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Research

Toxicity and Sublethal Effects of Flupyradifurone, a Novel Butenolide Insecticide, on the Development and Fecundity of *Aphis gossypii* (Hemiptera: Aphididae)

Ping-Zhuo Liang,[1] Kang-Sheng Ma,[1] Xue-Wei Chen,[1] Chun-Yan Tang,[1] Jin Xia,[1] Hsin Chi,[2] and Xi-Wu Gao[1,3]

[1]Department of Entomology, China Agricultural University, Beijing 100193, PR China, [2]Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University, 51240 Niğde, Turkey, and [3]Corresponding author, e-mail: gaoxiwu@263.net.cn

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Abstract

The cosmopolitan pest *Aphis gossypii* (Glover) causes considerable economic losses on various crops by its feeding damage and transmitting diseases around the world. Flupyradifurone is a novel butenolide pesticide; its toxicity on *A. gossypii* parent generation (F0) was estimated following treatment with LC_{25} concentration for 48 h. The adult longevity and fecundity of the F0 individuals treated by flupyradifurone showed no significant decrease in comparison with the control. Life table method was used to evaluate the sublethal effects on progeny population (F1). Results showed that the development time of the fourth instar and the preadult as well as the total pre-reproductive period were significantly prolonged, while their fecundity was significantly decreased compared with the control. Additionally, the intrinsic rate of increase (*r*), the finite rate of increase (λ), and the net reproductive rate (R_0) of F1 were all significantly lower in the group treated by LC_{25} than in the control group. These results reveal that the sublethal concentration of flupyradifurone could suppress the population growth of *A.* gossypii and indicate that this novel insecticide may be as a useful tool in pest management.

Key words: *Aphis gossypii*, flupyradifurone, sublethal effect, life table

The cotton aphid, *Aphis gossypii* (Glover), is a cosmopolitan and polyphagous pest with wide hosts, such as cotton, cucumber, citrus, and many other crops around the world. *Aphis gossypii* can cause serious damages via feeding and transmitting diseases (Carletto et al. 2010); moreover, it can secrete honey dew, on which the sooty mold grows and leads to photosynthesis and fiber problems (Slosser et al. 2002). The control of *A. gossypii* has primarily relied on chemical insecticides including organophosphates, carbamate, pyrethroids, and neonicotinoids (Shrestha and Parajulee 2013, Wei et al. 2017). However, the repeated and excessive use of pesticides has resulted in resistance and cross-resistance to multiple pesticides in many countries (Herron et al. 2001, Koo et al. 2014, Cui et al. 2016, Chen et al. 2017b).

Although pesticides commonly lead to direct mortality, the residues degrade over time in or on soil and plants resulted in sublethal exposure (Cutler 2013, Guedes et al. 2016) inducing sublethal effects on multiple biological traits of insects (Desneux et al. 2007). Some studies have demonstrated that the multi-faceted sublethal effects could impair physiological processes and behavioral changes, such as longevity and fecundity (Boina et al. 2009), and feeding and oviposition (Tan et al. 2012, He et al. 2013). Subsequently, long-term parameters of population dynamics are impacted, such as life span and development rate (Yuan et al. 2017). Thus, the identification of sublethal effects is essential for giving more accurate assessment on insecticides efficiency to optimize the application (Stark and Banks 2003). The approach of life table has been recommended as a suitable method to evaluate population dynamics that have been used in studies of multiple target and non-target insects (Biondi et al. 2013, Cira et al. 2017, Nawaz et al. 2017).

Flupyradifurone is the first member of the novel class of butenolide insecticides that were classified by the Insecticide Resistance Action Committee (IRAC) into IRAC class 4D (Colares et al. 2017). Flupyradifurone can provide fast and systemic protection by xylem-mobility (Barbosa et al. 2017). Flupyradifurone works as acetylcholine mimic in insect nervous system by binding reversibly to the post-synaptic nicotinic acetylcholine receptors (nAChRs) to keep them opened and finally lead to an uncontrolled excitation of the axon (Nauen et al. 2015, Colares et al. 2017). Though flupyradifurone is also targeted on nAChR, it exhibits difference from other nAChR agonists based on its structure–activity relationships (Jeschke et al. 2015). Furthermore, the safety profile of flupyradifurone has been demonstrated experimentally by Campbell et al. (2016) that it is compatible with honeybees and bumblebees when follow the directions. This novel insecticide has been reported effective in controlling populations in several sucking-pest species and even in resistant strains that emphasizes it a new tool for integrated pest management and a useful choice in resistance management strategies (Smith and Giurcanu 2013, Coy et al. 2016).

