Neonicotinoid resistance in rice brown planthopper, *Nilaparvata lugens*

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Abstract

BACKGROUND: Rice brown planthopper, *Nilaparvata lugens* Stål, is a primary insect pest of cultivated rice, and effective control is essential for economical crop production. Resistance to neonicotinoid insecticides, in particular imidacloprid, has been reported as an increasing constraint in recent years. In order to investigate the extent of resistance, 24 samples of *N. lugens* were collected from China, India, Indonesia, Malaysia, Thailand and Vietnam during 2005 and 2006. Their responses to two diagnostic doses of imidacloprid (corresponding approximately to the LC_{95} and 5 × LC_{95} of a susceptible strain) were examined.

RESULTS: Ten of the 12 samples collected during 2005 were found to be susceptible to imidacloprid, but two late-season samples from India showed reduced mortality at both diagnostic doses. All 13 strains collected in 2006 showed reduced mortality at both doses when compared with the susceptible strain. Dose–response lines showed resistance in one of the most resistant field strains to be approximately 100-fold compared with the susceptible standard.

CONCLUSION: The data demonstrate the development and spread of neonicotinoid resistance in *N. lugens* in Asia and support reports of reduced field efficacy of imidacloprid.

Keywords: *Nilaparvata lugens*; imidacloprid; insecticide resistance; neonicotinoid; Asia; rice; brown planthopper

1 INTRODUCTION

The rice brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae), is an important pest of rice crops throughout Asia.[1,2] It is a frequent target of insecticide applications aimed at reducing the severity of infestations. *Nilaparvata lugens* is a phloem feeder extracting nourishment directly from the plant. This induces complex plant responses, with direct and indirect deleterious effects including reduction in plant growth (root development, plant height and reproduction), wilting and leaf chlorosis.[3–6] All of these contribute to potentially dramatic losses in yield and can ultimately lead to plant death. Symptoms are collectively known as 'hopperburn', a non-contagious disease also caused by other planthoppers and leafhoppers including the sometimes coexisting white-backed planthopper, *Sogatella furcifera* (Horváth). It has been estimated that rice consumption accounts for 20% of the world's calorific intake,[7] and single-season losses due to *N. lugens* during 1990/1991 in Thailand and Vietnam were calculated at $US 30 million.[8]

Broad-spectrum insecticides used to control *N. lugens* over several decades were eventually compromised by insecticide resistance.[9,10] They were superseded by more selective chemistries including the neonicotinoid compound imidacloprid. Imidacloprid has since become one of the most common insecticides used against *N. lugens*, principally owing to its efficacy against previously resistant populations. Recently, however, reports of reduced efficacy have become more frequent and generally attributed to resistance development.[11,12] A reversion to more broad-spectrum chemistries is now of concern, as integrated pest management (IPM) strategies for rice that combine non-chemical approaches with complementary insecticide use would be severely compromised. Planthoppers, like other Hemipteran groups such as whiteflies and aphids, have a propensity for developing multiple insecticide resistance. *N. lugens* is no exception in this respect, but has the additional characteristic of being highly migratory.[13,14] Its annual cycle of long-distance migration is of great significance to both the spread of resistant genotypes and the transmission of viruses.[15] This study aimed to assess the potency and geographical distribution of imidacloprid resistance in *N. lugens* strains collected in 2005 and 2006 from six countries in Asia.

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(*Received 22 April 2008; accepted 23 May 2008*)
Published online 19 September 2008; DOI: 10.1002/ps.1635

 Pest Manag Sci 1526–498X/2008/$30.00

2 MATERIALS AND METHODS

2.1 Insect strains

Baseline susceptibility data were generated using a laboratory-maintained strain (S) of *N. lugens* provided by Bayer CropScience (Monheim, Germany). Bayer CropScience also organised the transfer to Rothamsted Research of 11 field strains collected in 2005 and a further 13 in 2006 from China, India, Indonesia, Malaysia, Thailand and Vietnam. All strains were reared in the laboratory on whole rice plants (*Oryza sativa* L. ssp. Japonica var. Nipponbare) under controlled environmental conditions (26 °C/16 h photoperiod).

