Sublethal Effects of Virtako™ on Life Table Parameters and Wing Formation of the Brown Planthopper (Homoptera: Delphacidae)[1]

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Abstract The brown planthopper, *Nilaparvata lugens* (Stål), is a major rice pest in many Asian countries. Virtako™ (Syngenta, Switzerland) is a relatively new mixture insecticide, containing 20% chlorantraniliprole and 20% thiamethoxam, which has low mammalian toxicity and high efficacy against Lepidoptera, Coleoptera, Diptera, Hemiptera and Orthoptera. The effects of this insecticide on life table parameters and wing formation of *N. lugens* were examined in the laboratory. The results showed that Virtako could significantly reduce the fecundity of *N. lugens*, demonstrating further activity against this pest in addition to direct toxicity. The fecundity (eggs produced per female) of *N. lugens* treated with the sublethal rates (LC_{10} and LC_{30}) of Virtako was 75.6% and 59.4% of the control in macropterous cohorts, and 73.0% and 59.3% in brachypterous cohorts, respectively. The relative fitness values were 0.56 and 0.31 for treatments with the LC_{10}, and 0.53 and 0.32 for treatments with the LC_{30} in macropterous and brachypterous cohorts, respectively. Treatment with sublethal concentrations of Virtako showed the significant induction of macropterous offspring in both macropterous and brachypterous cohorts, which are important in the control of *N. lugens*, particularly in the prediction of the population dynamics.

Key Words *Nilaparvata lugens*, Virtako, sublethal effects, reproduction, wing formation

The brown planthopper, *Nilaparvata lugens* (Stål), is a major insect pest of rice in Asia. Both nymphs and adults aggregate and feed on leaf sheaths at the basal portion of rice plants. Left uncontrolled, *N. lugens* populations, particularly nymphs, can quickly cause substantial yield loss, inducing symptoms commonly called 'hopper burn' (Backus et al. 2005, Wang and Wang 2007, Yin et al. 2008). Adult *N. lugens* have 2 forms, short-winged (brachypterous) and long-winged (macropterous). Macropterous adults migrate from northern Vietnam into China during the rainy period of early summer each year, making control of this pest difficult (Syobu et al. 2002, Huang et al. 2003, Otuka 2009).

Limited utility of alternative control methods has resulted in heavy reliance on chemicals to manage *N. lugens*. Recently, most China rice growers depend solely on chemicals to manage *N. lugens*. Such continuous and indiscriminate use of one insecticide results in rapid development of insecticide resistance and exhaustion of all insecticide options in many rice-growing regions. Since 2003, severe infestations of *N.*

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lugens have occurred in many areas including southern and Yangtze River regions of China, primarily because of insecticide resistance development and resurgence of pest populations (Dale 1994, Zhai and Cheng 2006, Gao et al. 2006, Yin et al. 2008).

One important factor causing resurgence is the stimulation of reproduction and survival by sublethal concentrations of insecticide (Ling et al. 2011, Wu et al. 2001a, b). For example, resurgence of *N. lugens* occurred during 2007 - 2008 in China because of resistance of the insect pest to fipronil and overuse of that insecticide. Ling et al. (2009) reported that applications of fipronil (1.25×10^{-2}µg and 7.50×10^{-3}µg) significantly stimulated the fecundity of *N. lugens* compared with the control. However, some researchers suggested that sublethal concentrations of insecticide could contribute to pest control. For example, a sublethal effect of reduced fecundity of *N. lugens* occurred after nitenpyram treatment at LC_{30} (Zhang et al. 2010). Sial and Bruner (2010) reported that the fecundity and fertility of *Choristoneura rosaceana* (Harris) were significantly reduced at a sublethal concentration of pyriproxyfen.

