Effects of Sublethal Concentrations of Insecticides on the Fecundity of Sogatella furcifera (Hemiptera: Delphacidae) via the Regulation of Vitellogenin and Its Receptor

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Abstract

White-backed planthopper (Sogatella furcifera, Hemiptera: Delphacidae) is an important migratory pest of rice. It causes severe economic losses by reducing crop production. Vg and VgR are important proteins that help in the successful reproduction of insects and have been studied in many insects. To understand the molecular mechanisms underlying the effects of insecticides on white-backed planthopper reproduction, we studied the expression profiles of SfVg, SfVg-like, and SfVgR in white-backed planthopper exposed to insecticides. SfVg and SfVgR silencing inhibited the ovarian development, number of eggs laid by, and hatching rate of white-backed planthopper. Thiamethoxam LC10 significantly inhibited the ovarian development, number of eggs laid by, and hatching rate of white-backed planthopper. Thiamethoxam LC10 significantly inhibited SfVg-like and SfVgR expression. In contrast, triazophos LC25 significantly promoted SfVg, SfVg-like, and SfVgR expression and increased vitellogenin content in white-backed planthopper. These results demonstrate that insecticides can regulate the reproduction of white-backed planthopper by altering the expression of SfVg and SfVgR, thereby affecting the population density of white-backed planthopper. These findings build a foundation for improving our understanding of the molecular mechanisms underlying the effects of insecticides on the reproduction and resurgence of pests.

Key words: white-backed planthopper, vitellogenin, vitellogenin-like, vitellogenin receptor, reproduction

Vitelin (Vn) plays an important role in the regulation of insect reproduction and is a prerequisite for oocyte maturation. Vitellogenin (Vg) was first detected in the hemolymph of Hyalophora cecropia (Telfer 1954) and was considered to be a precursor of Vn that could provide nutrients for egg development during oogenesis (Pan et al. 1969). Insect Vg is mainly produced in the fat body and is then secreted into the hemolymph. It finally enters the ovary via endocytosis mediated by its receptor (vitellogenin receptor, VgR), which is involved in oocyte development (Pan et al. 1969, Mukherjee et al. 1997).

Vg is an important protein that regulates insect reproduction and has been extensively studied in insects, being cloned from Blattodea, Orthoptera, Coleoptera, Diptera, Lepidoptera, Hemiptera, and Hymenoptera. In general, the amino acid structure and composition of insect Vg are highly conserved (Tufail and Takeda 2008), and an unknown functional domain (DUF1943). The number of Vg copies varies across insect species (Morandin et al. 2014). For example, Apis mellifera (Piulachs et al. 2003), Camponotus floridanus (Corona et al. 2013), Bombyx mori (Yano et al. 1994), and Blattella germanica (Martin et al. 1998) harbor only one Vg gene copy, whereas Periplaneta americana (Tufail et al. 2001) harbors two Vg gene copies. Recent studies have shown that Nilaparvata lugens harbors three Vg gene copies (Shen et al. 2019). The highest number at present is five in Aedes aegypti (Romans et al. 1995) and Linepithema humile (Smith et al. 2011, Corona et al. 2013). These differences in copy numbers are considered to be the result of gene duplication events. Gene duplication is an important mechanism of genomic evolution and serves as an important means of evolutionary novelty in gene expression patterns and functions (Lynch and Conery 2000). In this context, changes in Vg gene copy number may be related to the adaptation and evolution of insects (Lynch and Force 2000, Garcia et al. 2010). In white-backed planthopper (Sogatella
Of the white-backed planthopper (Sogatella furcifera), only one copy of Vg gene has been reported by Hu et al. (2019). Therefore, whether there are copies of multiple Vg genes in white-backed planthopper and whether their functions are the same remain unclear. All these problems warrant further study.

