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Fitness costs of laboratory-selected imidacloprid resistance in the brown planthopper, *Nilaparvata lugens* Stål

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Abstract: Imidacloprid has been used as a key insecticide to control the brown planthopper, *Nilaparvata lugens* Stål, for several years, but no obvious resistance has been identified in field populations as yet. To evaluate the risk, a field population was collected and selected with imidacloprid in the laboratory. After 37-generation selection a strain with 250-fold resistance had been successfully achieved. Fitness analysis by constructing life tables demonstrated that resistant hoppers had obvious disadvantages in their reproduction. The fitness of highly resistant hoppers had decreased dramatically (0.169 and 0.104) to only one-fifth to one-tenth of that of the susceptible strain. Hence it was concluded that the brown planthopper had the potential to develop high resistance to imidacloprid but that the lower fitness of resistant hoppers could result in a quick recovery of sensitivity when the population did not come into contact with imidacloprid. This means that a reasonable resistance management programme with less imidacloprid use may efficiently delay or even stop resistance development.

Keywords: *Nilaparvata lugens*; imidacloprid; fitness costs; insecticide resistance

1 INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is a major rice pest in many parts of Asia. Insecticides have been extensively used for control of this pest, and resistance to a number of them has been reported in different countries and areas.[1,2] Although this resistance develops slowly and is not very high,[3–5] it is enough to make the most convenient insecticides useless, because they are initially not very effective against this pest. Thus, when the highly efficient insecticide imidacloprid was introduced, it became almost the only insecticide used for control of this pest, and at present there seem to be no other available insecticides that can act as substitutes.

Imidacloprid had been used to control this pest for more than 10 years in China and is also used very widely in almost all BPH areas, but resistance in field populations is still very low.[5,6] However, high imidacloprid resistance has been reported in a range of other species, including field populations of silverleaf whitefly and Colorado potato beetle and laboratory-selected strains of potato peach aphid.[7–10] Therefore it is very important to evaluate the risk of resistance developing in BPH and to define reasonable resistance practices.

In the present work we have tried to evaluate the imidacloprid resistance risk in BPH by resistance selection and fitness analysis.

2 MATERIALS AND METHODS

2.1 Insects and insecticide

The susceptible strain (S) of BPH 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 the greenhouse for more than 10 years. The parental population (P) was collected from a field of hybrid paddy rice in Jiangpu (Jiangsu, China) in August 2000 and formed the original population for resistance selection. Selected hoppers T*n* were the populations selected for *n* generations.

All strains were reared on caged field rice from April to October and in laboratory cages with rice at 16/8 h light/dark and 28 ± 1 °C during winter.

Imidacloprid (97%) was purchased from Red Sun Group Corporation (Nanjing, China).

2.2 Resistance selection

About 50 seedling shoots (30 days old, 30 ± 2 cm high) were placed in a plastic box (20 cm × 15 cm ×

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6 cm) with all roots immersed in water containing imidacloprid emulsified with Triton X 100. About 100 larvae of the 2nd instar were inoculated and caged (50 cm × 38 cm × 38 cm nylon web) on the rice plants at 16/8 h light/dark and 28 ± 1 °C for 4 days. The surviving insects were transferred to another caged recovery box with fresh seedlings free from insecticide. The number of insects subjected to selection in each generation varied from 4000 to 8000, depending on the size of the population surviving from the previous selection.

The doses of imidacloprid used for each generation in the selection (0.05–8.00 mg·kg^{-1}) were determined by the LD_{50} of their parent generation, and the mortality was kept at about 40–60%.

2.3 Bioassay

The bioassay followed the microtopical application technique reported by Nagata.[1] Three- to 5-day-old macropterous adult females were used as test animals in this study. Imidacloprid was diluted to a series of concentrations with acetone. Under carbon dioxide anaesthesia a droplet (0.04 µl) of imidacloprid solution was applied topically to the prothorax notum of test hoppers with a hand microapplicator (Burkard Manufacturing Co. Ltd, Rickmansworth, UK). Thirty insects were treated at each concentration, and every treatment was repeated three times. The controls used acetone instead of imidacloprid solution. The treated insects were reared on seedlings cultured soilless in the rearing box at 25 ± 1 °C and 16/8 h light/dark. The results were checked after 24 h. LD_{50} values were determined on the basis of standard probit analysis[11,12] as adapted to personal computer use.[13]

