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Sublethal effects of sulfoxaflor on biological characteristics and vitellogenin gene (*AlVg*) expression in the mirid bug, *Apolygus lucorum* (Meyer-Dür)

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ABSTRACT

The mirid bug, *Apolygus lucorum* (Meyer-Dür) has evolved the resistance towards some traditional insecticides, especially pyrethroids and organophosphates. Sulfoxaflor, as a novel insecticide, is used for control of sap-feeding insects, like *A. lucorum*. Therefore, it is necessary to determine the acute toxicity and the potential sublethal effects of sulfoxaflor in *A. lucorum*. Here, the LD_{50} value of sulfoxaflor against *A. lucorum* was assayed as 3.347 ng/adult at 48 h via topical application. Besides, the effects of a sublethal dose (LD_{15}) of sulfoxaflor on biological characteristics of *A. lucorum* were estimated by comparison of the life table parameters. The longevities and fecundity of parent generation did not exhibited significant difference between both control and treatment groups after exposure to LD_{15} dose of sulfoxaflor (0.568 ng/adult) for 48-h. However, the parameters reflecting their progeny G1 generation population dynamics, including the intrinsic rate of increase (*ri*), the finite rate of increase (λ), the mean generation time (*T*), the net reproductive rate (R_0) and gross reproduction rate (GRR) significantly reduced in the treatment group compared to the control. Furthermore, the expression level of *AlVg* mRNA significantly decreased by 43.8% in the progeny whose parents were treated with LD_{15} dose of sulfoxaflor in comparison with the control transgenerational female adults. These results suggested that sublethal dose of sulfoxaflor adversely affect the development and reproduction of transgenerational *A. lucorum*. The downregulation of *AlVg* might have negative impacts on the fecundity of *A. lucorum*.

1. Introduction

Mirid bugs (Heteroptera: Miridae), as polyphagous pests, can damage various crops such as cotton, vegetables and fruit trees in many parts of the world [1]. In China, > 20 mirid species were recorded [2]. Of them, three dominant species, *Apolygus lucorum* and *Adelphocoris suturalis* were recorded, which distributed in the Yangze River and Yellow River regions, while another species, *Lygus pratensis*, in northwest of China [3]. A lot of chemical insecticides, such as organophosphates and pyrethroid insecticides, have been heavily applied for control of *A. lucorum*[4], which has gradually resulted in the development of resistance [5–8].

Sulfoxaflor developed by Dow AgroSciences in 2010 is the first commercial insecticide of the novel sulfoximine type [9,10]. Unlike the neonicotinoids and other nAChR-acting insecticides, sulfoxaflor has a unique action on nicotinic acetylcholine receptor nAChR in insect nervous system [11,12], and is classified into Group 4C by the Insecticide Resistance Action Committee [13]. Due to the special action mechanism, it has been demonstrated excellent control on sucking pests, lying within the families Aleyrodidiae, Aphididae, Delphacidae, Margarodidae, and Miridae even resistant populations [12,14]. In China, it has been registered as a 50% water dispersible granules (WDG) against *A. lucorum* in cotton crop (http://www.chinapesticide.gov.cn). Under field conditions, the relatively high longevity of *A. lucorum* adults (up to 30 days) [15] and insecticide degradation possibly induce sublethal effects [16].

Sublethal effects are defined as impacts (either physiological or behavioral) on survival individuals when exposed to a toxicant at low or sublethal concentration/dose [17]. These sublethal effects can be manifested as reductions in life span [18,19], development rates [20], fertility [21], and fecundity [19,22,23], and changes of sex ratio and behavior such as feeding [24], searching, and oviposition [17,25]. Various physiological and/or behavioral sublethal effects have been reported in *A. lucorum*[19,26–28]. Besides, the sublethal effects of sulfoxaflor on some other insect species have been determined [20,29,30]. However, little information is currently available with regard to the lethal and sublethal effects of sulfoxaflor on *A. lucorum*.

Age-stage life table analysis can comprehensively afford

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information about population dynamics to understand multiple sublethal effects of insecticides on insects [31,32]. In this context, the sublethal effects of sulfoxaflor on various biological traits and population dynamics of *A. lucorum* were assessed by age-stage life table. In addition, the sublethal effect of sulfoxaflor on the mRNA expression of *AlVg* was also investigated.

2. Materials and methods

2.1. Insects and insecticides

A colony of *A. lucorum* individuals was provided by the Langfang Experimental Station of the Chinese Academy of Agricultural Science. They were reared in the laboratory without exposure to insecticides since April 2008. *A. lucorum* was reared with fresh green beans (*Phaseolus vulgaris* L.) at standard environmental conditions (26 ± 1 °C, 65 ± 5% R.H., and 16:8 (L: D) h). Sucrose solution (5% w/v) was supplied for adults.

Sulfoxaflor (active ingredient 97.859% w/w) was provided from Dow AgroSciences Inc. (Indianapolis, IN, USA).

2.2. Bioassays

To examine the toxicity of sulfoxaflor against *A. lucorum* adults, the topical exposure procedure described in the reference [27] was adopted in this study.

