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# Susceptibility to Insecticides and Ecological Fitness in Resistant Rice Varieties of Field *Nilaparvata lugens* Stål Population Free from Insecticides in Laboratory

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**Abstract:** A population of rice brown planthopper (BPH) *Nilaparvata lugens* collected from a paddy field in Hangzhou was successively reared on susceptible rice Taichung Native 1 (TN1) in a laboratory free from insecticides for more than 14 generations. The changes in susceptibility to insecticides and ecological fitness on different resistant rice varieties were monitored in each generation. The resistance ratio to imidacloprid sharply declined with the succession of rearing generations without insecticides from 359.94-fold at $F_1$ to 6.50-fold at $F_{14}$ compared with the susceptible strain, and the resistance ratio to chlorpyrifos was from 9.90-fold at $F_1$ to 5.94-fold at $F_{14}$. Nymphal duration and weights of newly hatched female adults were significantly affected by rice variety, generation and their interactions, but nymphal survival was significantly affected by the generation only. The ratio of brachypterous adults in males was affected by the generation and generation × variety interaction, whereas no difference was found in females. Nymphal duration extended with increasing generations, and the female nymphal duration was shorter in the susceptible variety TN1 than those in the resistant varieties IR26 and IR36. In addition, the female adult weight in TN1 was higher than those in IR26 and IR36. These results indicated that the resistance of field BPH population to insecticides was reversed after several generations of no-exposure to insecticides, and the ecological fitness in TN1 was higher than those in IR26 and IR36. These findings suggested the rational and reduced use of insecticides in combination with the manipulation of resistant rice varieties would be effective for BPH management.
**Key words:** *Nilaparvata lugens*; insecticide-free; ecological fitness; insecticidal resistance; rice variety

The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is one of the most important long-distance migratory rice insect pests in paddy fields in China and other rice-growing countries of Asia (Cheng et al, 2008; Catindig et al, 2009; Liu et al, 2012). It showed increasing incidence and infestation area following the implementation of the green revolution, characterized by the adoption of high yielding varieties and application of chemical fertilizers and insecticides in the 1960s to 1970s (Xia, 2008; Heong, 2009; Peng et al, 2009). Resistant varieties and chemical insecticides are widely used to control BPH. Long-term misuse of chemical insecticides has resulted in insecticidal resistance and resurgence, as well as environmental pollution and other ecological problems (Jiang et al, 2005; Wang et al, 2013). By contrast, application of resistant varieties, an economically effective strategy to suppress BPH populations (Karban and Chen, 2007), has ensured the security of rice production, while substantially reduce the use of insecticides (Panda and Khush, 1995; Karban and Chen, 2007; Du et al, 2009; Heong, 2009; Norton et al, 2010). Therefore, wide scale use of resistant varieties plays a crucial role in the sustainable management of BPH (Medrano and Heinrichs, 1995; Lu et al, 2002; Singh and Dhaliwal, 2004; Chen et al, 2009).

Ecological fitness decreases in most insect pests with high resistance to insecticides, but it increases or remains unchanged in a few study cases (Yu et al, 1997; Ren et al, 2001; Arnaud and Haubruge, 2002; Berrticat et al, 2002; Boivin et al, 2003; Liu et al, 2008). These phenomena result from the interaction of

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insect pests and the environment. The fitness of imidacloprid-resistant BPH populations feeding on resistant rice varieties is significantly higher than that of imidacloprid-susceptible populations, whereas the fitness of resistant populations of BPH decreases with the succeeding generations of BPH (Chen et al, 2011). The decrease in the fitness of resistant populations may result in the instability of the resistance development of BPH to insecticides (Liu et al, 2001). Imidacloprid and chlorpyrifos belong to neonicotinoids and acetylcholinesterase inhibiting insecticides, respectively, and have high efficacies for BPH. Imidacloprid was a key insecticide in suppressing the BPH population before 2005 in China. Unfortunately, the field BPH population has developed high resistance to many insecticides because of the reliance on chemicals to control it (Liu et al, 2005; Matsmura et al, 2009; Wen et al, 2010). The Ministry of Agriculture of China has urged farmers to stop using imidacloprid for BPH control because of the high resistance to imidacloprid (Zhang et al, 2011).

In this study, the BPH population collected from rice fields was successively reared on susceptible rice TN1 insecticide-free in the laboratory, and was used to evaluate its changes in the susceptibility to insecticides and the ecological fitness to different rice varieties after ceasing application of insecticides. We aimed to provide practical tips optimizing BPH management with reduced pesticide application.