2.2 Bioassays

Adult macropterous (long-winged) female *N. lugens* of less than 10 days old were used for topical application bioassays. These consisted of three replicates per dose, each with 10–20 individuals. Diagnostic doses represented the LC_{95} (4 mg L^{-1}) and 5 × LC_{95} (20 mg L^{-1}) of the susceptible strain. Insects for testing were removed from rearing cages, lightly anaesthetised using carbon dioxide and dosed with 0.25 µL of technical imidacloprid dissolved in acetone (AR grade) on the pronotum using a microapplicator (Burkard Manufacturing Ltd, UK). Control insects were dosed with 0.25 µL acetone only. Treated individuals were transferred to ventilated plastic pots containing untreated, rooted rice stems and maintained under controlled environmental conditions (26 °C/16 h photoperiod). Mortality was assessed 48 h post-treatment.

2.3 Data analysis

When appropriate, bioassay data were subjected to probit analysis using Polo Plus© software (LeOra Software, Berkeley, California). This generated estimated LC_{50} values, and resistance ratios were calculated by dividing the LC_{50} of a field strain by that of the susceptible strain. Standard errors for mortalities at diagnostic concentrations were calculated using a binomial model.

3 RESULTS

Responses of 2005 samples of *N. lugens* to imidacloprid showed variation, particularly at the lower dose tested (Table 1). Strains 1, 4, 6 and 10 (originating from China, India or Malaysia) gave approximately 55–60% mortality at 4 mg L^{-1}. Two late-season samples from India (strains 7 and 8) gave the lowest mortalities (18 and 19%) at this dose and additionally showed approximately 20–30% survival at the higher dose of 20 mg L^{-1}. All other strains responded similarly to the susceptible strain at both doses.

In contrast, all 13 samples collected in 2006 showed reduced susceptibility to imidacloprid at both diagnostic doses (Table 1). Responses at 4 mg L^{-1} ranged from 0 to 58% mortality, and those at 20 mg L^{-1} from 0 to 97% mortality. The highest rates of survival occurred in strains 19 and 23, the former being a late-season sample from India, and the latter a mid-season sample from Vietnam. Little or no mortality was observed in these strains even at the higher dose of 20 mg L^{-1}. A comparison between dose–response data for the laboratory susceptible strain (S) and strain 19 showed near-parallel response lines (Fig. 1) with a resistance ratio of 95 at LC_{50} (Table 2).

Table 1. Mortalities (%) (± standard error)[a] for all *Nilaparvata lugens* strains at two diagnostic doses (LC_{95} and 5 × LC_{95} of susceptible strain) of imidacloprid topically applied to adult females

Strain	Year	Origin	4 mg L^{-1} (±SE)	20 mg L^{-1} (±SE)
S	–	–	91.43 (±4.48)	100.00 ± nc
1	2005	China	53.45 (±6.39)	100.00 ± nc
2	2005	India	85.21 (±4.74)	100.00 ± nc
3	2005	India	91.23 (±3.68)	100.00 ± nc
4	2005	India	59.32 (±6.09)	100.00 ± nc
5	2005	India	83.34 (±5.02)	100.00 ± nc
6	2005	India	59.66 (±7.57)	100.00 ± nc
7	2005	India	17.98 (±4.66)	81.50 (±4.74)
8	2005	India	18.63 (±4.79)	71.40 (±5.61)
9	2005	Indonesia	96.36 (±2.50)	100.00 ± nc
10	2005	Malaysia	54.03 (±6.91)	nt
11	2005	Thailand	87.01 (±4.20)	100.00 ± nc
12	2006	China	41.41 (±7.11)	46.20 (±10.4)
13	2006	China	23.34 (±6.24)	75.81 (±6.69)
14	2006	China	55.71 (±6.64)	75.11 (±6.92)
15	2006	China	35.00 (±6.88)	67.50 (±6.76)
16	2006	India	57.53 (±8.13)	97.14 (±2.36)
17	2006	India	50.00 (±7.45)	79.71 (±5.15)
18	2006	India	33.67 (±6.68)	48.04 (±5.97)
19	2006	India	0.00 ± nc	5.75 (±4.18)
20	2006	Malaysia	13.87 (±6.78)	33.07 (±5.23)
21	2006	Thailand	22.41 (±7.74)	35.71 (±8.10)
22	2006	Thailand	35.00 (±6.88)	67.50 (±6.76)
23	2006	Vietnam	2.27 (±2.52)	0.00 ± nc
24	2006	Vietnam	26.63 (±7.70)	42.11 (±7.81)