In most insects, Vg is synthesized by female fat body cells; however, in some insects, such as Cylorrhapha, Vns can also be synthesized by the ovarian follicular cells as a complementary vitellogenic organ (Relles 1998). Interestingly, in some insects, Vg is involved in both female reproduction and other developmental processes. For instance, Vg plays important roles in social ontogeny, behavior, life span, and immunity of honeybees (Apis mellifera; Harwood et al. 2016) as well as in the adaptability of insects to their host plants (Zhao et al. 2018). Vg is secreted into the hemolymph following synthesis and then into the developing oocytes via VgR-mediated endocytosis (Mukherjee et al. 1997), which is a key element in Vg uptake and mainly functions in oocyte maturation (Goldstein et al. 1979, Sappington et al. 1995, Richard et al. 2001). VgR is a member of the low-density lipoprotein receptor (LDLR) family. Insect VgR harbors two ligand-binding domains (LBDs) and two epidermal growth factor (EGF) precursor domains. LBDs possess multiple LDLR domain class A repeats, whereas EGF precursor domains possess multiple epidermal growth factor/factor-like domains and LDLR Tyr–Trp–Thr–Asp domains. RNA interference (RNAi)-mediated VgR gene knockdown in Aphis citricidus delayed the nymph–adult transition period, prolonged the prereproductive period, and shortened the reproductive period, resulting in suppressed embryonic development and reduced number of new-born nymphs (Shang et al. 2018). In Helicoverpa armigera, VgR depletion inhibited Vn deposition in the ovary, led to Vg accumulation in the hemolymph, and upregulated HaVg expression in the hemolymph, all of which ultimately decreased fecundity (Zhang et al. 2016).

White-backed planthopper is an important migratory pest of rice. White-backed planthopper adults or nymphs suck the phloem sap of the rice plant. The females lay eggs and spread the southern rice black-streaked dwarf virus (Zhou et al. 2008), which decreases rice production. Moreover, white-backed planthopper is a typical r-selected species, also called r-strategist; they are species whose populations are governed by their biotic potential (maximum reproductive capacity, r), showing high fecundity. At present, chemical pesticides are one of the most commonly used methods of white-backed planthopper control. When applied in the field, in addition to their lethal effects, insecticides produce sublethal effects on pest behavior, reproduction, development, and drug resistance of pests due to differential individual exposure of insects to insecticides as well as under exposure to insecticides were determined. In addition, their roles in white-backed planthopper reproduction were studied via RNAi. Based on our results, Vg and VgR are the potential targets for white-backed planthopper control.

Materials and Methods

Insects and Insecticides

As reported in previous studies (Zhou et al. 2019b), white-backed planthopper individuals were collected from a rice field in Huaxi, Guiyang, Guizhou, China, in 2013 and maintained in the laboratory on rice seedlings, without any exposure to insecticides at 25°C ± 1°C under 70% ± 10% relative humidity and a 16:8 h (light L:dark D) photoperiod. Most adult white-backed planthoppers are macropterous when raised in the laboratory. Therefore, macropterous white-backed planthoppers were used in this study, unless stated otherwise. Thiamehoxam (96%) was obtained from PFChem Co., Ltd (Nanjing, China) and triazophos (83.56%) was obtained from Guangxi Tianyuan Biochemistry Corp., Ltd. (Guangxi, China).

Insecticide Exposure

White-backed planthoppers were treated as per a previously reported method (Zhuang et al. 1999, Zhou et al. 2017) with slight modifications. First, thiamethoxam and triazophos were dissolved in acetone to prepare stock solutions. Then, distilled water containing 0.1% Triton X-100 was added to dilute the stock solution to the required concentration for use. In total, 100 third-instar nymphs were transferred to and reared separately in (300-mm high × 30-mm diameter) glass tubes that were open at both ends and contained rice seedlings dipped in sublethal concentrations (LC 10 and LC 25) of thiamethoxam and triazophos. Rice stems treated with distilled water containing 0.1% Triton X-100 were used as controls. Each treatment was repeated three times for a total of 300 nymphs. The treated insects were maintained at 25°C ± 1°C under 70% ± 10% relative humidity and a 16:8 h (LD) photoperiod in an artificial climate box. After 48 h, the surviving white-backed planthoppers were transferred to untreated rice seedlings for rearing and samples were collected until the second day of the fifth instar and the third day of the adult phase. The LC 10 and LC 25 values (Supp Table 1 [online only]) of thiamethoxam and triazophos against white-backed planthopper refer to previously described results (Liu et al. 2015, Liu et al. 2016).