For a comprehensive understanding of the sublethal effects of flupyradifurone to *A. gossypii*, it is necessary to consider its effect on the survival rate, developmental rate, and fecundity throughout the whole life span. In the present study, we evaluated the toxicity of flupyradifurone under laboratory condition, and we used life table analysis to estimate the sublethal and transgenerational effects of this new butenolide insecticide on *A. gossypii*. These results would be useful to optimize the application of flupyradifurone in the future IPM programs.

Materials and Methods

Insects and Insecticides

The individuals of cotton aphid (*A. gossypii*) used in this study were originally obtained from Binzhou, Shandong province, China, in July 2016 and reared on cotton seedlings without exposure to insecticides in a climatic chamber set at 22 ± 1°C, 65–75% relative humidity, and a photoperiod of 16:8 h (L:D), in the laboratory of China Agriculture University, Beijing, China.

The flupyradifurone (active ingredient 96% w/w) was obtained from Bayer CropScience Co. Ltd (Monheim am Rhein, Germany). Triton X-100 was purchased from Amresco Inc. (Solon, OH).

Bioassays

The toxicity of flupyradifurone to apterous adult of *A. gossypii* was assessed using the leaf-dipping method according to Moores et al. (1996). The insecticide was dissolved in acetone and diluted with distilled water containing 0.05% (v/v) Triton X-100 to the required concentrations. Cotton leave discs (20 mm in diameter) were dipped in insecticide solution for 15 s and air-dried by placing upside down for 30 min, then put into disposable 12-well cell culture plate, each cell contains 1 ml 2% agar to maintain humidity. Apterous adult aphids (30–35 individuals) were placed onto the discs, then the cells were covered with Chinese art paper to prevent escape. Leaf discs dipped in distilled water containing 0.05% (v/v) Triton X-100 were used as control. Three replicates were conducted for each concentration. Three times of bioassays were conducted separately. Mortality was assessed after 48-h continuous exposure to insecticide, and the insects that cannot move or only one leg vibrates slightly (after probed gently with a soft hair brush) were recorded as died (Moores et al. 1996). LC_{25}, LC_{50}, slope, and 95% confidence limits were calculated using software PoloPlus 2.00 (LeOra Software Inc., Petaluma, CA).

Sublethal Effects of Flupyradifurone on F0 Generation

Apterous aphid adults were treated with LC_{25} of flupyradifurone concentration. After 48 h, survived aphids were transferred individually to untreated fresh leaf discs in 12-well cell culture plates. Adults were observed daily for recording survival and numbers of newborn nymphs which were then removed. The total number of adults used in this study were 86 and 84 individuals in flupyradifurone and control treatments, respectively. The leaf discs were replaced every 4–5 d to prevent fungal growing until the adult aphids died.

Sublethal Effects of Flupyradifurone on F1 Generation

Newborn nymphs that produced within 24 h by F0 adults were collected as F1 generation and transferred to wells in plates independently. In both LC_{25} flupyradifurone and control treatments, the F1 generation aphids were reared on leaf discs without insecticide. The total number of newborn nymphs used in this study were 77 and 85 individuals in flupyradifurone and control treatments, respectively. Daily records of survival and development were conducted. During reproductive period of F1 generation, newly born aphids were counted and then removed. Fresh leaf discs and new agar beds were replaced every 4–5 d.

Life Table Analysis

The following parameters were analyzed for the description of stage differentiation, survival, and fecundity: age-stage-specific survival rate (s_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), and age-stage reproductive value (v_{xj}). In addition, the study estimates some other population factors, including adult pre-reproductive period (APRP), total pre-reproductive period (TPRP), intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), and mean generation time (T) (Huang and Chi 2012). The life expectancy (e_{xj}) was calculated according to Chi and Su (2006), while the reproductive value (v_{xj}) was calculated according to Tuan et al. (2014).

