Research Article

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Sublethal effects of four insecticides on the reproduction and wing formation of brown planthopper, *Nilaparvata lugens*

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Abstract

BACKGROUND: The brown planthopper (BPH), *Nilaparvata lugens* Stål, is a major rice pest in many parts of Asia. Neonicotinoids such as imidacloprid and dinotefuran are widely used for control of this pest, and resistance to these insecticides has developed in recent years. This pest has also been widely exposed to triazophos and fenvalerate, although these insecticides are not used for the control of the pest directly. Here, the effects of sublethal doses of these four insecticides on the reproduction and wing formation in BPH were examined.

RESULTS: Imidacloprid and dinotefuran reduced the fecundity of BPH to 68.8% and 52.4% in macropterous families, and to 57.9% and 43.1% in brachypterous families, when compared with the untreated controls. By contrast, triazophos and fenvalerate increased fecundity. In both macropterous and brachypterous families, sublethal doses of imidacloprid and dinotefuran showed significant induction of macropterous adults.

CONCLUSION: Imidacloprid and dinotefuran could reduce the fecundity of BPH to a significant extent, demonstrating further activity against this pest in addition to their direct toxicity. The significant induction of macropterous adults by sublethal doses of imidacloprid and dinotefuran is of importance in the management of this pest, particularly in the areas of predicting the size of emigrating populations and the numbers of insects likely to occur in newly colonized areas.

Keywords: *Nilaparvata lugens*; imidacloprid; dinotefuran; sublethal effects; reproduction; fecundity; wing formation

1 INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is a major rice pest in many parts of Asia. Wing polymorphism in this insect is known to be a common and ecologically important trait. The long-winged (macropterous) adults possess long-distance migration ability. 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.[1–3] Following the widespread use of imidacloprid in China for over 15 years, high levels of imidacloprid resistance were observed in BPH from 2005 onwards.[4] Imidacloprid-resistant populations demonstrate significant cross-resistance to other neonicotinoid insecticides, such as thiacloprid, nitenpyram, acetamiprid, thiamethoxam and clothianidin, but not to dinotefuran, a finding that is consistent with the results of previous resistance mechanism studies.[4,5] Owing to the development of resistance, there are now significant concerns over the viability of continuing to use imidacloprid and other neonicotinoids against BPH.

In previous toxicological studies on BPH, the effects on fecundity and wing formation caused by sublethal doses of some insecticides, such as imidacloprid, fenvalerate and dinotefuran, were observed.[6] The present paper sets out a detailed study reporting on these sublethal effects, which will potentially open the way to explore the diverse actions of these insecticides in BPH, including potential effects on the endocrine system.

2 MATERIALS AND METHODS

2.1 Insects and insecticides

The brown planthopper (BPH) was collected from paddy fields of the China National Rice Research Institute, Hang Zhou, China, in 2002 and has been reared in continuous laboratory culture since then. The insects were maintained in wooden cages (50 × 38 × 38 cm) with rice seedlings under conditions of $26 \pm 1\,^{\circ}$C with a 16 : 8 h light : dark photoperiod. Under laboratory conditions, the percentages of macropterous females and males were generally about 33% and 41% respectively.

Technical imidacloprid (98.0%) and fenvalerate (99.0%) were provided by Professor Toru Nagata of Ibaraki University, Japan. Technical dinotefuran (98.1%) was provided by Bayer CropScience

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Japan. Technical triazophos (98.0%) was purchased from the Shenyang Research Institute of the Chemical Industry, China.

2.2 Bioassay

Bioassays were undertaken using the microtopical application technique reported by Nagata.[2] Unmated macropterous adult females (1–2 days old) were used as test insects in this study. Insecticides were diluted to a series of concentrations in acetone. Under carbon dioxide anaesthesia, a droplet (0.04 μL) of insecticide 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 3 times. The control treatments used acetone only. The treated insects were reared on seedlings grown in the absence of soil in a rearing box at 26 ± 1 °C with a 16 : 8 h light : dark photoperiod. The results were checked after 48 h. The LD_{50} and LD_{20} values were determined on the basis of standard probit analysis, using PoloPlus software (LeOra Software, Berkeley, CA).[7–9] This procedure generated estimated LD_{50} values, and the relative toxicity of the insecticides was calculated as

$$[(LD_{50}\ \text{value of imidacloprid})/(LD_{50}\ \text{value of other insecticide})] \times 100$$