Virtako™ (Syngenta, Switzerland) is a relatively new mixture insecticide developed during the 2000s and contains 20% chlorantraniliprole and 20% thiamethoxam. Thiamethoxam is presently one of the most effective chemicals for the control of sucking pests, including aphids, whiteflies, thrips, some microlepidoptera, and a number of coleopteran species (Sharma and Lal 2002). Chlorantraniliprole is a new insecticide belonging to the anthranilic diamide class. It is a potent and selective activator of insect ryanodine receptors which are critical for muscle contraction (Lahm et al. 2007). Chlorantraniliprole has been registered recently for use against insect pests, such as Lepidoptera, Coleoptera, and Diptera in several crops (Xu et al. 2008). Virtako was recently labeled for use against rice pests in China, including *N. lugens, Cnaphalocrocis medinalis* (Güenée) and *Lissorhoptrus Oryzophilus* (Kuschel).

Numerous studies have demonstrated that chemicals may cause several sublethal effects, such as reduced survival, growth, and/or fecundity of a single cohort of insects (Cutler et al. 2009, Ling et al. 2009, Zhang et al. 2010). However, little knowledge exists regarding the effects of Virtako on rice-feeding insects, especially *N. lugens*. The purpose of this investigation was to gain insight into the potential impacts of sublethal concentrations of Virtako on *N. lugens* life table parameters and wing formation in the laboratory.

Materials and Methods

Plants. The highly susceptible rice species TN1 (no resistance gene) was provided by the Guangdong Academy of Agricultural Sciences (Guangzhou, China). TN1 rice seeds were sown every 15 d in identical cement troughs by using similar soil and fertilization. Rice plants at the tillering stage were used in all experiments.

Insects and insecticides. An insecticide-susceptible *N. lugens* strain was obtained from the Guangdong Academy of Agricultural Sciences (Guangzhou, China) and was maintained successively on nonresistant TN1 rice plants at 28 ± 1°C, 70 ± 5% RH, and a photoperiod of 16:8 (L:D) h for more than 3 yrs at the Institute of Entomology, Sun Yat-Sen University, Guangzhou, China, without any exposure to insecticide. Virtako with a purity of 40.0% (WG) was produced and provided by Syngenta AG (Switzerland).

Bioassay. A bioassay using the rice-stem dipping method (Zhuang et al. 1999, Wang et al. 2008b) was conducted using 5th-instar (1 - 2 days old) brown planthopper nymphs. Virtako was diluted in distilled water to 5 concentrations, each of which was half of the next highest concentration (16.0, 8.0, 4.0, 2, 1 mg AI/L). Rice plants (about 60 d old) at tillering phase were collected. Rice stems with roots were cut and washed thoroughly, and then air dried to remove residual water. Three rice stems were grouped and dipped into the respective Virtako solutions for 30 sec. After the rice stems had air dried, the rice roots were wrapped in moistened cotton. The treated rice stems were then placed into a 500 - ml plastic cup. Thirty 5^{th}-instar nymphs were introduced into each plastic cup using a vacuum device. A distilled water treatment was used as a control. Treatments were replicated 5 times. Mortality was recorded daily. The treated insects were maintained at 28 ± 1°C and a photoperiod of 16:8 (L:D) h. Individuals were considered dead if they failed to move after being gently prodded with a fine brush.

Sublethal effects on life table parameters and wing formation. 5^{th}-instar nymphs were collected and reared separately until adulthood. Unmated adults were then divided into 4 groups: macropterous females, macropterous males, brachypterous females, and brachypterous males. They were fed on rice stems treated with sublethal concentrations (LC_{10} and LC_{30} for instars V) of Virtako for 30 sec separately, and the surviving insects were collected after 48 h. The controls were treated with distilled water only. One macropterous female and 1 macropterous male were paired and released into plastic pots (30 cm high, 10 cm diam.) with rice seedlings under controlled conditions (28 ± 1°C; 16:8 [L:D] h photoperiod) as a macropterous cohort. Brachypterous cohorts also were established by pairing unmated brachypterous females and males. Ten cohorts for the control (both macropterous and brachypterous cohorts) and Virtako treatment (both macropterous and brachypterous cohorts) were established. The experiments were conducted with 5 replications. The sublethal effects of Virtako on the life table parameters of *N. lugens* were constructed according to the method of Liu and Han (2006). One hundred neonates were collected randomly from each cohort as founders of the experimental population and reared for a generation at 28 ± 1°C and a photoperiod of 16:8 (L:D) h. Insects were transferred to fresh rearing cages after the neonates developed into 3^{rd}-stage and 5^{th}-stage nymphs, and then the survival rate from neonate to 3^{rd}-stage nymphs (Su1) and from third to 5^{th}-stage nymphs (Su2) were recorded. The emergence rate (Emr) and female ratio (Fr) were recorded. Meanwhile, the emerged males and females were thereafter collected each day and paired into families (1 female plus 1 male, 10 families for the control and Virtako treatments), which were reared in glass tubes separately. When neonates of the new generation appeared, they were counted and removed until the female died. Then, the rice plants were checked thoroughly and the numbers of unhatched eggs were counted. Females which had no eggs produced were identified as failure in copulation, and the copulation rate (Cr) was recorded. The average number of eggs produced by the copulated females was recorded as fecundity (Fy), and the eclosion rate (Ecr) was calculated as (total neonates)/(total neonates plus all unhatched eggs).