## MATERIALS AND METHODS

### Insect and rice varieties

The field BPH population was collected from a paddy field in an experimental farm of Zhejiang Academy of Agriculture Sciences, Hangzhou, China in 2010. The population was successively reared on Taichung Native 1 (TN1, a susceptible rice variety to BPH) for generations without exposure to any insecticide in the laboratory, and labeled as $F_n$. The susceptible BPH strain, Pila population, has been reared on TN1 for many years without insecticides at the International Rice Research Institute (IRRI), the Philippines. The rice varieties TN1, IR26 (resistant to BPH) and IR36 (resistant to BPH) were provided by IRRI.

### Effect of successive rearing in the laboratory on BPH susceptibility to insecticides

Newly emerged (12 h) BPH female adults from $F_1$, $F_4$, $F_8$, $F_{10}$ and $F_{14}$ were subjected to the bioassays of imidacloprid and chlorpyrifos using micro-topical application technique. Technical insecticide was diluted with acetone, and five to six concentrations were prepared. The test insects were anesthetized by exposure to carbon dioxide gas for 15 s, and a droplet (0.04 μL) of insecticide solution was applied topically to the prothorax notum of the test hoppers using a hand microapplicator (Burkard Manufacturing Co., Ltd., Rickmansworth, UK). Thirty hoppers were treated at each concentration, and each treatment was repeated three times. Treated hoppers were reared routinely on TN1 at (26 ± 1) °C and 70% to 80% relative humidity (RH) under a photoperiod of 14 h light and 10 h dark. The number of deceased hoppers was recorded after 24 h.

### Effect of successive rearing in the laboratory on BPH ecological fitness in rice varieties

About 25 rice seeds were planted in a 240 mL plastic cup (height, 15 cm; diameter, 6 cm). Twenty-five newly hatched BPH nymphs were induced on 10-day-old rice seedlings. The plastic cups with BPH were covered to prevent BPH from escaping and kept at (26 ± 1) °C and 70% to 80% RH under a photoperiod of 14 h light and 10 h dark. The number of surviving nymphs were recorded every 24 h. Newly molted female adults were weighed, and wing patterns were identified. Each BPH population of $F_1$, $F_4$, $F_6$, $F_8$ and $F_{10}$ was tested on TN1, IR26 and IR36. Each rice variety was replicated five times.

### Data analysis

Bioassay data were subjected to probit regression analysis using POLO Plus software (LeOra software, CA, USA) with the natural response (control mortality) included as a model parameter. Ecological fitness data were shown as mean ± *SE*, and analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Multiple comparisons were analyzed using Tukey's test, and the percentage data were transformed by arcsin square before multiple comparison.

## RESULTS

### Changes in insecticidal susceptibility of BPH populations without exposure to insecticide

The bioassay results showed that the susceptibility of BPH to imidacloprid and chlorpyrifos increased with

**Table 1. Dynamics of insecticide susceptibility of brown planthopper population under free of insecticides for generations in the laboratory.**

| Insecticide | Generation | Slope ± *SE* | $LD_{50}$ (CL95%) (μg/g) | Ratio |
|---|---|---|---|---|
| Imidacloprid | $F_1$ | 0.995 ± 0.095 | 11.158 (6.062–19.560) | 359.94 |
| | $F_4$ | 0.960 ± 0.101 | 7.483 (4.597–11.688) | 241.40 |
| | $F_8$ | 0.989 ± 0.147 | 0.710 (0.376–1.253) | 22.92 |
| | $F_{10}$ | 0.693 ± 0.100 | 0.203 (0.093–0.040) | 6.54 |
| | $F_{14}$ | 0.725 ± 0.105 | 0.202 (0.095–0.039) | 6.50 |
| | Susceptible strain | 1.817 ± 0.285 | 0.031 (0.012–0.067) | 1.00 |
| Chlorpyrifos | $F_1$ | 1.921 ± 0.253 | 13.160 (8.103–19.435) | 9.90 |
| | $F_4$ | 2.948 ± 0.353 | 12.202 (10.126–14.641) | 9.18 |
| | $F_8$ | 2.244 ± 0.315 | 11.344 (6.719–17.160) | 8.54 |
| | $F_{10}$ | 3.244 ± 0.390 | 8.097 (6.785–9.670) | 6.09 |
| | $F_{14}$ | 2.710 ± 0.320 | 7.897 (6.467–9.614) | 5.94 |
| | Susceptible strain | 1.325 ± 0.166 | 1.329 (0.881–1.905) | 1.00 |