2.3 Effects of insecticides on reproduction and wing formation

Fifth-instar BPH nymphs were collected from the wooden cages and reared individually until adulthood. Unmated adults were divided into four groups: macropterous (long-winged with long-distance migratory ability) females, macropterous males, brachypterous (short-winged) females and brachypterous males. The calculated LD_{20} dose of each insecticide was topically applied to insects taken from these four groups, and the surviving insects were collected after 48 h. The controls were treated with acetone only. One unmated macropterous female and one unmated macropterous male were mated to initiate a macropterous family. A total of 30 macropterous families for the control and each insecticide treatment were constructed. Brachypterous families were also constructed by pairing unmated brachypterous females and males: 30 brachypterous families for the control and each insecticide treatment were constructed too. Each family was reared in glass bottles (11 cm high, 8 cm diameter) on rice seedlings under controlled conditions (26 ± 1 °C with a 16 : 8 h light : dark photoperiod).

When neonates of the new generation were produced, the families were checked every day and the neonates were counted and transferred into new glass bottles until the original female died. The rice shoots on which the adults had been feeding were then inspected thoroughly, and the numbers of unhatched eggs were recorded. Any females that had not produced eggs were considered to have failed to mate, and the rate of successful mating was recorded (the copulation rate). Fecundity was recorded as the average number of eggs produced by the mated females, and the viability was calculated as (total neonates)/(total neonates plus all unhatched eggs). The number of offspring per female was calculated as: copulation rate × fecundity × viability.

For the controls and all insecticide treatments, 20 neonates of the new generation were reared in glass bottles (11 cm high, 8 cm diameter) with rice seedlings under controlled conditions (26±1 °C with a 16 : 8 h light : dark photoperiod). Fresh rice seedlings were provided every 4 days until adult emergence. For the controls and all insecticide treatments, the numbers of macropterous females, macropterous males, brachypterous females and brachypterous males were recorded, and the percentages of the macropterous females and macropterous males were determined.

3 RESULTS

3.1 Relative toxicity of four insecticides against BPH

For the strain of BPH used in this study, imidacloprid gave the lowest LD_{50} (0.17 ± 0.01 ng insect^{-1} (Table 1). The toxicity of dinotefuran was similar to that of imidacloprid, but triazophos and fenvalerate showed much lower levels of activity, with relative toxicities close to 1, when compared with imidacloprid (100) and dinotefuran (58.62) (Table 1). The LD_{20} values were also calculated, and these were used as the sublethal doses in further experiments.

3.2 Sublethal effects of four insecticides on reproduction

Adult BPH were divided into macropterous and brachypterous groups. The brachypterous insects usually laid more eggs than the macropterous insects, so the sublethal effects of the insecticides on reproduction were divided into two groups: macropterous families (macropterous female + macropterous male) and brachypterous families (brachypterous female + brachypterous male). The results are shown in Table 2 for the macropterous families and in Table 3 for the brachypterous families.

To evaluate the effects on reproduction, the copulation rate, fecundity and egg viability were determined in macropterous families. None of the insecticides tested had significant effects on egg viability, and only the sublethal dose of dinotefuran had significant effects on the copulation rate in macropterous families. The four insecticides all showed significant effects on the fecundity in macropterous families at sublethal doses, which decreased in imidacloprid and dinotefuran, but increased in triazophos and fenvalerate. In addition, the macropterous families treated with dinotefuran laid fewer eggs than those treated with imidacloprid. The effects on the number of offspring per female were consistent with those on fecundity, which decreased in the imidacloprid and dinotefuran treatments and increased in the triazophos and fenvalerate treatments.

The effects of the four insecticides on reproduction in brachypterous families were similar to those in the macropterous families, and the major differences observed were in the copulation rate between brachypterous and macropterous families. Both imidacloprid and dinotefuran decreased, and triazophos significantly increased, the copulation rate in brachypterous families.