The experiments were conducted with 5 replications. The population growth index (I) was calculated as follows: $Nt = No \times Su1 \times Su2 \times Emr \times Fr \times Cr \times Fy \times Ecr$; $I = Nt/No$; relative fitness = I_T/I_C; where No is the number of individuals in the initial population, Nt is the number of individuals in the next generation, I_T is the population growth index of Virtako treatment, and I_C is the population growth index of control.

For control and Virtako treatments, 30 neonates of the new generation were reared in plastic pots (30 cm high, 10 cm diam) with rice seedlings under controlled conditions

(28 ± 1°C and 16:8 [L:D]h). Fresh rice seedlings were provided at intervals of 2 days until adult emergence. The numbers of macropterous females and males and brachypterous females and males were recorded, and the percentages of macropterous females and males were determined. The experiment was conducted with 5 replications.

Data analysis. All data were analyzed by SPSS®, version 17.0 (SPSS, Chicago, IL), and the sublethal concentration values were determined by probit analysis. Percentage data were arcsine square-root transformed before the analysis (Zar 1996). Significant differences between Virtako and control treatments were determined by using student's *t*-test and LSD test. Arcsine square-root transformed data were back transformed after analysis for presentation in text and tables.

Results

Relative toxicity of Virtako against *N. lugens*. The results revealed that the LC_{10}, LC_{30}, and LC_{50} values of instars V were 0.61, 1.85 and 3.95 mg/L (χ^2=0.52; df=3; $P > 0.05$) at 72 h after treatment, respectively ($P < 0.05$). The LC_{10} and LC_{30} levels were used as the reference of sublethal concentration in further experiments.

Effects of Virtako on life-table parameters of *N. lugens*. The results in Table 1 show that the *N. lugens* population of macropterous cohorts treated with the sublethal rates (LC_{10} and LC_{30}) of Virtako could increase 56.8 and 31.6 times in one generation, respectively, but in controls, populations increased 101.8 times; In brachypterous cohorts, populations increased 62.8 and 37.8 times in one generation after treatment with the sublethal concentrations of Virtako (LC_{10} and LC_{30}) respectively, whereas populations increased 119.3 times in control. The survival rate from neonates to 3rd-stage nymphs (Su1) and from 4th-stage to 5th-stage nymphs (Su2) in Virtako treatments (LC_{10} and LC_{30}) were significantly lower than those of the controls ($P < 0.05$). Also, there were significant differences ($P < 0.05$) in the emergence rate, copulation rate and fecundity compared with the control. Sublethal concentrations of Virtako (LC_{10} and LC_{30}) showed no significant differences in the eclosion rate and female ratio.

Sublethal concentrations of Virtako (LC_{10} and LC_{30}) significantly decreased reproduction ability of *N. lugens* ($P < 0.05$). The fecundity (eggs per female) of *N. lugens* treated with LC_{10} and LC_{30} of Virtako in both macropterous and brachypterous cohorts were 75.6% and 59.4%, and 73.0% and 59.3% of the control, respectively. The relative fitness values were 0.56 and 0.31, and 0.53 and 0.32 in both macropterous and brachypterous cohorts, respectively. This suggests that Virtako treatments could result in lower fitness values of *N. lugens*.

