

Mating pair combinations of insecticide-treated male and female *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) planthoppers influence protein content in the male accessory glands (MAGs) and vitellin content in both fat bodies and ovaries of adult females

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ABSTRACT

The brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is an insect pest in which offspring are produced by the mating of adult males with adult females. This species is a classic case in which pest resurgence is induced by insecticides. In the past, studies of resurgence mechanisms have focused on insecticide-induced stimulation of reproduction in adult females. To date, however, the role that males play in the resurgence mechanisms of *N. lugens* has not been investigated. The aim of the present study is to examine changes in protein levels in male accessory glands (MAGs) induced by the insecticides triazophos and deltamethrin and to determine their relationship with vitellin content in the fat bodies and ovaries of adult females in the context of mating pairs. Our results show that protein content in MAGs is significantly affected by male mating status, insecticide type, and insecticide concentration. Insecticide application induced increased protein levels in MAGs. A greater quantity of MAG products was transferred to females via mating. Thus, protein levels in MAGs significantly decreased after mating. Experimental matings indicate that vitellin content in both fat bodies and ovaries of adult females in mating pairs consisting of a treated male and an untreated female ($\text{♂}_t \times \text{♀}_{\text{ck}}$) is significantly greater than that of females in pairs consisting of an untreated male and an untreated female ($\text{♂}_{\text{ck}} \times \text{♀}_{\text{ck}}$). Under various concentrations of the two insecticides, vitellin levels are highest in mating pairs consisting of a treated male and a treated female ($\text{♂}_t \times \text{♀}_t$), followed by mating pairs consisting of an untreated male with a treated female ($\text{♂}_{\text{ck}} \times \text{♀}_t$). These findings demonstrate that (1) insecticides have an effect on males; (2) insecticide effect can be transferred to females; and (3) the reproductive effect of insecticides is strongest in mating pairs in which both the males and females are treated compared to pairs in which only one individual is treated. These findings provide valuable information about the role of males in pesticide-induced resurgence of *N. lugens*.

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1. Introduction

The brown planthopper *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is a classical insecticide-induced resurgent pest [1,2]. Among insecticides, pyrethroids (deltamethrin) and organophosphates (triazophos, methamidophos, parathion and diazinon) are known to induce population resurgence of this species [3–6]. Physiological mechanisms for the pesticide-induced resurgence of *N. lugens* involve stimulation of fecundity [4,6–8]. Thus, insecticide-induced stimulation of fecundity is one of the main causes of *N. lugens* resurgence. However, studies of insecticide-induced stimulation of pest fecundity have concentrated on the reproduction of

adult females while ignoring insecticide-induced reproductive effects in adult males. *N. lugens* reproduces sexually. Therefore, we hypothesize that insecticide-induced reproductive effects in males can be transferred to females via mating and hence this process influences female reproduction. This effect is not well understood.

For insects with sexual mating, several studies have examined the effect of male mating factors (accessory gland products, AGPs) on adult female reproductive performance. Males transfer not only sperm but also AGPs, which may be expensive to produce, and the amount or quality of these substances may decrease with increasing male mating frequency [9,10]. AGPs transferred during mating are known to affect several behaviors in insects, among which reduced female receptivity and increased oviposition are the most common [11,12]. In the variable field cricket *Gryllus lineaticeps*

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Stål, female life span is augmented by AGPs [13]. Male diet and larval host influence the duration of copulation, but male diet does not influence female fecundity. However, mating order and male larval diet influence female fertilization success [14]. Many studies of other insects have also demonstrated that male accessory gland peptides regulate reproductive performance. For example, the adult male accessory glands of *Drosophila melanogaster* Meigen synthesize and secrete a peptide that represses female sexual receptivity and stimulates oviposition [15]. Multiple mating in the female moth *Utetheisa ornatrix* (L.) increases fecundity but does not increase longevity or egg mass [16]. Lay et al. [17] have found a greater role of male *Locusta migratoria* (L.) in reproduction, with peptides from white secretions of the male accessory glands (MAGs) found in developing eggs. In addition, the fecundity of adult female *Spodoptera litura* F. increases when females mate with male moths with high quality sperm [18]. More interestingly, Pszczolkowski et al. [19] have found that in *Heliothis virescens* (F.), juvenile hormone (JH) stored in the MAGs is transferred to females via mating and that this process promotes JH synthesis and egg development in mated females. However, the effect of insecticides on adult male reproduction and the transference of this effect to the reproductive performance of adult females via mating have not been studied to date.

The present study is designed to examine the effects of sublethal doses of two common insecticides, deltamethrin and triazophos, that stimulate the fecundity of *N. lugens* on protein content in MAGs and on vitellogenin in fat bodies and vitellin in ovaries of adult female *N. lugens*. We utilize controlled matings between insecticide-treated males and females and control males and control females. Our aim is to understand the effect of insecticide-treated males on the reproductive performance of females via the mating process.

2. Materials and methods

2.1. Rice variety

Rice (*Oryza sativa* L.) variety Huai Dao 13 (japonica rice) was used in trials. This variety of rice was selected because it is commonly planted in Jiangsu Province, China. Seeds were sown outdoors in a standard rice-growing soil in cement tanks (height 60 cm, width 100 cm and length 200 cm). When seedlings reached the six-leaf stage, they were transplanted into 16-cm diameter plastic pots with four hills per pot and three plants per hill. Rice plants used in experiments had reached the tillering stage.

2.2. Insects

A laboratory colony of *N. lugens* that was originally obtained from the China National Rice Research Institute (CNRRI; Hangzhou, China) was reared in a greenhouse at an ecological laboratory at Yangzhou University.

2.3. Insecticides

Two insecticides were used in trials: the pyrethroid 2.5% deltamethrin EC (Yangnong Chemical Co. Ltd., Yangzhou, Jiangsu, China) and the organophosphate 20% triazophos EC (Changqin Pesticide Co. Ltd., Jiangdu, Jiangsu, China).

