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Changes in Methamidophos Resistance and Fitness of Hybrids in Different Strains of *Nilaparvata lugens*

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Abstract: A resistant strain selected successively in the laboratory for 17 generations had 198.63-fold resistance to methamidophos. The resistant levels and fitness of progenies from the resistant strain and susceptible strain or field population were closer to those of the resistant strain than those of the susceptible strain or field population. The changes in the resistant levels of the hybrid were propitious to the resistance development, however, the changes of the fitness went to the contrary. The effects of the migration on the development of methamidophos resistance in *Nilaparvata lugens* were discussed in the aspects of the migration of *Nilaparvata lugens*, the resistant levels of progenies and the changes of the fitness.

Key words: *Nilaparvata lugens*; hybridism; insecticide resistance; fitness

The brown planthopper, *Nilaparvata lugens* Stål (BPH), is one of the major destructive rice pests in many parts of Asia. Extensive use of insecticides has resulted in the development of resistance in the populations of this pest from different countries and areas [1-3]. Methamidophos is one of the most important insecticides for BPH control since the 1970s in China, and resistance to methamidophos in BPH had been reported up to 10-fold [4, 5]. The extensive use, in fact, has not resulted in high resistance to methamidophos and the other actual reasons have been discussed. Nagata reported that the populations from Japan and Southeast Asia had similar resistance level to methamidophos, considering migration was one of the most important reasons [2]. Wang et al also drew the same conclusion in China [6]. Additionally, the lower fitness of the resistant population, has been regarded as another important factor contributing to the lower methamidophos resistance in BPH [7]. In this paper, the resistance level and fitness of the progenies of the susceptible strain, field population and the resistant strain have been reported.

MATERIALS AND METHODS

Insect

BPH susceptible strain (SS) was provided by Jiangsu Academy of Agricultural Sciences. The field population (FP) was collected at the experimental rice fields of Jiangpu, Jiangsu Province, in July, 2002. The resistant strain (RS) was the 17th generation of the FP treated with methamidophos doses (LC_{50}–LC_{70}) and selected in laboratory. The hybrid crosses were F_1 progeny of ♀$_{RS}$×♂$_{SS}$, F_1' progeny of ♀$_{SS}$×♂$_{RS}$, T_1 progeny of ♀$_{RS}$×♂$_{FP}$ and T_1' progeny of ♀$_{FP}$×♂$_{RS}$.

Insecticide

Methamidophos (Bayer) with a concentration of 98.2%, was used in the bioassay; 72% of methamidophos (Suzhou Chemical Company), was used for resistance selection.

Bioassay

The bioassay followed the micro topical application technique as described by Nagata [2]. Macropterous adult females of 3- to 5-day-old were used in the present study. A droplet of 0.04 μL acetone solution of methamidophos was applied topically using a manual micro-applicator (Burkard Manufacturing Ltd., England) to the dorsal surface of the thorax of each female adult anesthetized with carbon dioxide. Thirty insects were treated at each concentration. Each treatment was repeated thrice. Insects treated with acetone alone severed as control. The treated insects were reared on the seedlings cultured in the rearing cage without soil under a regime of 25±1℃, 16 h light / 8 h dark. Mortality was investigated 24 h after treatment.

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Resistance selection

Resistance selection was carried out by spraying insecticides on seedlings infested with BPH as previously described [8]. The seedlings in soilless culture were placed in the selection cage (28 cm×28 cm×43 cm), then 100 to 200 3rd instar larvae were placed in the cage. Two hours later, the insecticide at about LC_{70} dosage was sprayed on the seedlings with insects by using the pocket sprayer (Hongxing Company, Zhejiang, China). The cage was placed in an observation room under a regime of 25±1℃ and 16 h light / 8 h dark. Ten to 20 replications were set up for each generation.

Life table construction

Life table construction was performed as described earlier [12]. In each strain or population, 100 neonates were collected randomly from the rearing box and kept rearing to the neonates of the next generation at a constant temperature. In this course, the survival rate from neonate to 2nd instar, survival rate from 3rd to 5th instar, larva duration, emergence rate, female ratio, copulation rate, fecundity, hatchability, egg duration and female duration were recorded. These recordings were used to compute the population trend index (I) and relative fitness as follows:

$$I = N_{n+1}/N_n \; ; \quad \text{Relative fitness} = I_{Other}/I_{CK}$$

Data analysis

Data analysis was performed using the software SAS 6.12 and Excel. Comparison of variance means was carried out at 95% level of significance.