Sublethal effects of Virtako on wing formation of *N. lugens*. Sublethal concentrations of Virtako (LC_{10} and LC_{30}) showed significant induction ($P < 0.01$) of macropterous adults in both macropterous and brachypterous cohorts compared with the controls (Table 2). The percentages of macropterous females and males in Virtako treatments(LC_{10} and LC_{30}) were significantly different ($P < 0.01$) from the control. The percentages of macropterous females and males in macropterous cohorts treated with Virtako (LC_{10} and LC_{30}) were 1.47-fold and 1.67-fold, 1.29-fold and 1.44-fold of the control, respectively. The percentages of macropterous females and males in brachypterous cohorts were 1.68-fold and 2.77-fold, 1.24-fold and 1.77-fold of the control in LC_{10} and LC_{30} treatment families, respectively. Sublethal concentrations of Virtako increased the percentages of macropterous females and males in both macropterous and brachypterous cohorts.

Table 1. Life tables of macropterous and brachypterous cohorts of *N. lugens* treated with sublethal concentration (LC_{10} and LC_{30}) of virtako.

	Macropterous female×Macropterous male (♀×♂)			Brachypterous female×Brachypterous male (♀×♂)		
Parameter	Control	LC_{10}	LC_{30}	Control	LC_{10}	LC_{30}
N_0	100	100	100	100	100	100
Su1 (%)	87.94 ± 1.64a	83.41 ± 1.36b	78.18 ± 0.73c	87.75 ± 1.13a	83.09 ± 0.86b	78.41 ± 0.77c
Su2 (%)	92.41 ± 1.34a	86.49 ± 0.83b	80.16 ± 0.78c	92.45 ± 1.62a	86.30 ± 0.92b	80.67 ± 0.90c
Emr (%)	89.36 ± 1.01a	83.74 ± 1.15b	78.68 ± 0.80c	89.48 ± 1.05a	84.00 ± 1.05b	79.33 ± 0.89c
Fr (%)	50.31 ± 0.55a	49.94 ± 0.56a	49.76 ± 0.43a	50.37 ± 0.66a	49.61 ± 0.60a	49.46 ± 0.48a
Cr (%)	91.12 ± 1.25a	82.96 ± 1.03b	74.36 ± 0.54c	91.67 ± 1.21a	82.98 ± 0.74b	75.47 ± 0.74c
Fy	356.53 ± 20.4a	269.62 ± 14.31b	211.67 ± 14.90c	414.53 ± 22.5d	302.59 ± 19.37ab	245.69 ± 19.09bc
Ecr (%)	85.79 ± 1.13ab	84.28 ± 1.20ab	81.96 ± 0.83a	85.89 ± 1.14b	83.70 ± 1.13ab	82.24 ± 0.70ab
Nt	10182.38	5678.36	3165.18	11933.77	6280.03	3784.60
I	101.82	56.78	31.65	119.34	62.80	37.85
Relative fitness	1	0.56	0.31	1	0.53	0.32

No, number of individuals in the initial population; Su1 and Su2, survival rate from neonate to third nymphs and from third to fifth nymphs separately; Emr, emergence rate; Fr, female ratio; Cr, rate of successful mating; Fy, fecundity (eggs per female); Ecr, eclosion rate; Nt, predicted number of offspring; I, population trend index. Mean ± SE is the mean of five replicates and standard error. Values in the same row with different letters show significant difference at $P < 0.05$ level (LSD test).

Table 2. Percentages of macropterous females and males in macropterous and brachypterous cohorts treated with sublethal concentration (LC_{10} and LC_{30}) of virtako.