2.4. Experiments

Four concentrations of each insecticide (1, 3, 6 and 12 ppm of deltamethrin and 10, 20, 40 and 80 ppm of triazophos) were selected based on previous results from a sublethal test [6,20]. Eighty

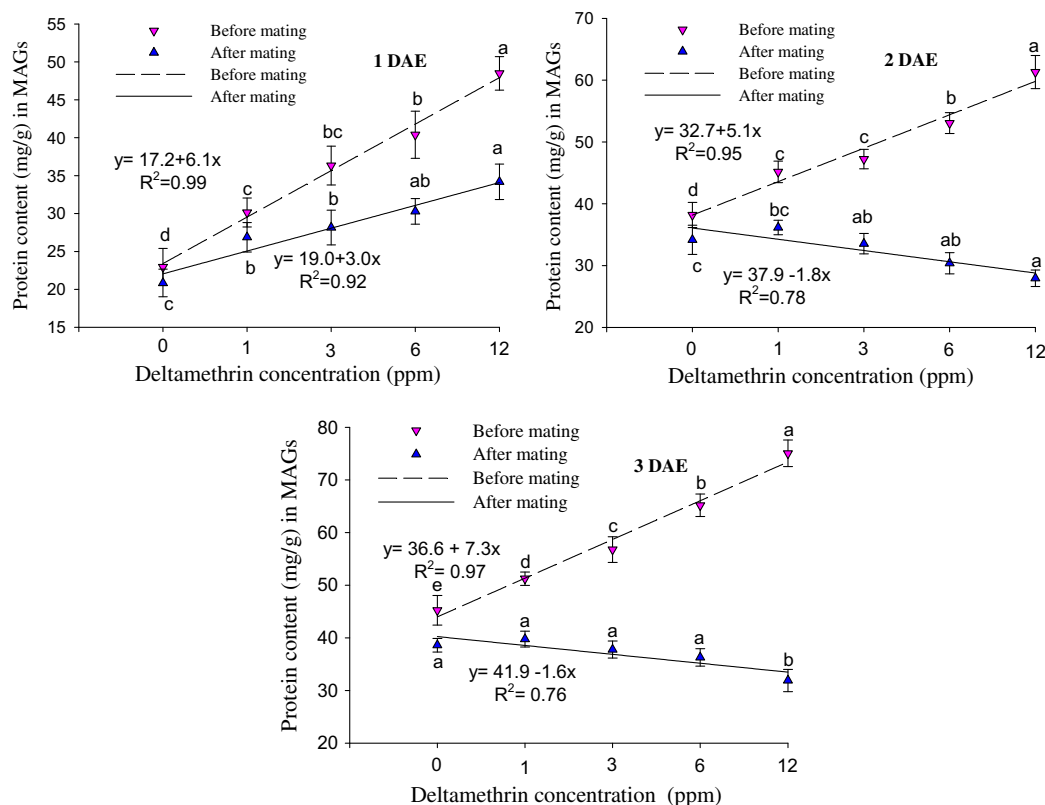


Fig. 1. Relationships between protein content in male accessory glands (MAGs) and deltamethrin concentrations. Means \pm SE followed by different letters in the same line are significantly different at the 5% level. DAE is days after adult emergence.

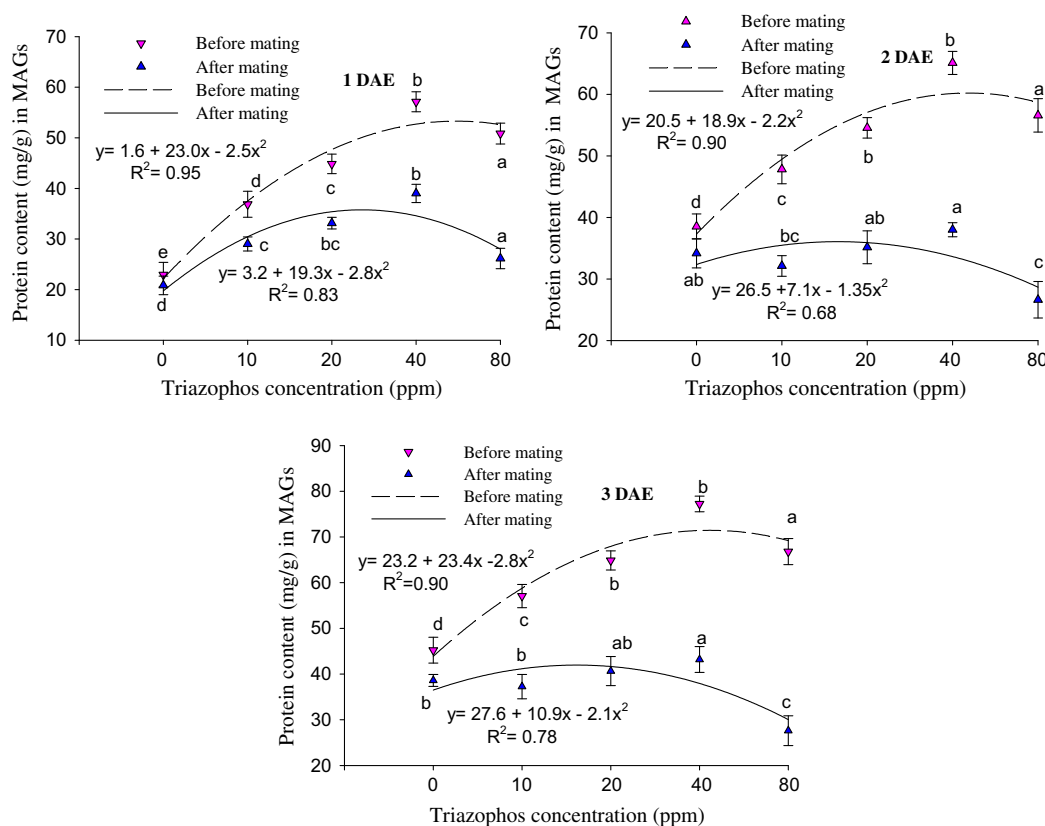


Fig. 2. Relationships between protein content in male accessory glands (MAGs) and triazophos concentrations. Means \pm SE followed by different letters in the same line are significantly different at the 5% level. DAE is days after adult emergence.

third-instar nymphs were released per hill. Rice plants at the tillering stage were sprayed 24 h after insects were released using a Jacto sprayer (Maquinas Agrícolas Jacto S.A., Brazil) equipped with a cone nozzle (1-mm diameter orifice, pressure 45 psi, flow rate 300 ml/min). Control plants at the same stage were sprayed with a mixture of emulsifiers and tap water similar to the composition of the insecticides but lacking their active components. Each treatment was replicated three times. The treated and control plants were covered with cages (screen size; 80-mesh). When the nymphs reached fifth (final) instar, nymphs on treated and control plants were collected and a single nymph from each plant was placed inside a glass jar (diameter 10 cm, height 12 cm) with untreated rice plants ($26 \pm 2^\circ\text{C}$ and 16L:8D).