Statistical Analysis

The probit analysis was conducted by PoloPlus 2.0 software (LeOra Software Inc., Petaluma, CA). Parameters for developmental time of different stages, adult longevity, and fecundity were analyzed by the TWOSEX-MSChart computer program (Chi 2018), according to the age-stage two-sex life table theory (Chi and Liu 1985) and the method mentioned in Chi (1988). The SEs of population parameters in life table were estimated by using 100,000 bootstrap replicates in TWOSEX-MSChart. The paired bootstrap test was used to evaluate the difference between control and treatment groups.

Results

Toxicity of Flupyradifurone on *A. gossypii* Adults

A total of 550 aphid adults were used in the bioassay and the slope ± SEM was 1.476 ± 0.114, χ^2 (df) was 22.01 (16), P value was 0.143. The LC_{50} value of flupyradifurone to *A. gossypii* adults after 48 h of exposure was 6.902 mg (a.i.)/liter (95% confident limits are 5.125–9.081 mg [a.i.]/liter), and the LC_{25} value was estimated as 2.410 mg (a.i.)/liter (95% confident limits are 1.552–3.376 mg [a.i.]/liter). The LC_{25} was used as the sublethal concentration for the following life table studies.

Sublethal Effects of Flupyradifurone on F0 Generation of *A. gossypii*

There were no significant differences in longevity and fecundity of *A. gossypii* adults after treated with sublethal concentration (LC_{25}) of flupyradifurone compared with the control group (Table 1).

Effects of Sublethal Flupyradifurone on F1 Generation of *A. gossypii*

The result exhibited that the development time of fourth instar nymphs (2.05 ± 0.09 d) was significantly prolonged in the treatment group compared to the control group (1.70 ± 0.07 d). No significant difference was observed in development time of the other three life stages, though there existed the similar increasing tendencies. Preadult development time was significantly increased in the LC_{25}-treated group of flupyradifurone compared to the control group, from 6.96 ± 0.15 d to 7.79 ± 0.18 d. LC_{25} treatment significantly extended the TPRP compared to the control aphids, from 8.38 ± 0.04 d to 7.36 ± 0.03 d, but the APRP showed no significant increase in the treatment group (Table 2).

Significant decreases for the group treated by flupyradifurone were observed in the intrinsic rate of increase (r) (0.191 ± 0.009/d), the finite rate of increase (λ) (1.210 ± 0.009/d), the net reproductive rate (R_0) (18.51 ± 1.52 offspring/individual), and the fecundity (24.57 ± 1.24 offspring/female) compared to control group (0.239 ± 0.009/d, 1.269 ± 0.009/d, and 22.42 ± 1.55 offspring/individual, 28.45 ± 1.16 offspring/female, respectively), while the mean generation time (T) in the treatment group (15.27 ± 0.36 d) was significantly increased compared to the control group (13.04 ± 0.27 d) (Table 3).

The curves of the age-stage survival rate (s_{xj}) for population show the probability that a newborn offspring will survive to age x and stage j (Fig. 1). Because the development rate varied among different individuals, it resulted in obvious overlaps between stages in both control group and treatment group. The probability that a newborn aphid will survive to the adult stage was adversely affected in the group treated by flupyradifurone (0.75 ± 0.05) compared to the 0.79 ± 0.04 in control group (Table 1 and Fig. 1). However, there were no significant differences in the preadult survival rate between treatments.

The age-specific survival rate (l_x), i.e., the simplified version of age-stage survival rate s_{xj}, showed a significant decrease within first 6 d in the LC_{25}-treated group (Fig. 2). Figure 2 reflected that the fecundity level in the treatment group was lower than that in control group. The peak value of m_x for the control group was higher than that in flupyradifurone-treated one.

The age-stage life expectancy (e_{xj}) (Fig. 3) of the control group is generally higher than that in LC_{25}-treated group. The age-stage reproductive value (v_{xj}) (Fig. 4) is considered a measure of contribution of individuals at different ages and stages to future population. The maximum value was significantly declined in the treatment group by LC_{25} (7.33/d) compared to that in the control group (8.74/d).