Treatment	Macropterous cohorts		Brachypterous cohorts	
	Females	Males	Females	Males
Control	39.48 ± 0.81a	48.89 ± 0.68a	18.84 ± 1.01a	23.94 ± 0.78a
LC_{10}	57.88 ± 0.70b	63.07 ± 0.73b	31.58 ± 0.61b	29.79 ± 0.66b
LC_{30}	65.89 ± 0.65c	70.40 ± 1.00c	52.22 ± 0.84c	42.50 ± 0.60c

Mean ± SE is the mean of five replicates and standard error. Values in the same column with different letters show significant difference at $P < 0.01$ level (Student's *t*-test).

Discussion

Previous studies report the sublethal effects of the constituent of Virtako (thiamethoxam or chlorantraniliprole) on insect pests. Knight and Flexner (2007) showed that chlorantraniliprole had significant disruption effect on mating behavior of *Cydia pomonella* (L.). Similar results were observed in our study in that both sublethal rates (LC_{10} and LC_{30}) of Virtako had significant effects on the copulation rate in both macropterous and brachypterous cohorts. Chen et al. (2011) demonstrated that chlorantraniliprole significantly decreased the egg eclosion rate of *Spodoptera exigua* (Hübner), but no significant impact on sex ratio of *S. exigua* was observed by Lai and Su (2011). In our study, there was no significant effect on the eclosion rate and female ratio in either macropterous or brachypterous cohorts with sublethal doses of Virtako, only slight reduction in egg hatch percentage was observed in high concentrate treatment.

Induction of insect fecundity under sublethal pesticide doses is a common phenomenon, particularly in mites and homopteran insects (Ge et al. 2010). Cho et al. (2011) showed that a sublethal dose of thiamethoxam increased fecundity of *Myzus persicae* (Sülzer). But in our study, the fecundity of *N. lugens* in both macropterous and brachypterous cohorts was significantly decreased with sublethal doses of Virtako, which were similar with the results on *Rhopalosiphum padi* (L.), *Aphis glycines* (Matsumura) and the imidacloprid-resistant strain of *Aphis gossypii* (Glover) treated with sublethal doses of thiamethoxam (Magalhaes et al. 2008, Shi et al. 2011, Daniels et al. 2009). In our study, the *N. lugens* population was decreased at least in the F_1 generation after application of sublethal doses of Virtako. These results suggest that fecundity seems to be highly dependent on the type of pesticide and the exposed insect species. Furthermore, our results suggest that Virtako could act as an effective alternative to conventional insecticides in the control of *N. lugens*.

The population of *N. lugens* with different wing forms had different dispersal and migration abilities. Macropterous of *N. lugens* possessed long-distance migratory ability and are adapted to search new habitats; whereas, brachypterous adult females had higher fecundity than macropterous females and were adapted to establish and remain in a suitable habitat (Bertuso and Tojo 2002, Zhang et al. 2004). Previous experiments have demonstrated that not only temperature, humidity, light, crowding, and host-plant nutritional factors could affect the wing formation of *N. lugens* (Kisimoto 1965, Bertuso et al. 2002, Syobu et al. 2002, Zhang et al. 2004), but also many insecticides induced a higher percentage of macropterous or winged offspring in *N. lugens* and some other insect pests (Ayoade et al. 1996, Conway et al. 2003, Wang et al. 2008a, Bao et al. 2009, Zhang et al. 2010). Some research shows that insecticide-induced increases in macropterous progeny in *N. lugens* might be caused by action on the endocrine system in a way similar to that of precocenes (Ayoade et al. 1996, Bao et al. 2009), or it impacted on the plant physiology, or a combination of these or other unknown mechanisms (Ayoade et al. 1996, Conway et al. 2003, Bao et al. 2009). In our research, both in macropterous and brachypterous cohorts, Virtako significantly increased the percentages of macropterous offspring of *N. lugens*, and also showed higher induction effects on macropterous adults in brachypterous than macropterous cohorts. The detailed mechanisms of Virtako-induced increased percentages of macropterous offspring of *N. lugens* should be investigated further. When some insecticides induce macropterous adults, the percentage of macropterous offspring and long-distance migration ability would increased, which creates difficulties in controlling this pest. Further investigations on mechanisms of Virtako-induced increased the percentages of macropterous offspring would be helpful to the rational application of pesticides to minimize their side effects.

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