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# Sublethal effects of triazophos on the life table parameters of *Sogatella furcifera* (Hemiptera: Delphacidae)

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## Abstract

*Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae), the white-backed planthopper, is a serious pest insect in rice (Poales: Poaceae) paddy fields in China and elsewhere in Asia, and it is primarily controlled using chemical methods. Triazophos is a broad-spectrum, non-systemic organophosphorus insecticide used for pest control in rice paddy fields. In this paper, we examined the sublethal effects of triazophos on nymphal duration and life table parameters of *S. furcifera* in the laboratory. The results showed that sublethal concentrations of triazophos could significantly prolong nymphal duration in *S. furcifera*. Relative to the control, exposure to LC10 and LC25 extended the nymphal period by 0.92 and 3.16 d, respectively. A fitness analysis was performed by constructing a life table in the F1 generation and showed that sublethal concentrations of triazophos could stimulate reproduction in *S. furcifera* populations. Various life table parameters were depressed under sublethal concentrations of triazophos, but fecundity was the exception. The fecundity rates (eggs per female) in LC10 and LC25 triazophos treatments were 116.2 and 131.9%, respectively, that of the control in the 2nd generation. The relative fitness of *S. furcifera* planthoppers treated with LC10 and LC25 of triazophos was 77.6 and 63.6%, respectively, compared with the control. Sublethal concentrations of triazophos inhibited population growth relative to the control group based on the predicted number of offspring. However, the possibility of oviposition stimulation at sublethal concentrations should be considered carefully if triazophos is applied to control white-backed planthoppers and other rice pests in a rice field habitat.

Key Words: insecticide; white-backed planthopper; fitness analysis; population growth; reproductive stimulation

## Resumen

*Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae), el saltador de plantas de dorso blanco, es un insecto plaga seria del arroz (Poales: Poaceae) en campos de arroz en China y otras partes de Asia, y se controla principalmente por medio de métodos químicos. Triazofos es un insecticida organofosforado no sistémico de amplio espectro, utilizado para el control de plagas en los campos de arroz. En el presente trabajo, hemos examinado los efectos subletales de triazofos sobre los parámetros de duración de ninfas y de la tabla de vida de *S. furcifera* en el laboratorio. Los resultados mostraron que las concentraciones subletales de triazofos podrían prolongar de forma significativa la duración de ninfas de *S. furcifera*. En relación con el control, la exposición a CL10 y CL25 extendió el período ninfal por 0,92 y 3,16 días, respectivamente. Se realizó un análisis de la aptitud mediante la construcción de una tabla de vida en la generación F1 y mostró que las concentraciones subletales de triazofos podrían estimular la reproducción de las poblaciones de *S. furcifera*. Varios parámetros de tabla de vida fueron deprimidos bajo concentraciones subletales de triazofos, pero la fecundidad fue la excepción. Las tasas de fecundidad (huevos por hembra) en los tratamientos de CL10 y CL25 triazofos fueron 116,2 y 131,9%, respectivamente, la del control en la segunda generación. La aptitud relativa de los saltadores de plantas *S. furcifera* tratados con LC10 y CL25 de triazofos fue de 77,6 y 63,6%, respectivamente, en comparación con el control. Concentraciones subletales de triazofos inhibió el crecimiento de la población con relación al grupo de control basado en el número previsto de progenies. Sin embargo, la posibilidad de estimulación de la oviposición en concentraciones subletales se debe considerar cuidadosamente si triazofos se aplica para controlar saltadores de las plantas de dorso blanco y otras plagas del arroz en el hábitat de arroz.