Discussion

Since insecticides are overused in pest management, *A. gossypii* shows resistance to many of them in various mode of actions (MOAs) (Moores et al. 1996, Peng et al. 2016; Wei et al. 2017). With the increase of resistances, insecticides are increasingly repeatedly applied, thus causing sorts of environment problems. Thus, the discovery and study of this novel alternative, flupyradifurone, is essential on delaying the development of resistance on *A. gossypii*. Sublethal effects of this new insecticide on population controlling have been studied on a broad range of sucking pests, including *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Chen et al. 2017a), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Smith and Giurcanu 2013), *Lygus hesperus* Knight (Hemiptera: Miridae) (Joseph and Bolda 2016), and so on, and its sublethal effects on *A. gossypii* was initially studied here. According to the results, LC_{50} (i.e., 6.902 mg [a.i.]/liter) of flupyradifurone on *A. gossypii* was lower than the recommended field rate (FR) reported in Barbosa et al. (2017), in agreement with the result on *D. citri* in Chen et al. (2017a) of which the calculated LC_{50} of flupyradifurone was also less than the FR.

In the present study, no significant decrease was shown in longevity and fecundity of *A. gossypii* adults directly exposed to LC_{25} of flupyradifurone, possibly because of the lower exposure concentration and the short period of treatment after which aphids were immediately transferred to untreated leaf discs. Although the action mode of flupyradifurone is the same as the other commercial insecticides of IRAC MoA4, such as neonicotinoid (IRAC MoA sub-group 4A) and sulfoxaflor (IRAC MoA sub-group 4C), however, the effects was dependent on difference of insecticides and species, such as imidacloprid for *D. citri* and sulfoxaflor for *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae) (Boina et al. 2009, Xu et al. 2016). The longevity and/or fecundity in the above researches showed significant decrease, but this type

Table 1. Sublethal effects of flupyradifurone on longevity and fecundity (mean ± SE) of F0 adults *A. gossypii*

Parameters	Control	Flupyradifurone	P
Adult longevity (days)	16.86 ± 0.63a	16.01 ± 0.63a	0.340
Fecundity (nymphs/female)	15.64 ± 0.87a	15.53 ± 0.82a	0.922

Means in a row followed by the same letter were not significantly different according to the paired bootstrap test with 100 000 resampling.

Table 2. Sublethal effects of flupyradifurone on developmental time, APRP, TPRP, and longevity (mean ± SE) of F1 generation *A. gossypii*

Stage	Control		Flupyradifurone		P
	n	Mean ± SE (days)	n	Mean ± SE (days)	
First instar (N1)	80	2.03 ± 0.07a	73	2.15 ± 0.06a	0.172
Second instar (N2)	71	1.69 ± 0.08a	64	1.81 ± 0.08a	0.166
Third instar (N3)	68	1.59 ± 0.09a	61	1.75 ± 0.09a	0.172
Fourth instar (N4)	67	1.70 ± 0.07b	59	2.05 ± 0.09a	0.005
Preadult duration	67	6.96 ± 0.15b	59	7.79 ± 0.18a	<0.001
Preadult survival rate	85	0.79 ± 0.04a	77	0.75 ± 0.05a	0.610
Adult longevity	67	23.97 ± 0.73a	59	23.33 ± 0.92a	0.580
APRP	67	0.40 ± 0.01a	59	0.59 ± 0.09a	0.717
TPRP	67	7.36 ± 0.03b	59	8.38 ± 0.04a	<0.001

Means in a row followed by the same letter were not significantly different according to the paired bootstrap test with 100 000 resampling.

Table 3. Sublethal effects of flupyradifurone on the population parameters (mean ± SE) of F1 generation *A. gossypii*

Population parameter	Control	Flupyradifurone	*P*
r (per day)	0.239 ± 0.007a	0.190 ± 0.009b	<0.001
λ (per day)	1.269 ± 0.009a	1.210 ± 0.009b	<0.001
R_0 (offspring/individual)	22.42 ± 1.55a	18.51 ± 1.52b	<0.001
Fecundity (offspring/female)	28.45 ± 1.16a	24.57 ± 1.24b	<0.001
T (days)	13.04 ± 0.27b	15.27 ± 0.36a	<0.001

Means in a row followed by the same letter were not significantly different according to the paired bootstrap test with 100 000 resampling.