Males and females were separated 1, 2, and 3 days after adult emergence, with 20 males and 20 females per replicate used separately to measure protein content in the male accessory glands (MAGs) and vitellin content in the fat bodies and ovaries of adult females. To examine the effect of mating pairs consisting of treated or untreated males and females on vitellin content in the fat bodies and ovaries of females, four mating combinations were designed: (1) treated males (δ_t) \times treated females (ϕ_t), (2) $\delta_t \times$ untreated females (ϕ_{ck}), (3) untreated males (δ_{ck}) \times ϕ_t , and (4) $\delta_{ck} \times \phi_{ck}$. For each of these combinations, we made three replicates of 20 males and 20 females each. Vitellin content in the fat bodies and ovaries of females was determined prior to and after mating (mated female is close to the male on

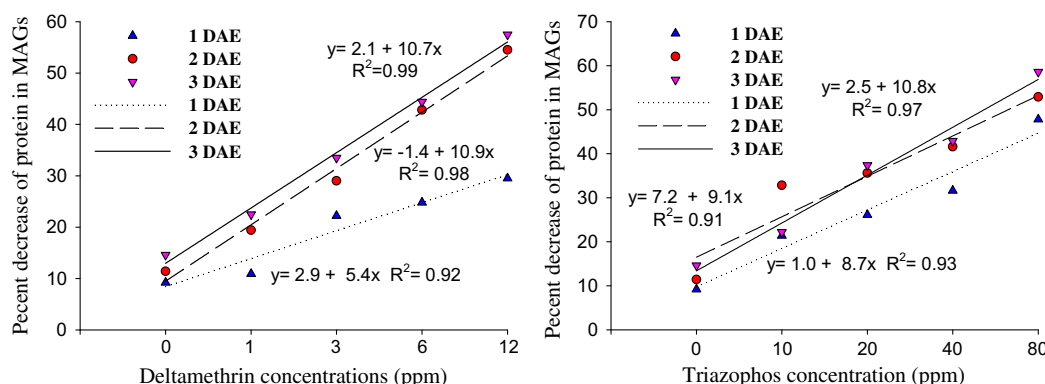


Fig. 3. Relationships between percent decrease of protein content in male accessory glands (MAGs) after mating and insecticide concentration.

Table 1
Analysis of variance for MAG protein content data shown in Figs. 1 and 2.

Source of variance	Df	1 DAE F-value	P-value	2 DAE F-value	P-value	3 DAE F-value	P-value
Insecticide (A)	1	59.7	0.0001	3.6	0.0001	12.2	0.0012
Concentration (B)	4	170.1	0.0001	63.1	0.0001	57.8	0.0001
Mating status (C)	1	351.4	0.0001	1394.9	0.0001	1543.1	0.0001
A × B	4	24.5	0.0001	32.8	0.0001	19.9	0.0001
A × C	1	23.5	0.0001	16.1	0.0001	6.1	0.0178
B × C	4	31.6	0.0001	80.4	0.0001	103.2	0.0001
A × B × C	4	2.7	0.0434	1.3	0.3038	3.4	0.0172

Df is degrees of freedom.

plants, while unmated female is far from the male according to our observation).

2.5. Extraction of protein from MAGs

Protein from MAGs was extracted using the methods of Chen et al. [15] and Smid et al. [21]. Individual adult males of *N. lugens*

were dissected under a zoom-stereomicroscope (model XTL20, Beijing Tech Instrument Co., Ltd., Beijing, China) in a cooled petri dish. MAGs were removed and placed in separate, pre-weighed, ice-cold eppendorf tubes. To each tube, 600 μl of mixed solution (methanol/distilled water/acetic acid/methyl thioethanol; 80:18:2:0.1; vol:vol:vol:vol) was added. The contents were then homogenized on ice and centrifuged at 12,000 rpm at 4 °C for 10 min. The super-