RESULTS

Toxicity determination

Table 1 showed the ratios of LD_{50F1}, $LD_{50F1'}$ to LD_{50SS} were 54.76 and 48.52, and the ratios of LD_{50RS} to LD_{50F1}, $LD_{50F1'}$ were 3.63 and 4.09, implying the resistance levels of the progenies of SS and RS were closer to that of RS. The similar results had been also showed in Table 1 for the progenies of FP and RS.

Fitness determination

Table 2 showed that the fitness of the progeny of F_1, F_1' and RS was significantly lower than that of SS with some disadvantages, including the lower larvae survival, emergence rate, copulation rate, fecundity and hatchability. The emergence rate, fecundity and hatchability varied among F_1, F_1' and RS. The ratios of the fitness of SS to that of F_1, F_1' were 2.96 and 2.81, and the ratios of the fitness of F_1, F_1' to that of RS were 1.92 and 2.02, showing that the fitness of the progenies of F_1 and F_1' was closer to RS and the progenies had significant disadvantage in the population development.

Table 3 showed that the fitness of the progeny of T_1, T_1' and RS was remarkably lower than that of FP with some disadvantages, including the lower levels of larvae survival, emergence rate, copulation rate, fecundity, hatchability and prolonged egg duration. The emergence rate, fecundity and hatchability varied among T_1, T_1' and RS. The ratios of the fitness of FP to that of T_1, T_1' were 2.27 and 2.13, and the ratios of the fitness of T_1, T_1' to those of RS were 1.75 and 1.86, revealing that the fitness of the progenies of T_1 and T'

Table 1. Responses of susceptible strain, resistant strain, field population and progenies of F_1, F_1', T_1, T_1' to methamidophos.

Population	*LD-p* line	LD_{50} (μg/pest)	Resistance ratio
SS	y=14.1022+3.8413x	0.0043	1.00
FP	y=10.5758+3.3192x	0.0209	4.86
RS	y=5.1768+2.5820x	0.8541	198.63
F_1	y=6.4859+2.3661x	0.2355	54.76
F_1'	y=6.9178+2.8174x	0.2086	48.52
T_1	y=5.8861+1.9572x	0.3526	87.01
T_1'	y=5.9914+2.1305x	0.3425	79.64

SS, Susceptible strain; RS, Resistant strain; FP, Field population; F_1, Progeny from ♀$_{RS}$×♂$_{SS}$; F_1', Progeny from ♀$_{SS}$×♂$_{RS}$; T_1, Progeny from ♀$_{RS}$×♂$_{FP}$; T_1', Progeny from ♀$_{FP}$×♂$_{RS}$.

Table 2. Life table and biological traits of F_1, F_1', resistant and susceptible strains of *N. lugens*.

Traits	SS	RS	F_1	F_1'
Neonate number	100	100	100	100
Survival rate from neonate to 2nd instar (%)	93.21±0.66 a	76.03±6.92 c	82.88±5.13 bc	84.13±3.58 b
Survival rate from 3rd to 5th instar (%)	97.46±0.57 a	85.11±3.46 b	86.72±6.06 b	85.07±5.15 b
Larva duration (d)	15.43±0.49 a	15.88±2.04 a	16.02±1.73 a	15.61±2.31 a
Emergence rate (%)	95.02±1.24 a	64.30±5.97 c	81.44±7.40 b	84.80±4.72 b
Female ratio (%)	47.84±2.98 a	48.39±3.51 a	48.04±3.01 a	47.92±2.27 a
Copulation rate (%)	91.06±2.40 a	70.11±7.27 b	74.97±6.67 b	78.65±5.85 b
Fecundity (No. of eggs)	585.10±61.27 a	267.54±33.31 c	381.57±54.52 b	410.54±41.34 b
Female duration (d)	22.13±0.67 a	16.97±2.14 b	18.82±1.69 b	19.04±3.06 ab
Egg duration (d)	8.53±0.59 a	11.91±2.17 b	11.37±2.22 b	12.02±1.50 b
Hatchability (%)	91.04±4.73 a	63.59±8.62 c	84.19±6.74 ab	75.87±5.43 b
Next generation larva number	20030.19	3534.52	6772.21	7124.74
I	200.30	35.35	67.72	71.25
Relative fitness	1.0000	0.1765	0.3381	0.3557

Different letters in the same rows showed significant difference at 0.05 level.

F_1, Progeny from ♀$_{RS}$×♂$_{SS}$; F_1', Progeny from ♀$_{SS}$×♂$_{RS}$.

Table 3. Life table and biological traits of T_1, T_1', resistant strain and field population of *N. lugens*.