the succession of generations without insecticide exposure in the laboratory (Table 1). The increased rate of susceptibility to imidacloprid was significantly higher than that to chlorpyrifos. Compared with the susceptible strain, the resistance ratio of imidacloprid sharply declined with the succession of rearing generations without insecticide exposure from 359.94-fold at $F_1$ to 6.50-fold at $F_{14}$. The resistance ratio of imidacloprid decreased faster at $F_4$ to $F_8$, and it became stable at $F_{10}$ to $F_{14}$. However, the resistance ratio of chlorpyrifos only slightly decreased from 9.90-fold at $F_1$ to 5.94-fold at $F_{14}$.

## Ecological fitness in rice varieties of BPH populations without insecticide exposure

Ecological fitness of BPH reared on TN1, IR26 and IR36 for 10 generations was observed. The nymph survival rate of the BPH population was significantly affected by the generation ($P < 0.001$), but not by rice variety ($P = 0.341$) and rice variety × generation interactions ($P = 0.360$). The survival rates of BPH on TN1 did not significantly differ among the generations, and the rates of $F_8$ were the highest in IR26 and IR36. The survival rate of BPH did not differ with the rice variety at the same generation, except $F_{10}$ (Table 2).

**Table 2. Nymph survival rates of brown planthopper under free of insecticides for generations in the laboratory.** %

| Generation | TN1 | IR26 | IR36 |
|---|---|---|---|
| $F_1$ | 70.67 ± 12.29 aA | 76.22 ± 3.05 aB | 66.00 ± 6.59 aB |
| $F_4$ | 84.66 ± 4.58 aA | 82.26 ± 5.71 aAB | 71.00 ± 6.82 aB |
| $F_6$ | 70.38 ± 6.03 aA | 78.00 ± 4.59 aB | 77.00 ± 5.79 aAB |
| $F_8$ | 91.00 ± 4.30 aA | 91.00 ± 2.45 aA | 93.00 ± 3.39 aA |
| $F_{10}$ | 89.60 ± 2.71 aA | 59.00 ± 7.55 bC | 68.00 ± 2.83 bB |

Data were shown as Mean ± *SE*. Data in the same column followed by different uppercases and data in the same row followed by different lowercases were significantly different at $P < 0.05$ level through Tukey's test.

Nymph durations of male and female BPH were significantly affected by rice variety (female, $P < 0.001$; male, $P < 0.001$), generation (female, $P < 0.001$; male, $P < 0.001$), and their interactions (female, $P < 0.001$; male, $P < 0.001$). Nymphal durations were prolonged with succeeding generations, but were shorter at $F_1$ and $F_4$ generations than at the others. Female nymph duration was shorter in the susceptible variety TN1 than those in the resistant varieties IR26 and IR36. Moreover, male nymphal duration in TN1 was longer than those in IR26 and IR36, except $F_4$ (Table 3).

Analysis of variance results showed that the weight of a newly hatched female adult was significantly affected by rice variety ($P < 0.001$), generation ($P < 0.001$), and their interactions ($P = 0.002$). The weights of female adult in TN1 did not significantly differ

**Table 3. Female and male nymph durations of brown planthopper under free of insecticides for generations in the laboratory.** d

| Generation | Female | | | Male | | |
|---|---|---|---|---|---|---|
| | TN1 | IR26 | IR36 | TN1 | IR26 | IR36 |
| $F_1$ | 10.73 ± 0.16 bD | 11.28 ± 0.18 aC | 11.21 ± 0.12 aC | 10.49 ± 0.27 aB | 10.78 ± 0.09 aB | 10.88 ± 0.10 aC |
| $F_4$ | 11.25 ± 0.08 bC | 11.76 ± 0.09 abC | 12.24 ± 0.20 aB | 10.69 ± 0.15 cB | 11.14 ± 0.14 bB | 11.72 ± 0.10 aB |
| $F_6$ | 13.81 ± 0.08 bAB | 13.98 ± 0.20 bB | 14.50 ± 0.12 aA | 13.40 ± 0.03 aA | 13.51 ± 0.15 aA | 13.72 ± 0.14 aA |
| $F_8$ | 14.15 ± 0.09 aA | 14.37 ± 0.14 aB | 14.19 ± 0.08 aA | 13.42 ± 0.37 aA | 14.01 ± 0.09 aA | 13.61 ± 0.11 aA |
| $F_{10}$ | 13.57 ± 0.17 bB | 15.56 ± 0.20 aA | 14.12 ± 0.44 bA | 13.52 ± 0.10 aA | 14.04 ± 0.38 aA | 13.26 ± 0.32 aA |

Data in the same column followed by different uppercases and data in the same row under the same sex followed by different lowercases were significantly different at $P < 0.05$ level through Tukey's test.