Sample Preparation

White-backed planthopper eggs, nymphs (first, second, and third instars; first and second days of the fourth instar; and first, second, third, and fourth days of the fifth instar), and female adults emerging at different time points (12 h and 1, 2, 3, 4, 5, and 6 d), accounting for 17 developmental stages, were collected to determine the expression profiles of SfVg, SfVg-like, and SfVgR. Each sample contained 15–40 white-backed planthoppers, and three biological replicates were set for each developmental stage. From females that emerged within 24 h, the gut and integument from 100 individuals, heads and fat bodies from 50 individuals, and ovaries from 30 individuals were dissected in phosphate-buffered saline (PBS). Three biological replicates were performed per tissue.
Total RNA Isolation and Reverse Transcription
Total RNA was isolated according to a previously reported method (Yang et al. 2019). The whole bodies or tissues of white-backed planthopper were placed in a grinding tube and lysed using Precellys 24 lysis/homogenizer (Bertin Technologies, France). Total RNA was extracted using the HP Total RNA Kit (Omega Bio-Tek Inc., Norcross, GA) according to the manufacturer’s protocol. The quality of the extracted RNA was determined using agarose gel electrophoresis, and RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The first strand of cDNA was synthesized using the PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara Bio., Dalian, China) according to the manufacturer’s instructions and amplified using polymerase chain reaction (PCR).

Amplification and Sequencing of SfVg, SfVg-like, and SfVgR
Based on white-backed planthopper genomic (Wang et al. 2017) and transcriptomic (Zhou et al. 2018) data, Vg, Vg-like, and VgR of N. lugens were used as templates to search for the Vg, Vg-like, and VgR genes of white-backed planthopper using BioEdit (v7.2.6.1, Isis Pharmaceuticals, Carlsbad, CA). Primer Premier 6.0 (Premier Biosoft, Palo Alto, CA) was used to design gene-specific primers (Supp Table 2 [online only]), and the obtained sequences were verified using reverse transcription–PCR (RT–PCR) or rapid amplification of cDNA ends (RACE). The specific RT–PCR amplification conditions were as follows: predenaturation at 95°C for 3 min, denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for approximately 1–3 min (for 30 cycles), and final extension at 72°C for 10 min. To obtain complete open reading frame (ORF) sequences, the 5′- and 3′-ends of the genes were amplified using the SMARTer RACE cDNA amplification kit (Takara Bio.). Specific steps were followed according to the kit instructions. After confirming the quality of the PCR product via electrophoresis, the amplicons were purified and inserted into a vector for cloning. The cloned product was then amplified and sequenced. The ORFs of SfVg, SfVg-like, and SfVgR were identified using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The compute pl/Mw tool of SWISS-PROT (ExPasy server; http://au.expasy.org/tools/pi_tool.html) was used to calculate the molecular weights and theoretical isoelectric points (pl) of SfVg, SfVg-like, and SfVgR proteins. SMART (http://smart.embl-heidelberg.de/) was used to identify the conserved domains of SfVg, SfVg-like, and SfVgR. The neighbor-joining method in MEGA X (Kumar et al. 2018) was used to construct the phylogenetic tree, with 1000 replicates.

Real-time Quantitative PCR
As previously reported (Zhou et al. 2019a), the mRNA levels of SfVg, SfVg-like, and SfVgR at different developmental stages, in different tissues, and under different insecticide treatments were measured via quantitative PCR (qPCR) using FastStart Essential DNA Green Master (Roche, Indianapolis, IN) on the CFX96 qPCR system (BioRad, Hercules, CA). The ribosomal protein L9 (RPL9; KM885285) and alpha 1-tubulin (TUB; KP735521) were used as internal controls. Gene-specific primers containing the T7 polymerase promoter sequence were designed online using E-RNAi (http://www.dkfz.de/signaling/e-rnai3/; Supp Table 2 [online only]). A dsDNA template was prepared using PrimeSTAR Max DNA Polymerase (Takara Bio.). The PCR program used was as follows: denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min (for 30 cycles). The synthesized dsRNA was purified using the GeneJET RNA Purification Kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. The concentration of the purified dsRNA was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific), and all concentrations were adjusted to 1000 ng/μl using ddH2O for subsequent use. dsRNA was stored at −80°C prior to use. dsGFP (dsRNA of the GFP gene; CAA58798) was used as a control.