Palabras Clave: insecticidas; saltahoja blanco respaldado; análisis de aptitud; crecimiento de la población; estimulación reproductiva

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Planthoppers, including the white-backed planthopper, *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae), are the most important sucking insects feeding on rice (*Oryza sativa* L.; Poales: Poaceae) (Tan et al. 2004). *Sogatella furcifera* is a typical large-scale migratory rice pest in Asia. It feeds on the phloem of rice plants and causes losses in yield. These specialist herbivores use stylets to pierce plant cells and consume large quantities of fluids as their source of nutrition. Feeding by a large number of planthoppers causes drying of the rice leaves and wilting of the tillers, a phenomenon called “hopperburn” (Tan et al. 2004). An additional problem is the infection of rice plants with south-

ern rice black-streaked dwarf virus transmitted to rice seedlings by feeding white-backed planthoppers. This virus was first discovered in Yangxi County, Guangdong Province, China, in 2001, and was limited to southern China from 2002 to 2008 (Zhou et al. 2008; Wang et al. 2010). Although great efforts were undertaken to control the disease, it damaged rice of more than 700,000 ha in 2011 and over 500,000 ha in 2012 in northern Vietnam and southern China. Moreover, the disease has now been found in some areas in Japan (Matsukura et al. 2013). Control measures based on protecting seedlings from *S. furcifera*, including seedbed coverage, chemical seed treatments, and chemical

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sprays, have proven effective against the spread of southern rice black-streaked dwarf virus in China (Zhou et al. 2013).

Resurgence of some pests after insecticide application on rice is becoming common in Asia. Insecticidal sprays are designed to be extremely effective at killing targeted pest insects but may also adversely affect non-target natural pest enemies (Gentz et al. 2010) through both lethal and sublethal effects. Sublethal effects include changes in the life history traits of insects, such as parasitism rate, longevity, egg viability, feeding rate, or behavior (Ruberson et al. 1998). Bao et al. (2009) performed a detailed study of these sublethal effects in an attempt to better explain the diverse actions of insecticides on *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), the brown planthopper, including potential effects on its endocrine system.

Organophosphorus insecticides are used against rice pests throughout Asia. In recent years, the most highly toxic and long lasting organophosphorus pesticides, for example, parathion, methyl parathion, and methamidophos, have been banned by the Chinese Ministry of Agriculture (Jin et al. 2012). Triazophos (O, O-diethyl O-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate) is a broad-spectrum organophosphate insecticide. It provides a good alternative to more harmful pesticides, is widely used on a variety of crops, and is now one of the most important pesticides for controlling cotton bollworms (*Helicoverpa armigera* Hübner; Lepidoptera: Noctuidae) (Liu 1999). Zhuang et al. (1999) found that triazophos increased the reproduction in macropterous and brachypterous adults of *S. furcifera* significantly. When triazophos was applied to control the rice stem borer and the paddy borer, the oviposition in *S. furcifera* increased. However, there are very few experimental studies on the effects of triazophos on *S. furcifera*. We therefore set out to study the sublethal effects of triazophos on the life table parameters of *S. furcifera* in order to evaluate triazophos use in rice fields.

## Materials and Methods

### INSECTS AND INSECTICIDES

*Sogatella furcifera* planthoppers were collected from rice fields and maintained at the Guizhou Key Laboratory for Agricultural Pest Management of Mountainous Region (Guiyang, China) over a 4 yr period without exposure to any insecticide. Third instar nymphs and macropterous adults of *S. furcifera* were tested in this study. Triazophos (83.56%, technical formulation) was obtained from Guangxi Tianyuan Biochemistry Corp., Ltd. (Guangxi, China).