**Table 4. Weight of female adult of brown planthopper under free of insecticides for generations in the laboratory.** mg

| Generation | TN1 | IR26 | IR36 |
|---|---|---|---|
| $F_1$ | 2.40 ± 0.07 aA | 1.94 ± 0.05 bAB | 2.05 ± 0.05 bA |
| $F_4$ | 2.36 ± 0.11 aA | 2.04 ± 0.04 bA | 2.08 ± 0.05 bA |
| $F_6$ | 2.37 ± 0.09 aA | 2.16 ± 0.09 abA | 2.02 ± 0.08 bA |
| $F_8$ | 2.36 ± 0.07 aA | 1.91 ± 0.07 bAB | 1.96 ± 0.05 bA |
| $F_{10}$ | 2.27 ± 0.07 aA | 1.86 ± 0.09 bB | 1.60 ± 0.04 cB |

Data in the same column followed by different uppercases and data in the same row followed by different lowercases were significantly different at $P < 0.05$ level through Tukey's test.

among all generations, and they were higher than those in IR26 and IR36 (Table 4). Moreover, the weight of a female adult nymph was higher at $F_{10}$ in IR26 than those at $F_4$ and $F_6$, and the lowest at $F_{10}$ in IR36.

The ratio of brachypterous adults in males were affected by the generation ($P < 0.001$) and generation × variety interactions ($P = 0.017$), with the highest differences observed in IR26 and IR36 at $F_4$ and $F_{10}$, and the lowest at $F_1$. By contrast, no difference was found in females (generation, $P = 0.470$; variety, $P = 0.279$; generation × variety, $P = 0.339$). The ratios of brachypterous adults in females were between 94% and 100% (Table 5).

## DISCUSSION

BPH management mainly depends on the application of highly efficient chemical insecticides, but the long-term abuse of these pesticides leads to insecticidal resistance, pest resurgence, environmental deterioration, decline in rice quality, and other social and ecological issues (Jiang et al, 2005). With the initiation of green plant protection and proposal of ecological civilization construction, the reduction in pesticide application has become one of the objectives of green plant protection (Yang et al, 2010; Yang and Zhao, 2012). Pest resistance to pesticides is an important concern in sustainable pest management. Delaying the development of pest resistance and increasing the susceptibility to pesticides have become important topics in reducing the use of chemical pesticides. Liu et al (2003) reported that the resistance of BPH to imidacloprid is clearly unstable and easily reversed. In this study, the field BPH population successively reared in the laboratory without insecticide exposure for several generations could reverse its resistance to imidacloprid and chlorpyrifos. The reversal range of resistance to imidacloprid was significant from the resistance ratio of 359.94-fold at $F_1$ generation to 6.50-fold at $F_{14}$ generation. Moreover, the resistance ratio became stable after the 10th generation. The resistance ratio of BPH to chlorpyrifos decreased from 9.9-fold at $F_1$ to 5.94-fold at $F_{14}$. The decline was insignificant, but the resistance ratio became stable. Wilson et al (2007) found that *Bemisia tabaci*, which is highly resistant to pyriproxyfen, reversed the resistance to pyriproxyfen after no exposure to pyriproxyfen for 13 generations accompanied with an increase in fitness. Keiding (1963) also investigated the reversal phenomenon of pest resistance to various insecticides. The reversal of resistance to pesticides in field populations may result from the increase in susceptible individuals within the population, or the resistant individual adapted to an insecticide- free environment and gradually lost its resistance (Keiding, 1963). The resistance levels and mechanisms in field populations and the ratio of resistant/susceptible individuals are important factors affecting resistance reversal. The stable resistance reversal of a field population indicates the quantitative homeostasis of resistant and susceptible individuals in an insecticide-free environment. Resistances to imidacloprid and chlorpyrifos in the field BPH populations differed, and the differences in action mechanisms of these two pesticides may be one of the reasons for the differences between the resistance reversal of imidacloprid and chlorpyrifos. Pesticide resistance reversal in field populations indicates that the rotation or non-application of pesticide has an important theoretical and practical significance in delaying resistance development.