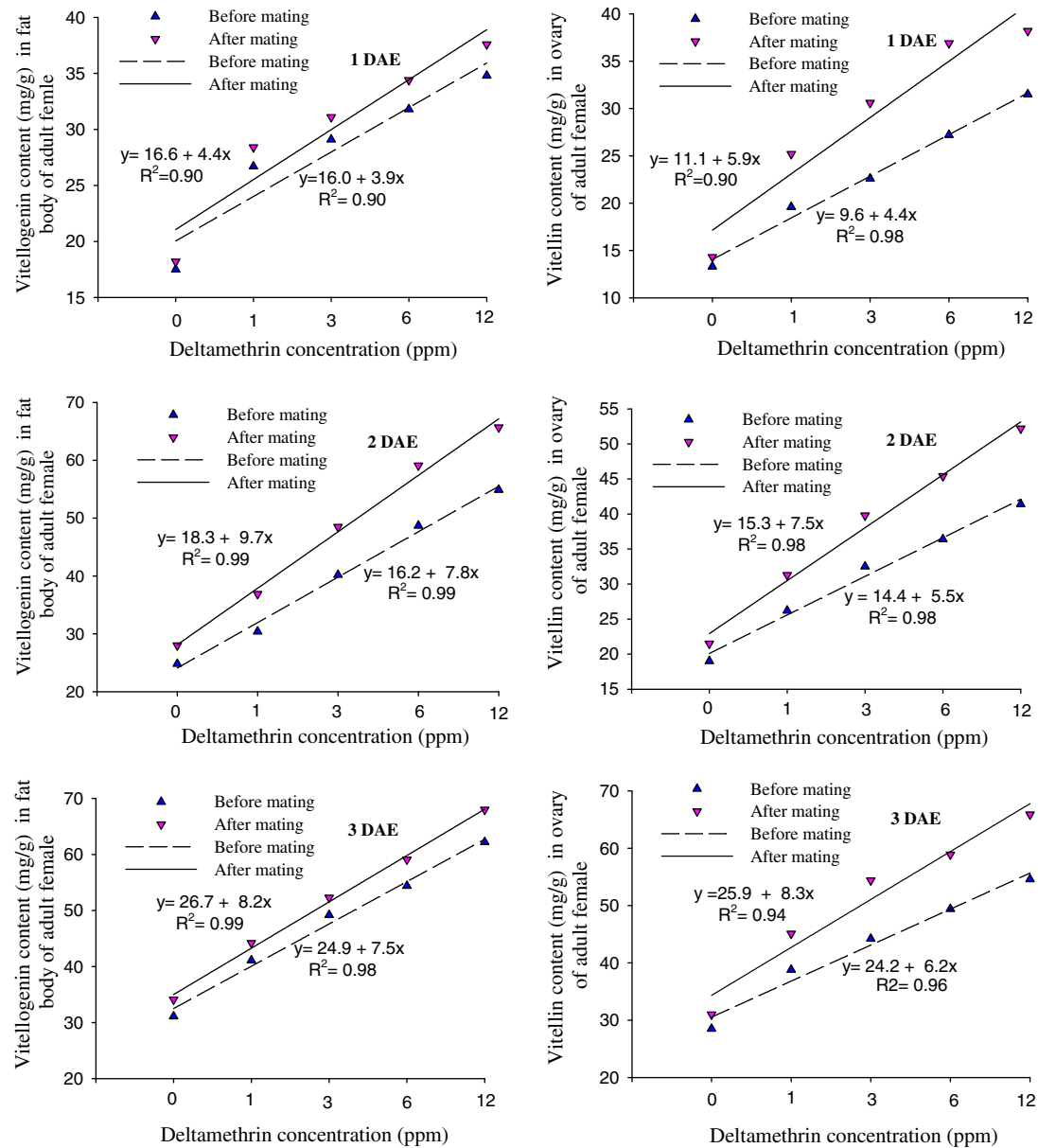


Fig. 4. Relationships between vitellin content in fat bodies (left) and ovaries (right) of *N. lugens* adult females before and after mating and deltamethrin concentration. DAE is days after adult emergence.

natant was collected after removing the upper fat layer. Four hundred microliters of mixed solution was added to the sediment in the tube, which was then centrifuged again and the supernatant collected.

2.6. Extraction of vitellin from fat bodies and ovaries of adult females

Vitellin was extracted from fat bodies and ovaries using a method similar to that of Gong et al. [22]. Individual adult males and females of *N. lugens* were dissected under a zoom-stereomicroscope (model XTL20, Beijing Tech Instrument Co., Ltd., Beijing, China) in a cooled petri dish. Ovaries and fat bodies of females were removed and placed in separate, pre-weighed, ice-cold centrifuge tubes and then re-weighed using a Mettler–Toledo electronic balance (EC100 model; 1/10,000 g sensitivity). A proportional amount of NaCl solution (0.4 M NaCl:1 M PMSF; vol:vol at a ratio of 20 ml NaCl solution to 1 g ovary or fat body) was added to the tube, homogenized on ice, and centrifuged at 12,000 rpm at 4 °C for 20 min. The

supernatant was collected after filtering the upper fat layer with glass fibers, placed at 4 °C overnight after adding ddH₂O (1 supernatant:10 ddH₂O; vol:vol), and centrifuged again at 5000 rpm at 4 °C for 20 min. The vitellin sediment was dissolved with 1.5 ml pre-cooled 0.4 M NaCl solution after removing the supernatant.

2.7. Measurement of protein content

We followed the procedure described in Li and Yu [23] to measure protein content using Coomassie Brilliant Blue R 250 (Shanghai Chemical Agent Co., Ltd., Shanghai, China). A standard curve was established based on a standard protein (bovine serum albumin, Shanghai Biochemistry Research Institute, Shanghai, China). The absorbance at 595 nm was determined in a UV755 B spectrometer (Shanghai Precision Instrument Co., Ltd., Shanghai, China). The protein content in the sample solution was calculated according to the standard curve.