3.3 Sublethal effects of four insecticides on wing formation

The effects of sublethal doses of the four insecticides on wing formation are shown in Table 4. In macropterous families treated with acetone only (controls), the percentages of macropterous females and males were 43.53% and 52.56% respectively. In brachypterous families, these percentages were 13.68% and 21.75%. Both in macropterous and in brachypterous families, imidacloprid and dinotefuran increased the percentages of macropterous females and males. Triazophos had no obvious effects on wing formation in macropterous and brachypterous families. Fenvalerate only increased the percentages of macropterous females and males in brachypterous families, but not in macropterous families.

Table 1. Toxicity of four insecticides against *Nilaparvata lugens*

Insecticide	Slope	LD_{50} (ng pest^{-1})[a]	Relative toxicity	LD_{20} (ng pest^{-1})[a]
Imidacloprid	3.16	0.17 (±0.01)a	100.00	0.09 (±0.01)a
Dinotefuran	2.92	0.29 (±0.03)b	58.62	0.15 (±0.02)b
Triazophos	2.86	16.60 (±1.44)c	1.02	8.43 (±1.05)c
Fenvalerate	2.55	24.71 (±1.73)d	0.69	11.56 (±0.86)d

[a] Values in the same column with different letters show significant difference at the $P < 0.05$ level.

Table 2. Effects on reproduction in macropterous families caused by sublethal doses (LD_{20}) of the four insecticides[a]

Treatment	Copulation rate (%)	Fecundity (eggs per female)	Viability (%)	Number of offspring per female
Control	82.31 (±4.56)a	333.65 (±52.77)b	88.20 (±4.07)a	242.22 (±34.22)b
Imidacloprid	76.44 (±5.09)ab	229.41 (±34.88)c	90.07 (±5.42)a	157.95 (±26.01)c
Dinotefuran	70.88 (±5.42)b	174.90 (±31.06)d	86.56 (±5.30)a	107.31 (±18.39)d
Triazophos	84.42 (±6.13)a	488.63 (±43.10)a	89.46 (±4.75)a	369.02 (±45.79)a
Fenvalerate	83.61 (±4.32)a	526.22 (±64.22)a	91.00 (±5.29)a	400.38 (±76.67)a

[a] Values in the same column with different letters show significant difference at the $P < 0.05$ level.

4 DISCUSSION

The phenomenon of reproductive stimulation of pests by sublethal doses of insecticides is known as hormoligosis.[10] This phenomenon has been observed in several pests treated with different insecticides, such as the increased fecundity seen in green peach aphids [*Myzus persicae* (Sulzer)] and cotton aphids (*Aphis gossypii* Glover) following azinphos-methyl and bifenthrin treatments respectively.[11,12] Similarly, increased oviposition rates have been observed in citrus thrips [*Scirtothrips citri* (Moulton)] treated with dicofol and malathion, and in two-spotted spider mites (*Tetranychus urticae* Koch) treated with either carbaryl, DDT or imidacloprid.[13–15] In the case of BPH, insecticide hormoligosis has been previously reported for insects treated with diazinon, monocrotophos, malathion, deltamethrin and triazophos.[16–19] In the present study, insecticide hormoligosis was also observed when BPH was treated with triazophos and fenvalerate, in both macropterous and brachypterous families. Although the use of fenvalerate is officially forbidden in paddy fields in mainland China, BPH may be exposed to fenvalerate occasionally when farmers illegally use this insecticide to control caterpillars in some areas. Triazophos is an important insecticide for the control of the rice stem borer, *Chilo suppressalis* (Walker), in China, so BPH can often become exposed to this insecticide, as the two pests often occur concurrently. To save money, farmers are frequently using insecticide doses that are lower than the recommended field rate. This practice, combined with the short residual toxicity of many commercial insecticides, will often lead to BPH being exposed to sublethal doses. In paddy fields, when BPH populations develop resistance to the insecticides, the effective dose becomes a sublethal dose, which provides another route by which BPH may be exposed to sublethal insecticide doses. In both these cases, low doses of some insecticides are likely to increase the reproductive rate of BPH and may eventually lead to the resurgence of this pest following treatment.[16,17]