[a] nt = not tested; nc = not calculable.

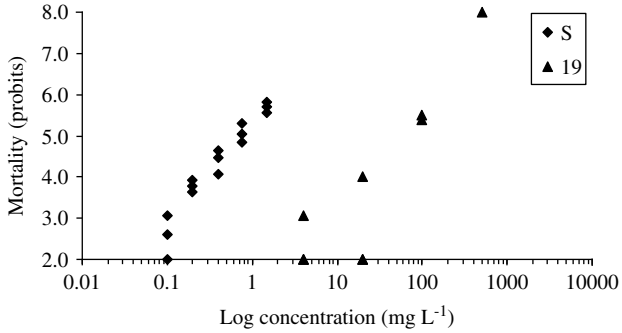

Figure 1. Dose–response data for the *Nilaparvata lugens* laboratory susceptible (S) and imidacloprid-resistant (19) strains against imidacloprid topically applied to adult females.

Table 2. Comparison between dose–response data for *Nilaparvata lugens* laboratory susceptible (S) and imidacloprid-resistant (19) strains against imidacloprid topically applied to adult females

Strain	*n*	LC_{50} (95% CL)	Slope (±SE)	RR[a] (95% CL)
S	367	0.61 (0.5–0.8)	1.8 (±0.26)	1
19	121	58 (31–92)	2.7 (±0.65)	95 (56–170)

[a] RR = resistance ratio (R/S).

4 DISCUSSION

In accordance with reports from growers, it appears that the low-level and localised imidacloprid resistance observed in strains of *N. lugens* collected in 2005 increased substantially in potency and geographical distribution by 2006. The four most resistant 2006 samples came from different countries (China, India, Malaysia and Vietnam), implying that resistance was not confined to a specific geographical region. This distribution is consistent with the migratory behaviour of *N. lugens*, and poses a serious threat to the sustainable use of imidacloprid and other compounds compromised by cross-resistance in this species. Further information on the spread of resistance to imidacloprid in south-east Asian populations of *N. lugens* is given in an accompanying paper in this In Focus group.[16]

Cross-resistance within the neonicotinoid class has not yet been investigated in detail for *N. lugens*, but in other neonicotinoid-resistant species it is known to extend across the majority of compounds, albeit to varying extents.[17–19] Research on *N. lugens* provided the first confirmed example of target-site resistance to neonicotinoids.[20] However, this involved a laboratory-selected strain that may not be representative of insects selected in the field. In the tobacco whitefly (*Bemisia tabaci* Gennadius) and the fruit fly (*Drosophila melanogaster* Meigen), increased detoxification of neonicotinoids by cytochrome-P450-dependent monooxygenases has been linked with neonicotinoid resistance.[17,21,22] Thus, both major types of resistance mechanism have been documented for neonicotinoids and require investigation for their occurrence in field strains of *N. lugens*.

In terms of resistance management, it now seems essential to reduce reliance on imidacloprid in favour of other compounds with different modes of action. Unfortunately, the supply of such compounds is limited and, as shown here for imidacloprid, without severe restrictions on overall pesticide applications there is scope in *N. lugens* for any form of resistance to spread rapidly.

ACKNOWLEDGEMENTS

The authors thank colleagues within Bayer CropScience, Nanjing Agricultural University and Rothamsted Research for detailed scientific discussion, and personnel in Asia for collecting and transporting insect samples. Rothamsted Research receives grant-aided support from the Biotechnology and Biosciences Research Council of the United Kingdom.

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