Using the IM-31 microinjector (Narishige, Tokyo, Japan), dsVg, dsVg-like, dsVgR, and dsGFP were injected into newly emerged (within 12 h) adult female white-backed planthoppers. Each female was injected with 0.1 μl of the sample per treatment. In total, 50 females were injected and three biological replicates were performed. The efficiency of gene knockdown resulting from RNAi was calculated as the ratio of gene expression between insects injected with target dsRNAs and dsGFP, determined 48 h after the injection. In addition, one female and two males (to eliminate the influence of males not being able to mate to lay eggs by females) were placed together after the injection. Each treatment included 15 groups, and three biological replicates were performed for each treatment. White-backed planthoppers reared in groups were supplied with fresh rice plants every 2 d, and the number of newly hatched nymphs was recorded daily. After 10 d, the rice seedlings were dissected to record the number of unhatched eggs, until the female adults died. The numbers of hatched nymphs, unhatched eggs, and eggs laid per female were counted, and fertility was calculated as the sum of the number of newly hatched nymphs and unhatched eggs. The hatching rate was determined as the ratio of the number of newly hatched nymphs and the number of eggs laid per female. After 6 d, the ovaries of the individuals injected with dsVg, dsVg-like, dsVgR, and dsGFP were dissected and observed. Morphological traits were observed using a stereoscopic microscope (SMZ25, Nikon, Tokyo, Japan).

Enzyme-Linked Immunosorbent Assay
The Insect VTG enzyme-linked immunosorbent assay (ELISA) Kit (Meimian, Jiangsu, China) was used to determine the Vg content in the fifth instar on the second day (insecticide treatment) and females on the third day (insecticide treatment and dsRNA injection). Whole bodies of the insects were first homogenized in PBS containing 0.05% Tween-20. Next, the homogenate was centrifuged at 10,000 × g at 4°C for 10 min, and the instructions of the Insect VTG ELISA Kit were followed for determining the Vg content. Absorbance was measured at 450 nm using a Multiskan FC (Thermo Fisher Scientific) microplate reader.

Statistical Analysis
Student t test was used to analyze the significance of the silencing effects (*P < 0.05 and **P < 0.01). Relative SfVg, SfVg-like, and SfVgR expressions at different developmental stages and in different
tissues were compared using one-way analysis of variance, followed by LSD (least-significant difference) test for multiple comparisons. Significance test level was $P < 0.05$. All data were analyzed using SPSS 22.0 (Chicago, IL).

**Results**

*SVg, SfVg-like, and SfVgR Sequences*

Based on the available transcriptomic and genomic data of white-backed planthopper as well as the verification results of RT–PCR and RACE, two Vg/Vg-like and one VgR gene copies were detected in white-backed planthopper, designated SVg (MN296092), SfVg-like (MN296091), and SfVgR (MN296093). This result indicates that there are two copies of Vg in the white-backed planthopper, Vg and VgR-like (Table 1).

The putative SfVg-like protein contains 2019 amino acids and a signal peptide (1–19); the predicted molecular weight of the protein is 227.68 kDa, and the theoretical pI is 7.11. SfVg-like possesses only two of the three conserved domains, namely, LPD_N and VWD, and has lost the DUF1943 domain (Fig. 1). Analysis of the Vg-like conserved domains of other Hemiptera (such as Zootermopsis nevadensis, Cryptotermes secundus, N. lugens, and Laodelphax striatellus) revealed that they all have lost their DUF1943 domain. Phylogenetic analysis based on Vg-like gene sequences of white-backed planthopper and other Hemiptera showed that the SfVg-like of white-backed planthopper was clustered on the same branch as the SfVg-like of N. lugens and Laodelphax striatellus, indicating that the three rice plant hoppers show a close evolutionary relationship.

*SfVg, SfVg-like, and SfVgR Expression Profiles*

Compared with the expression in other developmental stages, SVg was highly expressed in the female adult stage. With an increase in female emergence time, SVg expression also gradually increased, reaching the maximum level on the fifth day of female emergence (Fig. 2A). Although SVg-like was highly expressed in the adult female stage, it was not limited to this stage. SfVg-like was also highly expressed in eggs and the second-instar nymphs (Fig. 2B). The expression profile of SVgR was consistent with that of SVg, and it was also mainly expressed in the female adult stage. Conversely, SfVgR expression gradually increased with the increase in female emergence time (Fig. 2C). In addition, in all the tissues tested, the expression of SVg and SVg-like in the fat body was significantly higher than that in the other tissues (Fig. 2D and E). On the other hand, SfVgR had the highest expression in the ovary (Fig. 2F).

