. This order was not the same as for cross-resistance, but it seemed that there was some positive relationship, otherwise PBO could

Table 1. Toxicity of imidacloprid and other insecticides to S, R strains and a field population of *Nilaparvata lugens*

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Strains</th>
<th>Slope</th>
<th>LD\textsubscript{50} (ng per pest)</th>
<th>Resistance ratio (RR)\textsuperscript{a}</th>
<th>RR ratio\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>S</td>
<td>3.2517</td>
<td>0.120 (±0.011)</td>
<td>6.4</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.3303</td>
<td>0.770 (±0.084)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2.5172</td>
<td>8.740 (±0.896)</td>
<td>72.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Malathion</td>
<td>S</td>
<td>2.9732</td>
<td>19.74 (±0.95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.2852</td>
<td>280.2 (±11.5)</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2.3106</td>
<td>299.5 (±12.5)</td>
<td>15.2</td>
<td></td>
</tr>
<tr>
<td>Fenobucarb</td>
<td>S</td>
<td>3.1007</td>
<td>5.120 (±0.227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.5624</td>
<td>65.82 (±3.14)</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2.3613</td>
<td>71.63 (±3.30)</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>S</td>
<td>3.1017</td>
<td>6.728 (±0.194)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.3512</td>
<td>69.55 (±2.58)</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2.1853</td>
<td>84.51 (±18.50)</td>
<td>12.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Monosultap</td>
<td>S</td>
<td>2.4942</td>
<td>2143 (±52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.1703</td>
<td>7520 (±213)</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1.8006</td>
<td>10.790 (±1410)</td>
<td>5.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>S</td>
<td>3.1706</td>
<td>0.411 (±0.032)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.5564</td>
<td>2.069 (±0.140)</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1.9408</td>
<td>3.333 (±0.503)</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>JS598</td>
<td>S</td>
<td>3.0088</td>
<td>0.782 (±0.101)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.5772</td>
<td>2.429 (±0.322)</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1.9748</td>
<td>7.707 (±1.032)</td>
<td>9.9</td>
<td>3.2</td>
</tr>
<tr>
<td>JS599</td>
<td>S</td>
<td>2.5133</td>
<td>2.277 (±0.171)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.3600</td>
<td>9.279 (±0.518)</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1.7604</td>
<td>22.85 (±1.76)</td>
<td>10.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Resistance ratio (RR): LD\textsubscript{50} value of resistant strain or field population/LD\textsubscript{50} value of susceptible strain.

\textsuperscript{b} RR ratio: RR of resistant strain/RR of field population.
not depress LD50 values in the R strain to the same level of those in the F population.

4 DISCUSSION

The data reveal that continuous selection with imidacloprid for 25 generations can only increase resistance in *N. lugens* 11.35-fold. Even if the difference between the F population and the laboratory S strain was not taken into consideration, and using the LD50 on the S strain as standard, the total resistance ratio was only 72.83. In the field, imidacloprid is always used at the full recommended rate. Although uneven spraying and the decay with time may have some effect, the selection pressure for resistance is much less than the indoor experiment. Otherwise, the LD50 (8.74 ng per pest) for the resistant strain indicated that imidacloprid was still as effective as other conveniently used insecticides. This means that *N. lugens* can develop some resistance to imidacloprid under long-lasting selection pressure. However, the rate of selection and the resistance level is very low in the field. Considering that few applications of imidacloprid are made in a growing season, it would appear difficult for a field population to develop high resistance and cause control failure over a reasonable period.

The synergism study demonstrated that TPP and DEM had no effect on imidacloprid in any of the strains tested. This indicated that, in *N. lugens*, esterase and glutathione S-transferases play little role in imidacloprid detoxification. However, PBO, the inhibitor of P450-monooxygenases, displayed synergism, especially in the R strain, where the synergism ratio was as high as 2.93. These results indicate that P450-monooxygenases are key factors in imidacloprid detoxification and resistance development. This agrees with the reports on green peach aphid (*Myzus persicae* (Sulz)), in which P450-monooxygenase is also thought to be one of the important mechanisms for imidacloprid resistance.

Due to the broad substrate spectra, detoxification by P450 monooxygenases may potentially affect several classes of compound and thereby can confer cross-resistance to different insecticides. In this paper, our results showed that PBO had a synergistic effect on all the cross-resistant insecticides tested. There was some positive quantitative relationship between the cross-resistance and the synergism. It seemed that the cross-resistance might be resulting from a P450-monooxygenase detoxification mechanism. However, PBO could not depress LD50 values in the R strain to the same level as in the S strain or even in the field population. The synergism of PBO to insecticides did not follow exactly their cross-resistance. These results indicate the existence of mechanisms additional to the effects of detoxification enzymes.

Slow-down of penetration of the integument could be another mechanism, but the selection with rice seedlings with only roots immersed in imidacloprid solution would not selected for this factor. Otherwise, the selected resistant strain showed obvious cross-resistance to only the acetylcholine receptor targeting insecticides. The existence of target-site resistance could, therefore, be concluded, although target-site resistance for imidacloprid has not yet been found in any other pest species.

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REFERENCES