### BIOASSAY

The rice cultivar Taichung Native 1 (TN-1) was used in this study. Bioassays were undertaken using the rice stem dipping method (Zhuang et al. 1999; Wang et al. 2008) as follows. Five concentrations of triazophos were prepared in distilled water (60, 30, 15, 7.5 and 3.75 mg active ingredient [AI] per L). Tillering rice plants, about 50 d old, were collected, and stems with roots were cut to 12 cm long, washed thoroughly, and then air dried to remove residual water. Three rice stems were grouped and dipped into a given triazophos solution for 30 s. After the rice stems had dried, moistened absorbent cotton was wrapped around the rice roots. The treated rice stems were placed into a wide-mouth bottle (165 mm high × 80 mm base diameter, 500 mL volume). One hundred 3rd instar nymphs or macropterous adults were introduced into each wide-mouth bottle by using a suction trap. Rice stems treated with distilled water were used as a control. Both control and insecticide treatments were replicated 5 times. Mortality was recorded at intervals of 24 h. The treated insects were maintained

at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and a photoperiod of 16:8 h L:D. Individual planthoppers were considered dead if they showed no response after being gently prodded with a fine brush. To ensure that the fresh rice seedlings were healthy, they were cultivated in rearing cages. The rice seedlings in all tests were regularly replaced with fresh ones.

### LIFE TABLE CONSTRUCTION

The sublethal effects of triazophos on the life table parameters of *S. furcifera* were evaluated by the following experiment. About 600 neonates were collected and reared separately in hard glass tubes (300 mm high × 30 mm diameter) open at both ends with 10 neonates per tube until insecticide was introduced. The 3rd instar nymphs were fed on rice stems treated with sublethal concentrations (LC10 and LC25) of triazophos for 30 s, and the surviving insects (parental [P] generation for this experiment) were collected 48 h after feeding. The control nymphs were fed on rice stems treated with distilled water. The survivors were then collected and transferred to tubes stocked with healthy, untreated rice seedlings. After emergence of the surviving P adults, 1 macropterous female and 1 male adult were paired in glass tubes and reared on healthy, untreated rice seedlings under controlled conditions ( $25 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, photoperiod of 16:8 h L:D) in an artificial climate box. Ten pairs of the P adults for each replication were established for the control and each triazophos treatment. The experiment was replicated 5 times.

The sublethal effects of triazophos on the life table parameters of *S. furcifera* were analyzed using the method of Liu & Han (2006). One hundred neonates of the F1 generation for each replication were collected at random from each P pair as founders of the experimental population and reared in the control conditions described above. The planthoppers were transferred to fresh rearing tubes and assessed for survival rate from neonate to 3rd instar (Sr1) and from 4th to 5th instar (Sr2). The emerged male and female adults (F1 generation) were thereafter collected every day, and 10 pairs for each replication were established for the control and each triazophos treatment. Meanwhile, the emergence rate (Er) and ratio (percentage) of females (Fr) were recorded. Once the neonates of the new generation (F2) hatched, the pairs were checked every 2 d, and the neonates were counted and removed until the female died. The food rice shoots were then checked thoroughly, and the numbers of unhatched eggs (F2 generation) were recorded. The copulation rate (Cr) was calculated based on the proportion of females that produced neonates. Fecundity (Fd) was recorded as the average number of eggs produced by copulated females, and the hatchability (Ha) was recorded as the number of all neonates divided by the number of all neonates plus all unhatched eggs. The experiment was replicated 5 times.

The population trend index (I) and relative fitness were calculated as follows:

$$N_t = N_0 \times Sr1 \times Sr2 \times Er \times Fr \times Cr \times Fd \times ha$$

$$I = N_t/N_0$$

$$\text{Relative Fitness} = L_t/L_c$$

Where  $N_0$  is the number of individuals in the initial population,  $N_t$  is the number of individuals in the population of the next generation,  $I_t$  is the population growth index in the triazophos treatments, and  $I_c$  is the population growth index in the control. Relative fitness is the ratio of the population growth index in the triazophos treatments to the population growth index in the control.

### DEVELOPMENT DURATION

To assess the nymphal duration of *S. furcifera*, 100 neonates of the F1 generation were collected randomly from each of the adult pairs (of

the treated P generation) as founders of the experimental population. These neonates were reared on untreated, healthy rice seedlings at  $25 \pm 1^\circ\text{C}$  and a photoperiod of 16:8 h L:D. The instar number of the nymphs was recorded twice daily (at 1000 and 1700 h).