**Effects of SfVg, SfVg-like, and SfVgR on White-Backed Planthopper Reproduction**

According to the expression profiles of SVg, SVg-like, and SVgR at different developmental stages, RNAi experiments were performed in females before ovarian maturity (within 12 h of emergence). The expression levels of SVg, SVg-like, and SVgR were measured 48 h after the injection. After dsRNA injection, the expression levels of SVg, SVg-like, and SVgR were significantly inhibited, being downregulated by 88.15%, 89.28%, and 78.93%, respectively (Fig. 3A). The number of eggs laid by females and their hatching rate were significantly lower in the treatment group injected with dsSVg and dsSVgR than in the control group. However, the number of eggs laid and their hatching rate were not significantly different between the treatment group injected with dsSVg-like and the control group (Fig. 3B and C). ELISA showed that the Vg content in the whole bodies of females was significantly lower in the treatment group injected with dsSVg than in the control group; however, the Vg content in the whole bodies of females did not significantly differ between the treatment groups injected with dsSVg-like and dsSVgR and the control group (Fig. 3D). In addition, the Vg content in the female ovary was significantly lower in the treatment group injected with dsSVgR than in the control group (Fig. 3E). Taken together, these results indicate that dsSVgR injection did not affect Vg synthesis in white-backed planthopper but significantly affected Vg uptake in the ovary.

On the sixth day after dsRNA injection, banana-shaped mature oocytes were observed in dissected individuals treated with dsGFP and dsVg-like; this was not observed in the dsSVg and dsSVgR treatment groups (Fig. 3F). Therefore, dsSVg and dsSVgR knockdown significantly affected ovarian development in white-backed planthopper.

**Effects of Insecticides on SVg, SfVg-like, and SVgR Expression**

Triazophos LC$_{25}$ significantly induced SVg, SVg-like, and SVgR expression in the third instar nymphs, whereas thiamethoxam LC$_{25}$ significantly induced SVg-like expression (Fig. 4C–E). However, thiamethoxam LC$_{10}$ and LC$_{25}$ treatments showed no significant effects on SVg expression. Thiamethoxam LC$_{10}$ significantly inhibited SVg-like and SVgR expression. After treatment with thiamethoxam and triazophos, Vg contents in the fifth-instar nymphs did not significantly change; however, after treatment with thiamethoxam LC$_{25}$, Vg content in female adults was significantly reduced (Fig. 4A and B). After triazophos LC$_{10}$ treatment, Vg content in treated female adults was significantly higher than that in controls.

**Discussion**

In all insects, successful reproduction depends on the biosynthesis of Vg, a precursor of Vn, and its accumulation in the oocytes (Raikhel and Dhadialla 1992). With the rapid development of genomic sequencing technologies, the complete genomes of over 300 insect species have been sequenced, greatly promoting entomological research. As a result, multiple copies of Vg have been found in many insects. To date, multiple Vg/Vg-like transcripts have been identified in various Hemiptera insects, such as N. lugens, Bemisia tabaci, Plautia stali, Bactericera cockerelli, and Riptortus clavatus (Shinoda et al. 1996, Lee et al. 2000, Leshkowitz et al. 2006, Ibanez et al. 2018, Shen et al. 2019). A previous study by Hu et al. (2019) reported only one Vg gene copy in white-backed planthopper.
however, in this study, two Vg copies in white-backed planthopper, SfVg and SfVg-like, were cloned. The amino acid structure of insect Vg’s is highly conserved. They typically contain three conserved domains: LPD_N, VWD, and DUF1943. SfVg harbors all three conserved domains, whereas SfVg-like harbors only two of the three conserved domains and has lost the DUF1943 domain. In addition,
the expression profiles of SfVg and SfVg-like at different development stages and in different tissues of white-backed planthopper were significantly different. Hu et al. (2019) reported that SfVg possesses three conserved domains and is involved in ovarian development. The present study further revealed that SfVg knockdown significantly inhibited oviposition, hatching rate, and ovarian development and reduced the Vg content in white-backed planthopper. However, targeted SfVg-like knockdown did not affect oviposition and hatching rate. These results indicate that SfVg orthologs have different functions following replication. In addition, the results of our study are comparable with those for N. lugens, in which NlVg-like2 lost the DUF1943 domain and its function was significantly different from that of NlVg (Shen et al. 2019). In addition, Ibanez et al. (2018) have suggested that the two Vg genes of Bactericera cockerelli show different functions: BcVg1-like is a typical Vg—a precursor of Vn—that provides nutrition during oocyte development, whereas BcVg6-like is involved in lipid and molecular transport and the immune response. Studies on social insects have also found that Vg and Vg-like are associated with reproduction as well as with the caste system and polyethism (Morandin et al. 2019). The expression profile of SfVg-like has revealed that SfVg-like also has higher expression in eggs. At the same time, after silencing SfVg-like, it has been found that 9.91% of the eggs fail to hatch, indicating that SfVg-like has an important role in the embryogenesis of the white-backed planthopper (Fig. 3). These results indicate that insect Vg orthologs have acquired novel functions that are rather diverse from their conventional functions during duplication events in the course of evolution, such as transporting lipids and/or other molecules and embryogenesis.