**Table 5. Ratio of brachypterous brown planthopper under free of insecticides for generations in the laboratory.** %

| Genaration | Female | | | Male | | |
|---|---|---|---|---|---|---|
| | TN1 | IR26 | IR36 | TN1 | IR26 | IR36 |
| $F_1$ | 97.92 ± 2.08 aA | 98.72 ± 1.28 aA | 100.00 ± 0.00 aA | 16.82 ± 8.01 aB | 22.11 ± 5.38 aC | 29.17 ± 8.21 aC |
| $F_4$ | 97.78 ± 4.97 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 79.98 ± 5.72 aA | 77.46 ± 4.75 aAB | 83.81 ± 3.63 aAB |
| $F_6$ | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 52.92 ± 6.24 aA | 51.03 ± 10.56 aB | 65.82 ± 7.28 aB |
| $F_8$ | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 100.00 ± 0.00 aA | 63.13 ± 9.92 aA | 66.91 ± 2.54 aB | 42.20 ± 6.58 aC |
| $F_{10}$ | 100.00 ± 0.00 aA | 94.29 ± 5.71 aA | 100.00 ± 0.00 aA | 55.87 ± 12.05 bA | 88.36 ± 7.92 aA | 93.70 ± 2.96 aA |

Data in the same column followed by different uppercases and data in the same row under the same sex followed by different lowercases were significantly different at $P < 0.05$ level through Tukey's test.

Application of resistant rice varieties is one of the most economic ways to control BPH. In the 1970s, wide adoption of the resistant varieties IR26 and IR36 successfully controlled the damage caused by BPH in Asia, and achieved remarkable economic and ecological benefits (Lu et al, 2002). The adaptation of pests to different rice varieties is one of the important factors affecting the development of pest populations after ceasing or reducing the use of pesticides. Our findings showed that nymphal duration and adult female weight of BPH from fields reared in the laboratory without insecticide exposure over generations were significantly affected by the rice varieties, generations, and rice variety × generation interactions, whereas nymphal survival was affected only by the generation number. In the susceptible rice variety TN1, the adult female weight showed no significant differences among the generations, but the weights of female adults at $F_{10}$ in resistant rice varieties IR26 and IR36 were significantly lower than those at other generations. Liu and Han (2006) screened imidacloprid-resistant and -susceptible BPH strains in the laboratory, and found that the fitness of BPH strains with high resistance to imidacloprid is only 1/10 of that of susceptible strains. Liu et al (2003) also reported that the fitness of imidacloprid-resistant BPH strains decreases compared with that of the susceptible strains, resulting in decreased low-instar nymph survival, adult emergence rate, mating rate, fecundity and hatchability, and shorter female adult longevity. Wen et al (2010) found that fitness significantly declines by 0.191-fold of that of susceptible strains when BPH acquires resistance to imidacloprid, which indicates that the resistance of BPH populations to imidacloprid is unstable and will gradually decline if pesticide exposure is removed. We found that the survival and body weight of BPH did not increase in the resistance reversal generations. In the studies of Liu and Han (2006) and Wen et al (2010), the fitness decreased in the BPH population, which was under continuous selection pressure and insecticide-free environment. In this study, the fitness of BPH slowly changed. The field population of BPH adapted on TN1 with certain fitness costs, and likewise, after continuous rearing on TN1, BPH overcame the fitness cost and adapted to the resistant varieties IR26 and IR36. Chen et al (2011) found that the relative fitness of resistant and susceptible BPH to imidacloprid in the resistant variety IR36 is lower than that in the susceptible variety TN1, and the relative fitness of BPH transferred from TN1 to IR36 sharply declines and recovers with the succession of generations. Changes in the insecticidal resistance of BPH can influence the fitness of a BPH population in a rice variety.

Our study on the insecticidal resistance and fitness to rice variety of BPH without insecticide exposure for generations will promote the understanding of resistance reversals in BPH, and guide the rational use of insecticides and sustainable management of BPH. Resistance management is an important aspect in BPH management, and many methods can delay resistance development, including the judicious use of pesticides. In recent years, the Ministry of Agriculture of China recommended that farmers stop the use of imidacloprid because of the high resistance of BPH (Zhang et al, 2011). Our results suggest that the insecticidal susceptibility of BPH population increased with the number of rearing generations free of insecticide, whereas the ecological fitness of BPH in resistant rice varieties did not increase. Rational and reduced use of insecticides, combined with the application of resistant rice varieties, should be considered in the sustainable management of BPH.

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