Widiarta *et al.*[20] reported that a sublethal dose of imidacloprid could reduce the fecundity of two green leafhopper species, *Nephotettix virescens* (Distant) and *Nephotettix cincticeps* Uhler, with treated insects producing less than half the number of eggs laid by untreated individuals. This phenomenon of reduced fecundity induced by sublethal doses of imidacloprid was also observed in the cotton aphid.[21] In the present study, lower fecundity was also observed in BPH treated with sublethal doses of imidacloprid and dinotefuran. In macropterous families, the fecundities (eggs per female) of BPH treated with imidacloprid and dinotefuran were ca 69% and 52% of the untreated control insects. In brachypterous families, these values fell to ca 58% and 43% respectively. The results presented here show that the sublethal effects of these two insecticides on fecundity in the brachypterous families are more marked than in the macropterous families. Moreover, the effects of dinotefuran on fecundity are

Table 3. Effects on reproduction in brachypterous families caused by sublethal doses (LD_{20}) of the four insecticides[a]

Treatment	Copulation rate (%)	Fecundity (eggs per female)	Viability (%)	Number of offspring per female
Control	84.15 (±3.94)b	416.74 (±61.16)b	87.43 (±5.16)a	306.61 (±42.91)b
Imidacloprid	75.49 (±5.28)c	241.16 (±30.44)c	88.27 (±7.25)a	160.70 (±23.38)c
Dinotefuran	72.22 (±4.13)c	179.49 (±42.16)d	86.42 (±5.70)a	112.02 (±17.50)c
Triazophos	91.76 (±3.99)a	522.22 (±59.40)a	91.36 (±4.29)a	437.79 (±65.74)a
Fenvalerate	89.88 (±4.26)ab	576.07 (±63.28)a	90.41 (±6.47)a	468.12 (±73.82)a

[a] Values in the same column with different letters show significant difference at $P < 0.05$ level.

Table 4. Percentages of macropterous females and males in macropterous and brachypterous families treated with sublethal doses of four insecticides[a]

Treatment	Females in macropterous families	Males in macropterous families	Females in brachypterous families	Males in brachypterous families
Control	43.53 (±3.26)a	52.56 (±3.57)a	13.68 (±1.47)a	21.75 (±2.42)a
Imidacloprid	65.27 (±4.22)b	66.23 (±3.29)b	35.77 (±4.02)c	33.28 (±2.57)c
Dinotefuran	74.19 (±5.37)c	72.01 (±3.32)c	43.19 (±3.21)d	38.72 (±2.79)d
Triazophos	46.24 (±4.70)a	48.88 (±2.95)a	15.23 (±2.18)a	23.06 (±3.39)a
Fenvalerate	48.84 (±6.79)a	55.71 (±4.34)a	22.49 (±4.76)b	28.49 (±3.15)b

[a] Data in the table show the percentages of macropterous females in macropterous families (column 2) and in brachypterous families (column 4), and the percentages of macropterous males in macropterous families (column 3) and in brachypterous families (column 5). Values in the same column with different letters show significant difference at the $P < 0.05$ level.

more significant than those of imidacloprid in both macropterous and brachypterous families. These results provide convincing arguments in support of the continued use of neonicotinoid insecticides in BPH control, especially in the case of dinotefuran.

Another interesting phenomenon observed in the present study is the significant effect of imidacloprid and dinotefuran on wing formation in BPH. Both in macropterous and in brachypterous families, sublethal doses of imidacloprid and dinotefuran caused significant induction of macropterous adults (both females and males), when compared with the untreated controls. Imidacloprid and dinotefuran showed higher induction effects of macropterous adults in brachypterous families than in macropterous families, and also showed more marked effects when applied to macropterous females than when applied to macropterous males in all families. In all treatments, dinotefuran produced higher rates of macropterous adult induction than imidacloprid. Wing polymorphism in BPH is known to be a common and ecologically important trait. The long-winged (macropterous) adults possess long-distance migration ability. Although emigration decreases the population within the original area, the migrants initiate populations in new areas. When sublethal doses of insecticides, such as imidacloprid and dinotefuran, induce macropterous adults, the proportion of the population possessing long-distance migration ability increases and may initiate populations in new areas, which may create difficulties in controlling this pest. Additionally, the extra macropterous adults induced by insecticides could result in inaccurate predictions of pest incidence and ineffective control. If the migrant populations show some resistance to insecticides, and farmers in the colonized areas are unaware of this, the recommended field rate previously used may only be sublethal and serve to increase the pest problem.