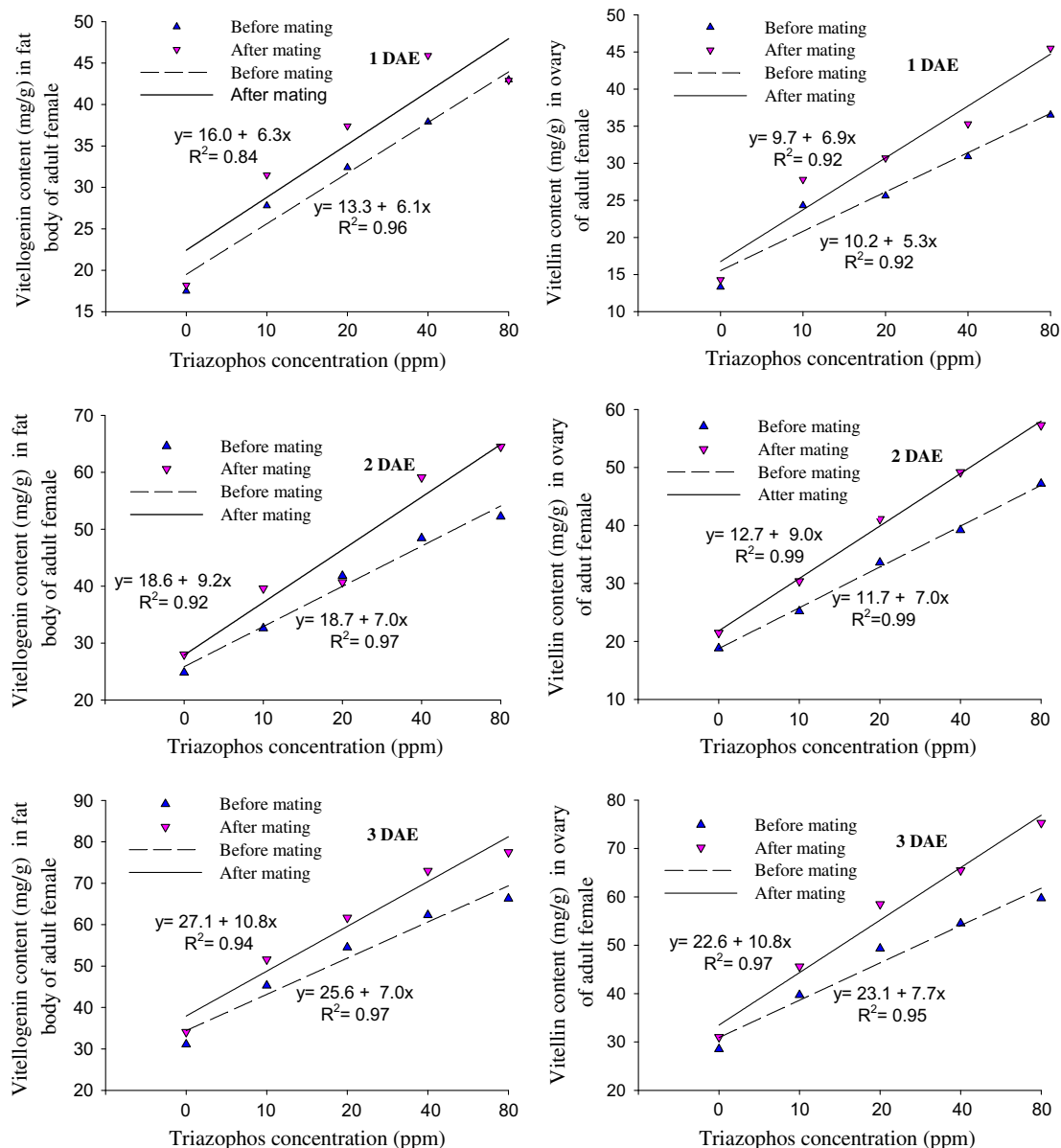


Fig. 5. Relationships between vitellin content in fat bodies (left) and ovaries (right) of *N. lugens* adult females before and after mating and triazophos concentration. DAE is days after adult emergence.

2.8. Statistical analysis

Data were evaluated for normality and homogeneity of variance using Bartlett test [24]. Based on these evaluations, no transformations were needed. The protein content of male accessory glands and vitellin content of fat bodies and ovaries of adult females were analyzed using Analysis of Variance (ANOVA) of three factors (insecticide type, insecticide concentration and mating status or mating pair combinations). Multiple comparisons of means were conducted using Fisher's Protected Least Significant Difference (PLSD) test. Regression analyses were conducted to determine the relationship between insecticide concentration and protein content in MAGs and vitellin content in fat bodies and ovaries of adult females. All analyses were conducted using the GLM procedure (SPSS Inc., 2002) [25].

3. Results

3.1. Relationship between insecticide concentration and protein content in MAGs before and after mating

For both insecticides, protein content in MAGs prior to mating increased with insecticide concentration from the first day after adult emergence (1 DAE) to 3 DAE (Figs. 1 and 2). The relationship between protein content and insecticide concentration fits a linear regression equation with a significant correlation coefficient both before and after mating. However, protein content showed a certain decline at the highest concentration of triazophos. Therefore, there was a parabolic relationship between protein content and triazophos concentration. Protein content in MAGs significantly decreased after mating compared to before mating (by 25.2%, 34.7% and 36.1% for grand means of protein levels after mating at one, two and three DAE, respectively). This reduction increased with insecticide concentration (Figs. 1–3), as shown by significant linear regressions (Fig. 3), especially at one and two DAE. This indicates that more MAG products were transferred to adult females via mating with increasing insecticide concentration.

ANOVA of the data shown in Figs. 1 and 2 showed that insecticide type, insecticide concentration and mating status significantly influenced protein content in MAGs (Table 1). Grand means of protein content in MAGs for triazophos were significantly different

from those for deltamethrin, increasing by 23.9%, 14.2% and 10.3% at one, two and three DAE, respectively. Multiple comparisons of means indicated that protein content in MAGs prior to mating was significantly greater for all concentrations of both insecticides than for untreated controls. Protein content in MAGs after mating at one DAE was significantly greater than in untreated controls, but protein content at two and three DAE varied with insecticide type, insecticide concentration and DAE.

3.2. Changes in vitellin content in both fat bodies and ovaries of adult female *N. lugens* before and after mating

Vitellin content in both fat bodies and ovaries of adult females increased with insecticide concentration whether it was measured before or after mating, with a significant linear regression between protein content and insecticide concentration (Figs. 4 and 5). Fat content in both fat bodies and ovaries was significantly greater after mating than before mating, indicating that mating and insecticide treatments promoted the synthesis of vitellin in fat bodies and the transfer of vitellin to ovaries.

ANOVA of the data shown in Figs. 4 and 5 showed that all main effects (insecticide type, insecticide concentration and mating status) and most second-order interactions significantly influenced vitellin content in both fat bodies and ovaries, except for vitellin in fat bodies at two DAE (Table 2). For vitellin content in both fat bodies and ovaries, grand means for triazophos treatment were significantly greater than those for deltamethrin treatment, except for vitellin in fat bodies at two DAE. All grand means for insecticide treatments were significantly greater than those for untreated controls; those after mating were significantly greater than those prior to mating.

3.3. Comparisons of vitellin content in fat bodies and ovaries of adult female *N. lugens* in different mating pairs

Insecticide type, insecticide concentration and mating pair combinations significantly influenced vitellin content in both fat bodies and ovaries of *N. lugens* (Figs. 6 and 7, Table 3). Grand means of main effects in ANOVA showed that vitellin content in both fat bodies and ovaries was significantly greater for triazophos treatment than for deltamethrin treatment. Grand means for high

Table 2
Analysis of variance of vitellin content data shown in Figs. 4 and 5.