Developmental period	FP	RS	T_1	T_1'
Neonate number	100	100	100	100
Survival rate from neonate to 2nd instar (%)	92.42±2.17 a	76.03±6.92 b	79.57±5.51 b	83.60±7.32 b
Survival rate from 3rd to 5th instar (%)	92.66±2.42 a	85.11±3.46 b	82.42±4.17 b	85.76±3.49 b
Larva duration (d)	15.08±1.94 a	15.88±2.04 a	16.03±1.92 a	15.78±1.70 a
Emergence rate (%)	90.05±2.57 a	64.30±5.97 c	81.57±5.00 b	83.68±6.27 b
Female ratio (%)	48.84±2.39 a	48.39±3.51 a	49.13±2.86 a	48.76±3.03 a
Copulation rate (%)	88.67±2.45 a	70.11±7.27 c	78.54±5.12 bc	83.29±8.01 ab
Fecundity (No. of eggs)	479.72±51.04 a	267.54±33.31 c	374.72±59.71 b	328.96±71.62 bc
Female duration (d)	21.17±1.09 a	16.97±2.14 b	18.66±1.68 b	19.13±2.02 ab
Egg duration (d)	9.65±1.26 a	11.91±2.17 b	11.06±1.60 ab	11.24±1.87 ab
Hatchability (%)	87.29±5.16 a	63.59±8.62 c	79.80±4.98 b	82.02±7.95 ab
Next generation larva number	13984.49	3534.52	6172.54	6574.11
I	139.84	35.35	61.73	65.74
Relative fitness	1.000	0.2528	0.4414	0.4701

Different letters in the same row showed significant difference at 0.05 level.

T_1, Progeny from ♀$_{RS}$×♂$_{SS}$; T_1', Progeny from ♀$_{SS}$×♂$_{RS}$.

was closer to RS and the progenies experienced a large disadvantage in the population development.

DISCUSSION

Insecticide resistance in BPH developed slowly and had not reached a high level in the last decades, except 1984 and 1985 in Japan [9]. A general standpoint is that the migration of BPH results in a dilution of the resistant gene and holding back the resistance development [6]. From the viewpoint of migration, the level of insecticide resistance and development mode are based on several factors, in which the most important factors are considered as the population amount and resistant level in the field population, the population size and resistant level of the immigratory population and the inheritance of insecticide resistance. Based on our results, the fitness of the progeny should be also taken into account. The disadvantages in the development and reproduction of the progeny in comparison with the relatively susceptible strain or population might result in slow development of insecticide resistance.

The development of methamidophos resistance in BPH can be viewed out in the following four aspects: 1) the population size and resistance level of the field population and immigratory population; 2) the population growth of these two populations; 3) the resistance level and population growth of the progenies from the same population; 4) the resistance level and population growth of the progenies from different populations. If we assume the size of the field population and the immigratory population was Q_1 and Q_2 and the female ratio was r (the present results indicated that there were no significant differences among different populations), then the size of the crosses of ♀$_{RS}$ × ♂$_{RS}$, ♀$_{RS}$ × ♂$_{FP}$, ♀$_{FP}$ × ♂$_{RS}$, ♀$_{FP}$ × ♂$_{FP}$ will be:

$$q_1=[r/(1+r)]\times Q_1\times[Q_1/(Q_1+Q_2)];$$
$$q_2=[r/(1+r)]\times Q_1\times[Q_2/(Q_1+Q_2)];$$
$$q_3=[r/(1+r)]\times Q_2\times[Q_1/(Q_1+Q_2)];$$
$$q_4=[r/(1+r)]\times Q_2\times[Q_2/(Q_1+Q_2)];$$

And the average resistance level is calculated as follows:

$$LD_{50A}=(LD_{50RS}\times I_{RS}\times q_1+LD_{50T1}\times I_{T1}\times q_2 + LD_{50T1'}\times I_{T1'}\times q_3+LD_{50FP}\times I_{FP}\times q_4)/(I_{RS}\times q_1+I_{T1}\times q_2+I_{T1'}\times q_3+I_{FP}\times q_4)$$

If RS and FP are treated as the field population and the immigratory population, respectively, then Q_1 and Q_2 would be 353 500 and 1 398 400 according to the results of the Table 2 and Table 3, denoting that the female ratio r is 0.486. LD_{50A} calculated based on the above equations is 0.0920 μg/pest. The ratios of LD_{50RS} to LD_{50A} and LD_{50A} to LD_{50FP} are 9.28 and 4.40, indicating that the average of resistant level was closer to FP. Therefore, the overall results of the fitness and resistance inheritance were propitious to the decline of insecticide resistance.

However, many other factors such as rice variety (genotype), insecticide used in field, immigration, and natural enemy, contributed unevenly to the population growth and resistance development in different populations. Hence, the more accurate equations in predicting the resistance development should be improved and modified further.

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