In cotton aphids, a sublethal dose of imidacloprid has also been shown to induce alate (winged) offspring.[21] The induction of alate offspring in cotton aphids and macropterous adults in BPH might be caused by the insecticide acting on the endocrine system in a manner similar to that of the precocenes.[22–24] Alternatively, the insecticides may be affecting plant physiology, or the phenomenon may be caused by a combination of these and other, unknown mechanisms.[22,23] Juvenile hormone (JH), synthesized and released from the corpora allata (CA) endocrine glands, plays an important role in insect metamorphosis, including wing formation in BPH.[25,26] Recent studies have shown that glutamate-gated chloride channels and ionotropic glutamate receptors occur in insect CA cell membranes and mediate juvenile hormone synthesis.[27–29] There is, therefore, the potential that some insecticides targeting these membrane channels or receptors could influence JH synthesis and eventually have an effect on wing formation, such as has been shown for avermectin.[30] Imidacloprid and dinotefuran may similarly influence insect JH synthesis and result in effects on wing formation, as the insect nicotinic acetylcholine receptors (nAChRs) targeted by neonicotinoid insecticides are also important membrane receptors. However, it is not known at the present time whether nAChRs exist in the cell membrane of the CA, and ongoing studies into wing formation in BPH are currently seeking to clarify this issue.

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REFERENCES

1 Nagata T, Masuda T and Moriya S, Development of insecticide resistance in the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Appl Entomol Zool* **14**:264–269 (1979).
2 Nagata T, Insecticide resistance and chemical control of the rice planthopper, *Nilaparvata lugens* Stål. *Bul Kyushu Nat Agric Exp Station* **22**:49–164 (1982).
3 Gao HH, Wang YC, Tan FJ and You ZP, Studies on the sensitivity-level of the brown planthopper, *Nilaparvata lugens* Stål, to insecticides. *J Nanjing Agric Univ* **4**:65–71 (1987).
4 Tang ZH, Tao LM and Li Z, Resistance of insect pests to neonicotinoid insecticides and management strategies. *Chinese J Pestic Sci* **8**:195–202 (2006).
5 Liu Z, Williamson MS, Lansdell SJ, Han Z, Denholm I and Millar NS, A nicotinic acetylcholine receptor mutation (Y151S) causes reduced agonist potency to a range of neonicotinoid insecticides. *J Neurochem* **99**:1273–1281 (2006).
6 Liu Z, Han Z, Zhang L and Dong Z, The sustainable impacts of imidacloprid with lower dosages on the population growth of *Nilaparvata lugens*. *Plant Prot Technol Extension* **22**:29–33 (2002).
7 Bliss CI, The calculation of the dosage–mortality curve. *Ann Appl Biol* **22**:134–167 (1935).
8 Finney DJ, *Probit Analysis*, 3rd edition. Cambridge University Press, Cambridge, UK (1971).
9 Raymond M, Présentation d'un programme Basic d'analyse logprobit pour micro-ordinateur. *Cah ORSTOM Ser Entomol Med Parasitol* **23**:117–121 (1985).
10 Luckey TD, Insecticide hormoligosis. *J Econ Entomol* **61**:7–12 (1968).

11 Lowery DT and Sears MK, Stimulation of reproduction of the green peach aphid (Homoptera: Aphididae) by azinphosmethyl applied to potatoes. *J Econ Entomol* **79**:1530–1533 (1986).

12 Kerns DL and Stewart SD, Sublethal effects of insecticides on the intrinsic rate of increase of cotton aphid. *Entomol Exp Appl* **94**:41–49 (2000).