2.4 Life table construction

One hundred neonates were collected randomly from the susceptible strain (S) and the offspring of the survivors from two selected generations (T25 and T35) as the founders of the experimental population and reared for a generation at 25 ± 1 °C and 16/8 h light/dark. When they had developed into 3rd and 5th instars, the hoppers were transferred to fresh rearing cages and assessed for survival rate from neonate to 3rd instar (Sr1) and from 3rd to 5th instar (Sr2). The emerged males and females were thereafter collected every day and coupled into families (one female plus one male, about ten families for each replication, in total about 30 for each strain) which were reared in glass tubes separately. At the same time the emergence rate (Er) and female ratio (Fr) were recorded. When the neonates of the new generation appeared, the families were checked every 2 days and the neonates were counted and removed until the female died. The food rice shoots were then checked thoroughly and the numbers of unhatched eggs were recorded. The females which had not produced any neonates were considered to have failed in copulation, and the copulation rate (Cr) was accounted accordingly. The fecundity (Fd) was recorded as the average number of eggs produced by copulated females, and the hatchability (Ha) was recorded as (all neonates)/(all neonates plus all unhatched eggs). The experiments were carried out with three replications. The population trend index (I) and relative fitness were calculated as follows:

$$N_t = N_0 \times \mathrm{Sr1} \times \mathrm{Sr2} \times \mathrm{Er} \times \mathrm{Fr} \times \mathrm{Cr} \times \mathrm{Fd} \times \mathrm{Ha}$$

$$I = N_t / N_0$$

$$\text{relative fitness} = I_{\mathrm{T}n} / I_{\mathrm{S}}$$

where N_0 is the number of individuals in the initial population, N_t is the number of individuals in the population of the next generation, $I_{\mathrm{T}n}$ is the increase trend index of the resistant population and I_{S} is the increase trend index of the susceptible population.

3 RESULTS

3.1 Imidacloprid resistance selection

Based on the root-dipping method featuring the systemic uptake of imidacloprid through roots, imidacloprid resistance in BPH was continuously selected for 37 generations. The results are shown in Fig. 1.

The results in Fig. 1 show that the development of imidacloprid resistance in BPH was uneven over time. In the first nine generations, selection resulted in little increase in resistance ratio (from 6.4 to 15.8). After T9 the resistance increased steadily until T22 with a resistance ratio of 73.0-fold. Then, after stagnating for three generations (T23–T25), it increased quickly again after T26. The resistance ratio of T33 reached 247.1-fold, after which the resistance ratio stayed at 250-fold with little variation.

3.2 Influence of imidacloprid resistance on the fitness of *Nilaparvata lugens*

With the laboratory susceptible strain as control, the relative fitnesses of two representative generations (T25 and T35) were analysed by constructing their life tables. The results are presented in Table 1.

The results in Table 1 show that in the laboratory the susceptible population could increase 147 times in one generation, but the resistant populations T25 and T35 only increased 25 and 15 times respectively. The fitness of the selected resistant hoppers decreased dramatically (0.169 and 0.104) to only one-fifth to one-tenth of that of the susceptible strain. Their larval survival rate, adult emergence rate, copulation rate, fecundity and hatchability were all significantly lower. Both susceptible and resistant strains used in this experiment were laboratory strains originally collected in the same migration area, so it can be concluded that imidacloprid resistance in this pest results in high fitness costs.