Prior to the treatment, 4-day-old *A. lucorum* adults were exposed to carbon dioxide (CO_2) to anaesthetize the individuals, and 0.6 μL of the insecticide in an acetone solution was applied on the dorsum (thorax) of each mirid bug. Insecticide was applied with a semiautomatic dropper (PB600-1, Hamilton Company of America, Reno, NV, USA). Each concentration was performed three replicates, and at least 10 mirid bugs were treated per replicate; each bioassay consisted of six concentrations. Control mirid bugs were treated with acetone. After topical exposure, the adults were placed into vials with a 2-cm-long section of green bean pod for food and kept for 48 h in growth chambers at 26 ± 1 °C, 65 ± 5% R.H. and a 16:8 (L: D) h photoperiod. At the end of this period, mortality was examined, i.e., adults not moving when touched with a fine brush were considered dead. The mortalities of all of the control samples were lower than 5%. Each whole bioassay was conducted three times on different days.

The LD_{15} and LD_{50} values (see below) were calculated on the basis of standard probit analysis with PoloPlus software (LeOra Software Inc., Petaluma, CA).

2.3. Sublethal effects of sulfoxaflor on A. lucorum

Topically exposed to LD_{15} of sulfoxaflor or acetone (control) via the method above [27], the mortalities of *A. lucorum* adults were calculated at 48 h posttreatment. The survivors were chosen for further sublethal effects of sulfoxaflor.

2.4. Sublethal effects on life history traits of the G0 generation

26–40 mating pairs (1 male and 1 female) were placed in a polyethylene cup (3-cm-diameter, 10-cm-high) and provided an insecticide-free green bean pod (as food and oviposition substrate). Old green bean pods were replaced with new ones per day and checked under a stereo microscope to count the number of eggs laid by each female. Individual survival was recorded daily, and the number of eggs deposited on per bean pod by each mating pair was counted daily. The individual G0 longevity of adult *A. lucorum* was determined, and fecundity was quantified for each mating pair. Individuals that died during the process of the experiment were removed and not replaced.

2.5. Transgenerational sublethal effects of sulfoxaflor on the G1 generation

After counting the number of deposited eggs per bean pod from various mating pairs, each bean pod was tagged and transferred to a polyethylene box (4 cm × 4 cm × 2 cm) under rearing conditions. Egg hatching was recorded daily and newly emerged nymphs were removed daily from containers, and then each individual transferred to a new petri dish (3-cm-diameter) with bean pod (as food). Bean sections were changed on a daily basis and nymphal survival and development were recorded simultaneously. Within 24 h of molting into adults, individuals from the same mating group were paired in the polyethylene cups. > 30 pairs were mated in each treatment. The longevity of individual G1 adults was determined and fecundity of each mating pair was quantified.

The number of eggs laid by G1 female adults was counted and then transferred them to a polyethylene box. The total number of emerged (G2) nymphs was counted and the egg-hatching rate was determined.

All experiments were conducted at 26 ± 1 °C, 65 ± 5% R.H., and a photoperiod of 16:8 (L: D) h.

2.6. Life table data analysis

The raw data of the life table for all of *A. lucorum* individuals in the study were analyzed according to an age-stage, two-sex life table [31,33] via the TWOSEX-MSChart computer software [34]. In our study, population parameters: age-stage-specific survival rates (s_{ij}), age stage-specific fecundity (f_{ij}), age-specific survival rate (l_x) and age-specific fecundity (m_x), the adult pre-oviposition period (APOP), the intrinsic rate of increase (ri), the finite rate of increase (λ), the net reproductive rate (R_0), the mean generation time (T), and the gross reproduction rate (GRR) were calculated. The means and standard errors of these parameters were evaluated with the bootstrapping techniques with 10,000 replications [35] implemented in the TWOSEX-MSChart.

2.7. Relative quantitation of AlVg mRNA expression by RT-qPCR

Samples of *A. lucorum* abdomen were collected after 48 h, including LD_{15} sulfoxaflor treated female adults, acetone-exposed female adults, and G1 female adults of 6-day-age. Total RNA were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's specifications, and cDNA was synthesised by total RNA using PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara, Dalian, China). Platinum SYBR Green qPCR SuperMix-UDG kit (Invitrogen, Carlsbad, CA) was used to conduct the RT-qPCR reactions. The *AlVg* primers were 5′-AGACCGTCATGCTCGGAGAT-3′ and 5′-CTG GGATTGGGAGGGACA-3′, and the reference gene β-actin primers used for data normalisation were 5′-ACCTGTACGCCAACACCGT-3′ and 5′-TGGAGAGAGAGGCGAGGAT-3′ [36].

RT-qPCR reactions were carried out using the ABI 7500 qPCR System (Applied Biosystems 7500). RT-qPCR reactions of each treatment were replicated 3 times, and non-template control reactions were performed in triplicate for each primer pair. The relative *AlVg* gene expression levels were represented by relative quantification (RQ) values calculated by the math formula method [37].

2.8. Statistical analysis

Differences in life history traits, population parameters and *AlVg* mRNA expression between *A. lucorum* treated and untreated with sulfoxaflor at the dose of LD_{15} were compared by Student's *t*-test in SPSS version 19.0 (IBM Inc., USA). *P*-value of < 0.05 ($P < 0.05$) was considered to be statistically significant. Survival rate, fecundity, reproductive value curves, and the graph about the relative *AlVg* expression were generated using SigmaPlot 12.0 (Systat Software Inc., San Jose, CA).