STATISTICAL ANALYSES

The data were analyzed using Statistical Product and Service Solutions 16.0 (SPSS 16.0; IBM, New York, New York), and the sublethal concentration values were determined using Probit model analysis. Significant differences between the triazophos treatments and the control were tested using Student's *t*-test and the least significant difference (LSD) test.

Results

RELATIVE TOXICITY OF TRIAZOPHOS TO *S. FURCIFERA*

The sensitivity of the 3rd instar nymphs and adults was determined for each rice stem impregnation method, and detailed results are shown in Table 1. Based on this method and 5 replications, the LC50, LC25, and LC10 values of triazophos to 3rd instars 72 h after treatment were 13.63, 5.86, and 2.74 mg [AI] per L, respectively ( $P < 0.05$ ) (Table 1). The sublethal concentrations LC25 and LC10 were used as the reference in further experiments (Table 1).

NYMPHAL DURATION OF *S. FURCIFERA*

The F1 progeny in populations derived from adults that had been treated as 3rd instar nymphs with sublethal concentrations of triazophos had longer development periods compared with the control population (Table 2). The entire nymphal periods in F1 populations after treatment of the P generation with LC10 and LC25 were delayed by 0.92 and 3.16 d, respectively, compared with the control population. Compared with the control, every nymphal instar at LC10 was prolonged, except for the 3rd instar. The development period of every instar at LC10 was shorter than of those at LC25. The nymphal durations at LC25 were significantly longer than at LC10 and the control ( $P < 0.05$ ). These results indicated that sublethal concentrations had a more negative effect on *S. furcifera* nymphal duration at LC25 than at LC10 when the parental generation was fed on rice seedlings treated with triazophos.

INFLUENCE OF TRIAZOPHOS ON THE LIFE TABLE PARAMETERS OF *S. FURCIFERA*

The results in Table 3 show that the *S. furcifera* populations from F1 to F2 increased 33.69 and 27.63 times when the parental generation had been treated with triazophos at LC10 and LC25, respectively, whereas the population increased 43.44 times in the control. The survival rates of neonates to 3rd instar and from 4th to 5th instar were sig-

nificantly lower when the parental generation had been treated with triazophos compared with the control ( $P < 0.05$ ).

The emergence rate and copulation rate were both reduced after the parental generation had been treated with sublethal concentrations of triazophos. The emergence rate of white-backed planthoppers whose parents had been treated with LC10 showed no significant variation compared with the control and LC25 treatments, whereas the control and LC25 treatments showed significant differences between them ( $P < 0.05$ ). The copulation rates of white-backed planthoppers whose parents had been treated with triazophos at LC10 and LC25 were 73.6 and 68.8%, respectively, and were significantly lower than in the control ( $P < 0.05$ ). The female ratio showed no obvious variation ( $P < 0.05$ ) and was approximately 50% in both treatments and the control (Table 3). The percentage of hatching neonates in the F2 generation was 86.9, 79.6, and 77.2% for the control, LC10, and LC25, respectively. The variation in emergence rate between the control and both treatments was similar.

The fecundity (eggs per female) of white-backed planthoppers whose parents had been treated with distilled water, LC10, and LC25 was 154.1, 179.0, and 203.2, respectively. Sublethal concentrations of triazophos significantly increased the reproductive parameters, especially at the higher sublethal concentration (LC25) ( $P < 0.05$ ). These results show that sublethal concentrations of triazophos can stimulate the reproductive output of mated adults. However, the relative fitness of white-backed planthoppers whose parents had been treated with LC10 and LC25 decreased to 0.78 and 0.64, respectively. The relative fitness reached four-fifths and three-fifths of that of the control and suggested that treatment with triazophos could result in decreased fitness of *S. furcifera* relative to the control.