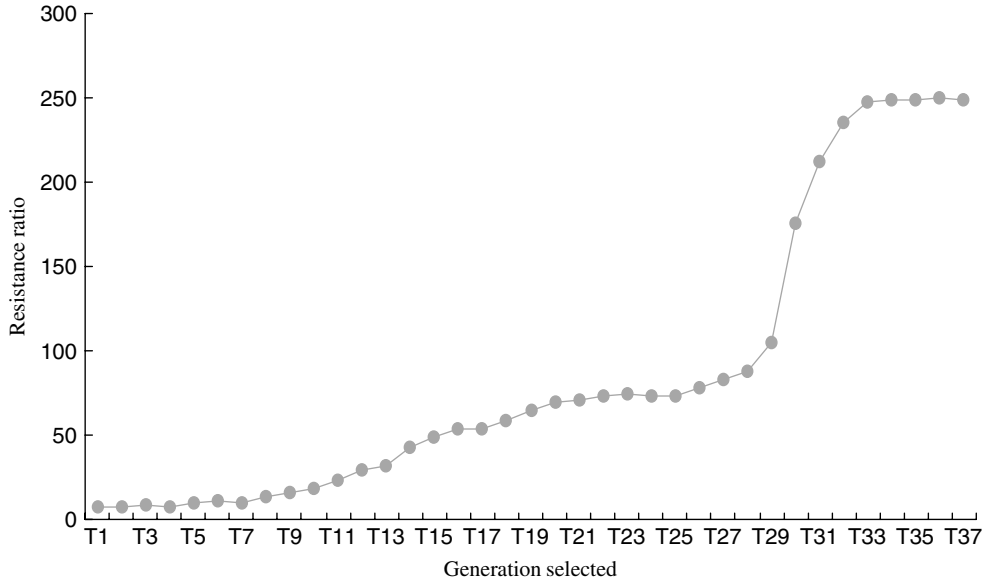

Figure 1. The dynamics of imidacloprid resistance in *Nilaparvata lugens* during resistance selection. T*n* = generation selected.

Table 1. Life tables of the susceptible (S), T25 and T35 strains of *Nilaparvata lugens*[a]

Parameter	S	T25	T35
Neonate number	100	100	100
Survival rate from neonate to 3rd instar (%)	91.6 (±1.7) a	70.2 (±5.4) b	71.1 (±11.9) b
Survival rate from 3rd to 5th instar (%)	93.7 (±1.1) a	86.6 (±9.4) ab	78.0 (±9.7) b
Emergence rate (%)	92.1 (±1.9) a	73.6 (±8.6) b	69.6 (±14.2) b
Female ratio (%)	49.2 (±1.3) a	49.1 (±3.1) a	48.8 (±3.1) a
Copulation rate (%)	87.5 (±4.1) a	70.4 (±9.7) b	64.9 (±8.4) b
Fecundity (eggs per female)	491.3 (±65.1) a	284.9 (±51.4) b	217.9 (±38.2) c
Hatchability (%)	88.2 (±4.6) a	56.4 (±9.2) b	57.3 (±11.6) b
N, predicted number of offspring	14 746.3	2485.2	1526.3
I, population trend index	147.5	24.9	15.3
Relative fitness	1	0.169	0.104

[a] Values in the same row with the same letter do not differ significantly at $\alpha = 0.05$.

4 DISCUSSION

Imidacloprid acts as an agonist of insect nicotinic acetylcholine receptors (nAChRs), has high effects against many insect pests and is safe to non-target animals. Thus it has been extensively used in many countries and become the primary insecticide for the control of piercing sucking insects on rice, cotton, wheat, vegetables and fruit trees.

Nowadays, various species have been reported as developing resistance to imidacloprid, including western flower thrips (resistance ratio 14-fold), Colorado potato beetle (110.8-fold), tobacco whitefly (116-fold), peach aphid (7-fold), tobacco aphid (10-fold) and small brown planthopper (18-fold).[8,9,14,15] In Colorado potato beetle and tobacco whitefly, even complete failure in control has been reported.[9,16] However, there are no reports of imidacloprid resistance in field populations of BPH, even after being used for more than 10 years.

In this study, laboratory selection demonstrated that BPH could develop high resistance to imidacloprid. However, imidacloprid resistance loaded BPH with higher fitness costs. The fitness of resistant hoppers was only one-fifth to one-tenth of that of susceptible ones, so the competition from susceptible hoppers in the field could surely result in a quick recovery of population sensitivity when the use of imidacloprid was suspended. On the other hand, long-distance migration could efficiently dilute the resistance developed in control areas. In conclusion, BPH has the potential to develop high resistance to imidacloprid, but migration and the low fitness of resistant hoppers can delay resistance development in field populations.

Previous work had demonstrated that imidacloprid-resistant BPH showed no cross-resistance to other non-AChR-targeting insecticides and that resistance resulted mainly from increased microsomal P450 monooxygenase (MFO) activity and insensitive target.[17] Thus, if imidacloprid is used rationally (rotational use with other kinds of insecticides and mixed use with MFO inhibitors) and a preventive strategy is carried out for resistance management, resistance development in *N. lugens* may be delayed or even stopped.

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