DAE	Source of variance	Df	Fat body F-value	P-value	Ovary F-value	P-value
1	Insecticide (A)	1	147.2	0.0001	51.2	0.0001
	Concentration (B)	4	387.3	0.0001	424.9	0.0001
	Mating pairs (C)	1	81.6	0.0001	207.2	0.0001
	A × B	4	26.6	0.0001	7.0	0.0002
	A × C	1	16.5	0.0002	0.9	0.3407
	B × C	4	5.9	0.0008	12.0	0.0001
	A × B × C	4	2.1	0.0953	1.4	0.2474
2	A	1	1.0	0.328	67.6	0.0003
	B	4	721.3	0.0001	895.9	0.0001
	C	1	328.9	0.0001	330.4	0.0001
	A × B	4	3.0	0.0272	17.8	0.0001
	A × C	1	0.4	0.5501	8.9	0.0049
	B × C	4	10.9	0.0001	13.1	0.0001
	A × B × C	4	0.07	0.9891	0.9	0.4454
3	A	1	171.9	0.0001	68.1	0.0001
	B	4	729.8	0.0001	729.4	0.0001
	C	1	154.8	0.0001	361.9	0.0001
	A × B	4	14.2	0.0001	10.5	0.0001
	A × C	1	16.9	0.0002	0.9	0.3601
	B × C	4	4.9	0.0025	18.4	0.02
	A × B × C	4	1.4	0.2608	1.2	0.3321

Df is degrees of freedom.

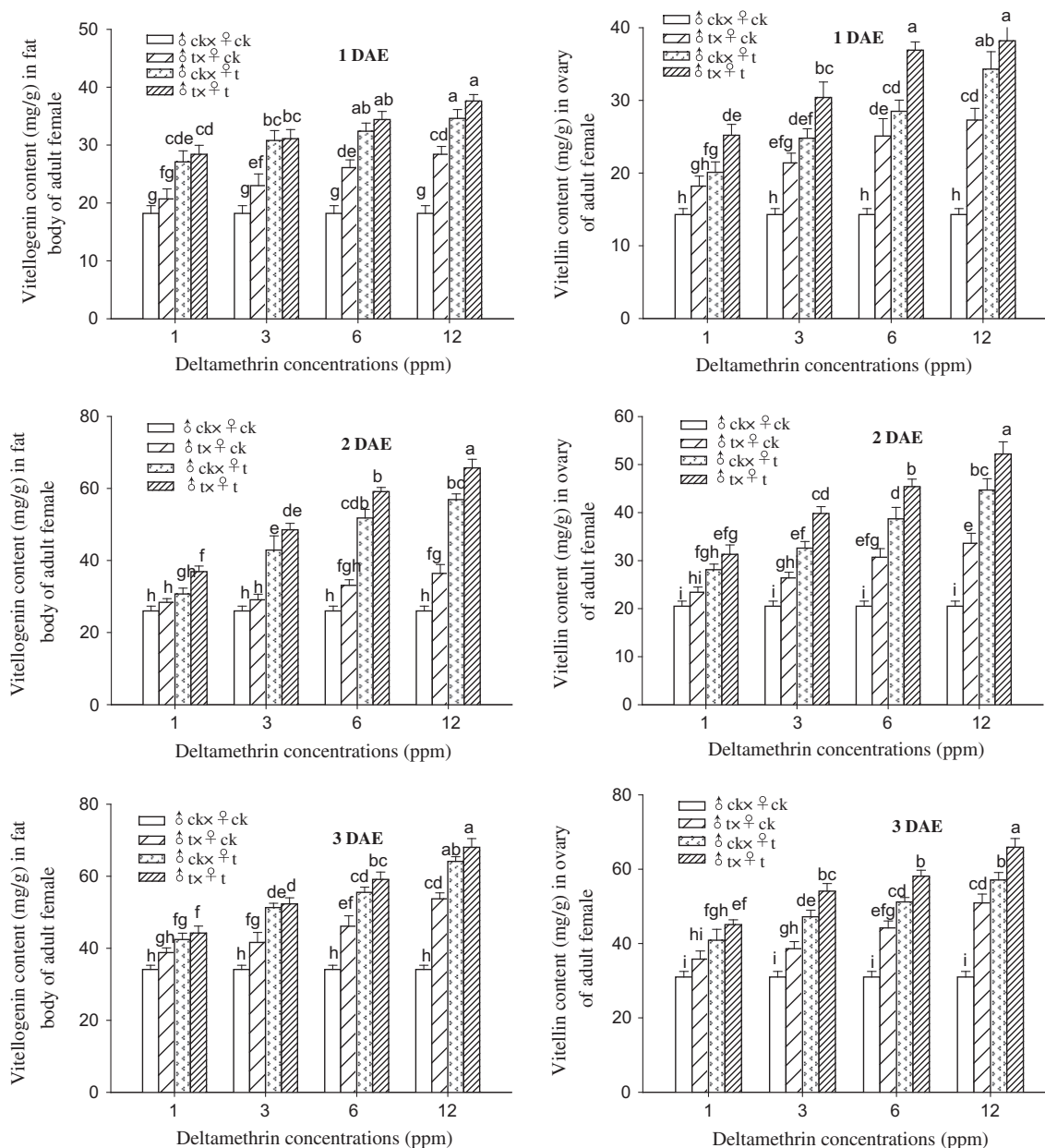


Fig. 6. Comparisons of vitellin content (mg/g) in fat bodies (left) and ovaries (right) of *N. lugens* adult females in mating pairs consisting of deltamethrin-treated or untreated males and deltamethrin-treated or untreated females. DAE is days after adult emergence. Bars with different letters within graphs are significantly different at the 5% level.