13 Morse JG and Zareh N, Pesticide-induced hormoligosis of citrus thrips (Thysanoptera: Thripidae) fecundity. *J Econ Entomol* **84**:1169–1174 (1991).

14 Dittrich V, Streibert P and Bathe PA, An old case reopened: mite stimulation by insecticide residues. *Environ Entomol* **3**:534–540 (1974).

15 James DG and Price TS, Imidacloprid boosts TSSM egg production. *A Monthly Report on Pesticides and Related Environmental Issues of Washington State University* **89**:1–11 (2002).

16 Haque NMM, Haq MHM and Karim ANMR, Insecticide hormoligosis on brown planthopper, *Nilaparvata lugens* (Stål), in resistant and susceptible rice varieties of Bangladesh. *Pakistan J Biol Sci* **5**:915–918 (2002).

17 Chelliah S, Influence of insecticide sprays on brown planthopper resurgence. *Internat Rice Res Newsl* **5**:10–11 (1980).

18 Alam S and Karim ANMR, Brown planthopper situation and its management in Bangladesh, in *Proceedings of the Workshop on Experiences with Modern Rice Cultivation in Bangladesh, 8–11 April 1984*. BRRI, Gazipur, Bangladesh, p. 218 (1986).

19 Zhuang Y, Shen J and Chen Z, The influence of triazophos on the productivity of the different wing-form brown planthopper *Nilaparvata lugens* (Stål). *J Nanjing Agric Univ* **22**:21–24 (1999).

20 Widiarta IN, Matsumura M, Suzuki Y and Nakasuji F, Effects of sublethal doses of imidacloprid on the fecundity of green leafhoppers, *Nephotettix* spp. (Hemiptera: Cicadellidae) and their natural enemies. *Appl Entomol Zool* **36**:501–507 (2001).

21 Conway HE, Kring TJ and McNew R, Effect of imidacloprid on wing formation in the cotton aphid (Homoptera: Aphididae). *Fla Entomol* **86**:474–476 (2003).

22 Hardie J, Nong G, Timar T, Sebok P and Honda D, Procecenes derivatives and aphid morphogenesis. *Arch Insect Biochem Physiol* **32**:493–501 (1996).

23 Ayoade O, Morooka S and Tojo S, Induction of macroptery, precocious metamorphosis, and retarded ovarian growth by topical application of Precocene II with evidence of its non-systemic allaticidal effects in the brown planthopper, *Nilaparvata lugens*. *J Insect Physiol* **42**:529–540 (1996).

24 Ayoade O, Morooka S and Tojo S, Metamorphosis and wing formation in the brown planthopper, *Nilaparvata lugens*, after topical application of Precocene II. *Arch Insect Biochem Physiol* **32**:485–491 (1996).

25 Bertuso AG and Tojo S, The nature and titer of juvenile hormone in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), in relation to wing morphogenesis and oocyte development. *Appl Entomol Zool* **37**:117–125 (2002).

26 Bertuso AG, Morooka S and Tojo S, Sensitive periods for wing development and precocious metamorphosis after precocene treatment of the brown planthopper, *Nilaparvata lugens*. *J Insect Physiol* **48**:221–229 (2002).

27 Chiang AS, Lin WY, Liu HP, Pszczolkowski MA, Fu TF, Chiu SL, *et al*, Insect NMDA receptors mediate juvenile hormone biosynthesis. *Proc Natl Acad Sci USA* **99**:37–42 (2002).

28 Chiang AS, Pszczolkowski MA, Liu HP and Lin SC, Ionotropic glutamate receptors mediate juvenile hormone synthesis in the cockroach *Diploptera punctata*. *Ins Biochem Mol Biol* **32**:669–678 (2002).

29 Liu HP, Lin SC, Lin CY, Yeh SR and Chiang AS, Glutamate-gated chloride channels inhibit juvenile hormone biosynthesis in the cockroach, *Diploptera punctata*. *Ins Biochem Mol Biol* **35**:1260–1268 (2005).

30 Cully DF, Vassilatis DK, Liu KK, Paress PS, Van der Ploeg LH, Schaeffer JM, *et al*, Cloning of an avermectin-sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. *Nature (London)* **371**:707–711 (2002).