In insects, Vg is mainly synthesized in the fat body and enters the oocyte via VgR-mediated endocytosis, transforms into Vn, and plays important roles in oocyte formation and development (Mukherjee et al. 1997). Many studies have shown that VgR inhibition can significantly reduce Vg entry into oocytes, thereby inhibiting oocyte development and reducing female fecundity. For example, in Bemisia tabaci, siRNA-mediated BtA1VgR silencing significantly reduced BtA1Vg content in oocytes compared with that in controls, although its content in hemolymph was significantly increased.
In a study on *N. lugens*, targeted *NlVgR* silencing decreased *NlVg* content in the ovary, resulting in *NlVg* accumulation in the hemolymph, and inhibited ovarian development (Lu et al. 2015). Consistent results were noted in a study on *Helicoverpa armigera* (Zhang et al. 2016). Moreover, Hu et al. (2019) found that the lack of *SfVgR* in white-backed planthopper significantly inhibited ovarian development. Our study further revealed that targeted *SfVgR* silencing significantly inhibited *SfVg* entry in the ovary of white-backed planthopper, thereby suppressing ovarian development and female fecundity. Overall, these studies indicate that the lack of VgR prevents Vg from entering the oocytes, hindering nutrition required for oocyte development and maturation. Therefore, we believe that Vg and VgR can only ensure the normal reproduction of insects when they are present together. Thus, both the proteins are indispensable.

Sublethal concentrations of insecticides may affect insect population dynamics by impairing developmental and reproductive traits (Desneux et al. 2007). Several studies have shown that sublethal concentrations of insecticides can affect insect reproduction. For example, sublethal concentrations of thiamethoxam and imidacloprid significantly inhibited white-backed planthopper reproduction (Yang et al. 2017, Zhou et al. 2017), whereas lethal concentrations of triazophos and deltamethrin significantly stimulated white-backed planthopper reproduction (Liu et al. 2016, Zhou et al. 2019b). However, whether Vg and VgR, as the primary proteins involved in white-backed planthopper reproduction, are also affected by insecticides remains unknown. Therefore, in this study, we selected an insecticide to inhibit the reproduction and another insecticide to stimulate the reproduction of white-backed planthopper and examined *SfVg*, *SfVg*-like, and *SfVgR* expression and Vg content in the whole body of insects. Thiamethoxam LC 10 significantly inhibited *SfVg*-like and *SfVgR* expression. However, triazophos LC 25 significantly stimulated *SfVg*, *SfVg*-like, and *SfVgR* expression and increased Vg content in white-backed planthopper. In *N. lugens*, triazophos and deltamethrin significantly induced *NlVg* expression (Ge et al. 2010), but indoxacarb and chlorantraniliprole significantly inhibited *NlVg* expression and reduced Vg content in the ovary and fat body of female adults; therefore, the two insecticides suppressed Vg synthesis in the fat body and Vg transfer to the ovary (Liu et al. 2012). In *Chilo suppressalis*, the sublethal concentration of chlorantraniliprole significantly inhibited *CsVg* expression (Huang et al. 2016). In *Conopomorpha sinensis*, the sublethal concentration of emamectin benzoate significantly inhibited Vg expression (Yao et al. 2018). Overall, these results indicate that insecticides affect insect reproduction via stimulation or inhibition by regulating Vg gene expression and synthesis and its transfer to the ovary. However, it is not clear whether other
genes are involved in the regulation process during this process, warranting further research.

In conclusion, SfVg and its receptor SfVgR are indispensable for the successful reproduction and oocyte maturation of white-backed planthopper. Therefore, SfVg and SfVgR can serve as potential targets for RNAi-based white-backed planthopper control. In addition, insecticides affect white-backed planthopper reproduction by regulating SfVg and SfVgR expression. Therefore, combined with the results of previous studies (Liu et al. 2016), it can be said that the insecticides-induced expression of SfVg and SfVgR is an important molecular mechanism underlying the stimulation of white-backed planthopper reproduction and their resurgence.

Supplementary Data
Supplementary data are available at Journal of Insect Science online.

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