insecticide concentrations were significantly greater than for low concentrations. The lowest mean vitellin content in fat bodies and ovaries was found in mating pairs consisting of untreated males and females, while the greatest mean vitellin content was found in control females mated to treated males. For the four mating pair combinations in multiple comparisons, vitellin content in fat bodies and ovaries of adult females was significantly greater for all mating pairs involving at least one treated individual ($\sigma_t \times \varphi_t$, $\sigma_t \times \varphi_{ck}$ and $\sigma_{ck} \times \varphi_t$) than for mating pairs involving untreated males and females ($\sigma_{ck} \times \varphi_{ck}$). In the three combinations involving treated males and/or females, vitellin content in fat bodies and ovaries varied with pair combination, insecticide type and insecticide concentration. The vitellin levels of most combinations involving treated females were significantly higher than those of mating pairs in which only the males were treated. No significant differences in vitellin content were found among most mating pairs involving treated males compared to pairs in which

only the females were treated. Vitellin content for the three treated pair combinations ($\sigma_t \times \varphi_t$, $\sigma_t \times \varphi_{ck}$ and $\sigma_{ck} \times \varphi_t$) showed an increasing trend with concentration of both insecticides. For most pairs, the mating of treated males with untreated females ($\sigma_t \times \varphi_t$) resulted in the greatest vitellin content in fat bodies and ovaries, followed by the mating of untreated males with treated females ($\sigma_{ck} \times \varphi_t$) and the mating of treated males with untreated females ($\sigma_t \times \varphi_{ck}$). Also, vitellin levels increased with insecticide concentration.

4. Discussion

Our previous experiments show that fecundity is elevated in *N. lugens* females developed from nymphs fed on rice plants treated with triazophos and deltamethrin [6]. However, insecticide-induced reproductive effects in adult males have received little

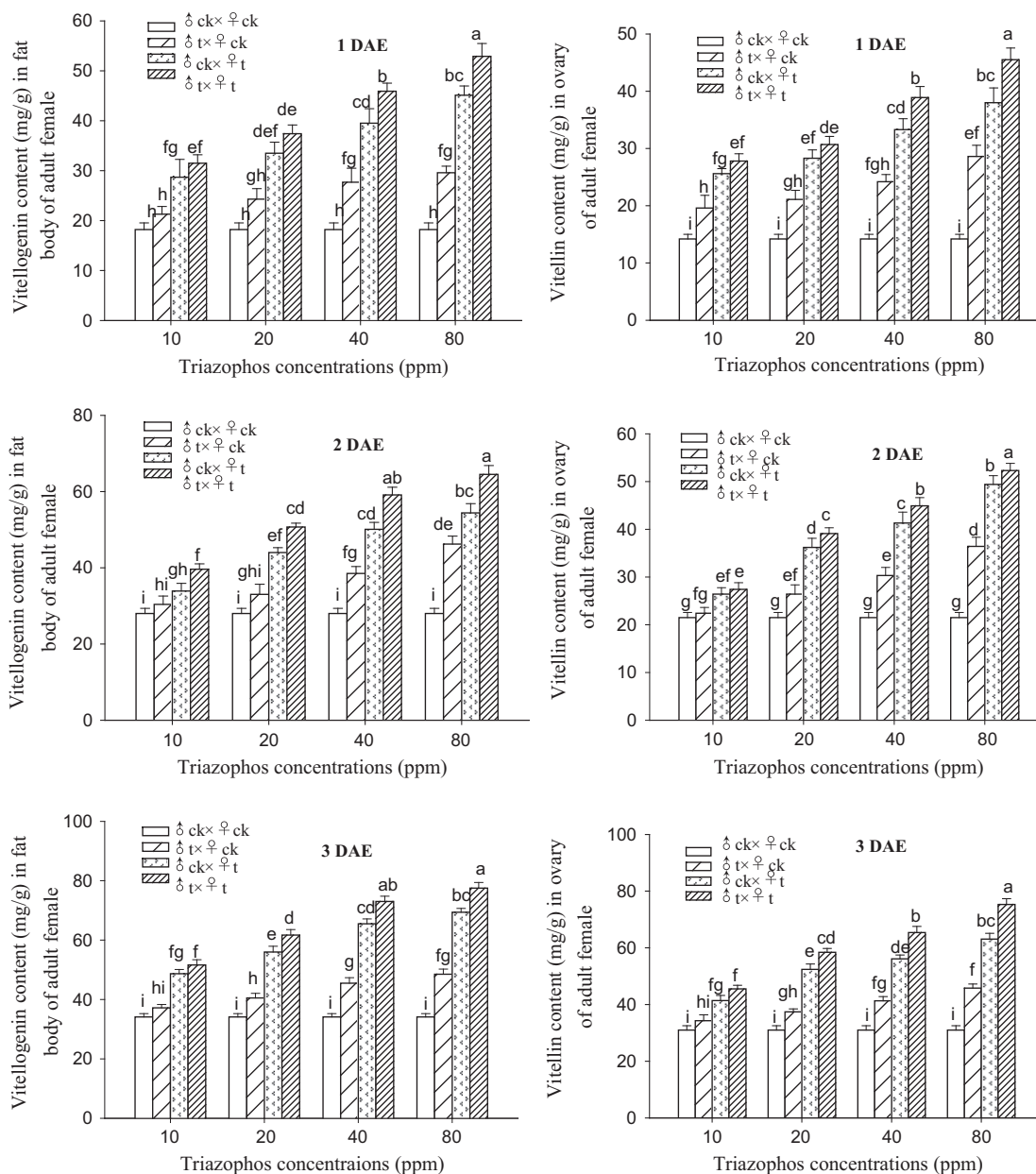


Fig. 7. Comparisons of vitellin content (mg/g) in fat bodies (left) and ovaries (right) of *N. lugens* adult females in mating pairs consisting of triazophos-treated or untreated males and triazophos-treated or untreated females. DAE is days after adult emergence. Bars with different letters within graphs are significantly different at the 5% level.

attention. The present study indicates that protein content is significantly increased in MAGs of *N. lugens* males developed from nymphs fed on insecticide-treated rice plants and that the insecticide-induced reproductive effect on males is transferred to adult females via mating. This demonstrates that insecticide-induced stimulation of fecundity in *N. lugens* is associated not only with females, but also with males. We consider the present findings to indicate insecticide-induced sexual reproduction advantages. However, numerous studies have shown that sublethal doses of insecticides are disadvantageous to the reproductive performance of insects. Other reports also demonstrate that sublethal doses of insecticides have deleterious effects on insect sex pheromone communications [26–30]. Sublethal doses of some insecticides result in declines in female fecundity. For example, the fecundity of female cockroaches treated with deltamethrin and propoxur decreases with increasing sublethal doses of both insecticides [31]. This indicates that the effects of sublethal doses of insecticides on repro-

ductive performance vary with insect species and insecticide type. However, stimulation of insect fecundity induced by sublethal doses of insecticides is a common phenomenon, particularly in Homopteran insects and mites.