Discussion

Rice planthopper control programs rely primarily on the spraying of chemical insecticides. The effects of insecticides on insects are twofold: mortality resulting from the direct toxic effect of the compound; and the effect of sublethal doses of insecticides on insect life history parameters and behavior (Kumar & Chapman 1984). Sublethal effects can be defined as physiological or behavioral in nature and act on individuals that survive exposure to a toxic compound at sublethal concentrations (Desneux et al. 2007). Sublethal effects result when insects absorb insufficient amounts of the pesticide to cause death. Sublethal doses of insecticides have been shown to cause latent toxicity, enzyme induction, stimulatory and inhibitory effects on reproduction, and altered behavior and physiology (Moriarty 1969).

Contrasting results to ours were found in tests of toxicity of insecticides to 3rd instar nymphs of *N. lugens* using the rice stem-dipping method and various concentrations of insecticide (Samer et al. 2009). LC2.5 and LC10 concentrations of imidacloprid clearly stimulated the reproduction in *N. lugens*. However, fecundity at LC50 and LC90 was significantly less than in the control (Samer et al. 2009). Stimulation of insect fecundity induced by sublethal concentrations of insecticides

Table 1. Toxicity of triazophos to *Sogatella furcifera*.

Development stage	LC90 (mg [AI] L <sup>-1</sup> ) (95% CL)	LC50 (mg [AI] L <sup>-1</sup> ) (95% CL)	LC25 (mg [AI] L <sup>-1</sup> ) (95% CL)	LC10 (mg [AI] L <sup>-1</sup> ) (95% CL)	Slope ± SE	χ <sup>2</sup>	df
Nymph	67.73 (51.49–98.69)	13.63 (11.51–16.01)	5.86 (4.45–7.23)	2.74 (1.80–3.71)	1.84 ± 0.17	0.55	3
Adult	73.32 (56.18–105.61)	16.51 (14.11–19.30)	7.54 (5.91–9.10)	3.72 (2.57–4.87)	1.98 ± 0.18	3.55	3

CL, Confidence interval. χ<sup>2</sup>, Chi-squared value.

**Table 2.** Nymphal duration of *Sogatella furcifera* populations exposed to water (control) or sublethal concentrations (LC10, LC25) of triazophos.

Population	Nymphal stage duration (d) $\pm$ SE <sup>a</sup>					Total
	1st instar	2nd instar	3rd instar	4th instar	5th instar	
Control	3.28 $\pm$ 0.21a	2.28 $\pm$ 0.11a	2.46 $\pm$ 0.17a	2.43 $\pm$ 0.15a	3.37 $\pm$ 0.20a	13.81 $\pm$ 0.15a
LC10	3.44 $\pm$ 0.20a	2.62 $\pm$ 0.06ab	2.44 $\pm$ 0.05a	2.58 $\pm$ 0.22a	3.66 $\pm$ 0.28a	14.73 $\pm$ 0.68a
LC25	4.22 $\pm$ 0.14b	2.87 $\pm$ 0.17b	3.06 $\pm$ 0.17b	2.78 $\pm$ 0.15a	4.04 $\pm$ 0.26a	16.97 $\pm$ 0.67b

<sup>a</sup>Mean values ( $\pm$  SE) in the same column followed by different letters denote significant differences at the  $P \leq 0.05$  level using the LSD test.

is a common phenomenon, particularly in homopteran insects and in mites (Ge et al. 2010). The present study showed that sublethal concentrations of triazophos can increase fecundity in *S. furcifera*. The fecundity (eggs per female) of the planthoppers treated with LC25 was 131.9% that of the control, suggesting that sublethal concentrations of triazophos could significantly stimulate reproduction in *S. furcifera*. The mechanisms by which the fecundity of *S. furcifera* is increased by triazophos application should be considered if its use in controlling rice stem borers and paddy borers is to be continued. Qin et al. (2013) deduced that the relative fitness values of *N. lugens* were 0.55, 0.41, 0.21, and 0.09 following treatment with LC20, LC30, LC40, and LC50, respectively, of the insecticide paichongding (1-[(6-chloropyridin-3-yl)methyl]-7-methyl-8-nitro-5-propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2- $\alpha$ ] pyridine).

Pesticide-induced homeostatic modulation has been suggested as a term to broadly include both hormesis and stimulatory effects of pesticides on non-target pests. The specific role played by pesticide-induced homeostatic modulation in inducing pest outbreaks in agroecosystems is difficult to evaluate because other complex environmental factors are probably also involved (Cohen 2006). Hormesis is a biphasic dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition (Calabrese 2008). It is a dose- and time-dependent phenomenon in which effects are displayed as a result of exposure to a certain sublethal dose range of an applied pesticide. Our study shows that treatment with various concentrations of insecticide has differing effects, including changes in nymphal duration and life table parameters. Although, on the whole, the population of the next generation of *S. furcifera* may decline, various sublethal concentrations of triazophos significantly stimulated reproduction. Resurgence in arthropod pest numbers following applications of chemical pesticides could be the result of disruptions of the biological equilibrium by elimination or drastic

reduction of arthropod species that regulate the pest population or compete for food or space within a given ecological niche, and thus represent either direct or indirect effects on the pest population (Hardin et al. 1995).

In conclusion, the organophosphorus insecticide triazophos could stimulate reproduction in *S. furcifera* as demonstrated by treatments with specific sublethal concentrations in the laboratory. This effect may be due to stimulatory effects on the physiological and biochemical mechanisms involved in reproduction and growth. Alternatively, insecticide applications to insect host plants are known to result in improvement of the plants' nutritional quality, thus leading to better reproduction in the insects feeding on them (Suri & Singh 2011). Field application of insecticides may result in sublethal levels at some time after application. Understanding how individual insects and their populations respond to insecticides should help in the selection of appropriate and improved treatment plans. In particular, future research should focus on exploring the molecular mechanisms that stimulate reproduction in *S. furcifera* planthoppers when treated with sublethal concentrations of triazophos.

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**Table 3.** Life table of *Sogatella furcifera* after treatment of the parental generation with water (control) or sublethal concentrations (LC10, LC25) of triazophos.

Parameter	Control	LC10	LC25
$N_0$	100	100	100
Sr1 (%)	91.40 $\pm$ 2.06a	86.80 $\pm$ 3.92ab	78.20 $\pm$ 3.95b
Sr2 (%)	94.11 $\pm$ 0.47a	88.32 $\pm$ 1.20b	81.99 $\pm$ 2.80c
Er (%)	90.24 $\pm$ 1.61a	86.16 $\pm$ 4.01ab	78.91 $\pm$ 2.04b
Fr (%)	49.30 $\pm$ 1.76a	48.66 $\pm$ 2.59a	50.60 $\pm$ 2.19a
Cr (%)	84.80 $\pm$ 2.94a	73.60 $\pm$ 4.12b	68.80 $\pm$ 3.20b
Fd	154.06 $\pm$ 12.86a	178.98 $\pm$ 14.49ab	203.22 $\pm$ 15.31b
Ha (%)	86.90 $\pm$ 2.00a	79.57 $\pm$ 3.65ab	77.19 $\pm$ 2.66b
$N$	4344.44	3368.90	2762.91
$I$	43.44	33.69	27.63
Relative fitness	1.00	0.7755	0.6360

$N_0$ , number of individuals in the initial population; Sr1 and Sr2, survival rate from neonate to 3rd instar and from 4th to 5th instar, respectively; Er, emergence rate; Fr, female ratio; Cr, rate of successful mating; Fd, fecundity (eggs per female in F2); Ha, hatchability;  $N$ , predicted number of offspring;  $I$ , population trend index. Mean ( $\pm$  SE) of 5 replicates. Values in the same row with different letters denote significant differences at  $P \leq 0.05$  (LSD test).

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