The mating pair experiments reported here demonstrate that vitellin content in fat bodies and ovaries of females is greater in mating pairs consisting of insecticide-treated males and females than in mating pairs including only one treated individual, indicating that insecticide treatment of both males and females has a strong interaction effect on the stimulation of female reproduction. The causes and consequences of the effect may be attributed to the transfer of a greater quantity of MAG products by treated males into females via mating compared to untreated males. In insects, MAG secretions are known to improve a male's chances of siring offspring by a variety of mechanisms, such as sperm mobility, sperm storage, stimulation of ovulation/oviposition, and egg protection [12]. For example, Garcia-Gonzalez and Simmons [32] have

Table 3

Analysis of variance of vitellin content data shown in Figs. 6 and 7.

DAE	Source of variance	Df	Fat body <i>F</i> -value	<i>P</i> -value	Ovary <i>F</i> -value	<i>P</i> -value
1	Insecticide (A)	1	105.1	0.0001	38.2	0.0001
	Concentration (B)	3	560.2	0.0001	714.4	0.0001
	Mating pairs (C)	3	113.8	0.0001	169.3	0.0001
	A × B	3	30.5	0.0001	11.2	0.0007
	A × C	3	9.6	0.0001	2.5	0.0713
	B × C	9	15.9	0.0001	22.9	0.0001
	A × B × C	9	3.2	0.0031	1.8	0.103
2	A	1	16.9	0.0001	15.2	0.0002
	B	3	857.4	0.0001	848.8	0.0001
	C	3	312.9	0.0001	326.6	0.0001
	A × B	3	11.2	0.0001	4.0	0.0008
	A × C	3	0.4	0.7654	6.4	0.0111
	B × C	9	50.3	0.0001	46.6	0.0001
	A × B × C	9	3.1	0.0037	1.4	0.208
3	A	1	113.1	0.0001	20.2	0.0001
	B	3	1276.9	0.0001	1092.1	0.0001
	C	3	348.3	0.0001	276.0	0.0001
	A × B	3	69.0	0.005	24.7	0.0001
	A × C	3	4.8	0.0043	2.7	0.0501
	B × C	9	49.1	0.0001	39.3	0.0001
	A × B × C	9	1.5	0.1848	2.8	0.0072

Df is degrees of freedom.

demonstrated that sperm viability is positively correlated with proportion of offspring sired for the cricket *Teleogryllus oceanicus* (Leguillou). Identification of the types of protein that are elevated after exposure to insecticides could also assist in determining the physiological processes involved. Gillott [12] has placed accessory gland secretions into three groups: small peptides (e.g., sex peptides in *D. melanogaster*), molecules of 200–400 amino acids that are commonly glycosylated, and large proteins (e.g., structural proteins such as the sperm-storage protein Acp36DE of *D. melanogaster* [33]). MAG proteins have not yet been characterized in *N. lugens*.

Numerous studies have shown that male mating in sexually reproducing insects influences the reproductive behavior and processes of females [15–17,34–37]. For example, MAG proteins may increase oviposition rate, reduce sexual receptivity and decrease female life span in crickets [37]. Multiple mating in female *U. ornatrix* increases fecundity, but not longevity or egg mass [16]. However, the fecundity of a *Sphenarium purpurascens* female mated repeatedly with the same male is not affected by the number of matings [35]. Male and female condition influences mating performance, sexual receptivity and reproduction of females [14,41]. Male accessory gland proteins (Acps) mediate a variety of effects that benefit males, such effects include stimulation of female egg protection and reduction of receptivity after mating in *D. melanogaster* [42–44]. In the present study, increased protein content in MAGs was induced by insecticides. The mechanism of insecticide-induced increase in MAG protein content in *N. lugens* may be associated with the accumulation of more energy in insects developed from nymphs fed on rice plants treated with triazophos and deltamethrin [6]. A previous study by Yin et al. [6] has shown both an increase in the soluble sugar and crude fat content in adults and the number of eggs laid by adults after exposure of third-instar nymphs to rice plants treated with either deltamethrin, triazophos, or imidacloprid. The present study and that of Yin et al. [6] both support the hypothesis that sublethal rates of insecticide-treated plants can be beneficial to *N. lugens* through the accumulation of biochemical substances in the insect body.

Thus, mating pairs involving treated males and females resulted in the promotion of reproduction in the female. The amount of sap sucking by *N. lugens* is significantly greater on rice plants treated with pesticides than on control plants [38], indicating that treated

plants are beneficial to *N. lugens* feeding. The quantity and quality of food ingested by a male affect the quality and quantity of its ejaculate [39]. Host quality has been shown to affect sperm numbers in Indian meal moths *Plodia interpunctella* Hübner [40]. Male diet and larval host influence other aspects of insect reproductive performance [14,41].

To further substantiate the role of *N. lugens* males in pesticide-induced resurgence, it must be demonstrated that when males with greater MAG protein content mate, not only are fat body and ovary protein levels increased, but also a greater number of eggs are laid. In addition to MAG proteins, other compounds are influenced by pesticide exposure. Effects of insecticide-treated male on fecundity and vitellin gene expression will be reported in successive findings. The economic impact of this significant pest of rice will necessitate further studies to elucidate the roles of both males and females in the population dynamics of pesticide-induced resurgence.

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