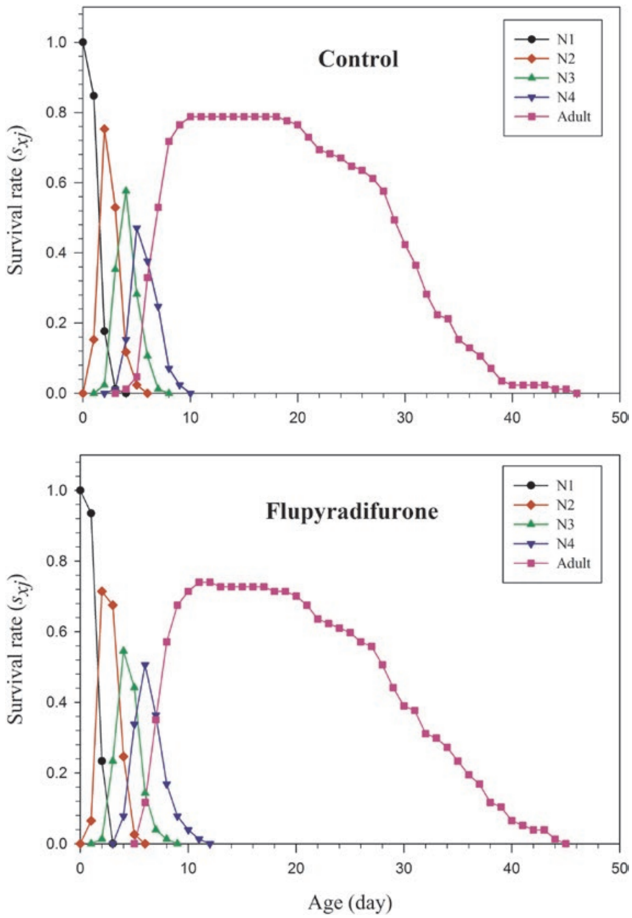

Fig. 1. Age-stage-specific survival rate (s_{xj}) for F1 generation *A. gossypii* from initial adults exposed to sublethal flupyradifurone concentration.

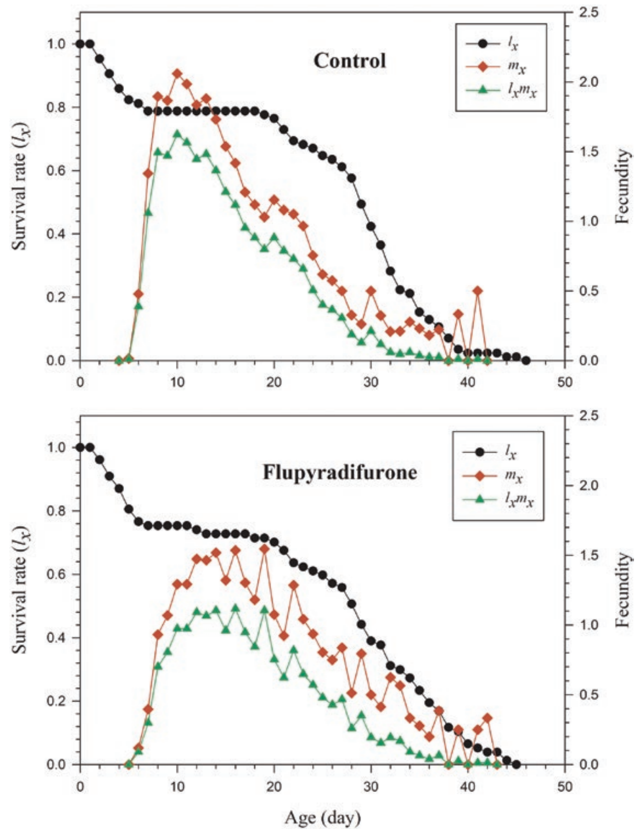

Fig. 2. Age-specific survival rate (l_x), age-specific fecundity of total population (m_x), and age-specific maternity (l_xm_x) for F1 generation *A. gossypii* from initial adults exposed to sublethal flupyradifurone concentration.

of decrease was not observed in *B. tabaci* exposed to imidacloprid (He et al. 2011) and in *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae) exposed to cycloxaprid (Pan et al. 2014). Moreover, the decrease trend of longevity and fecundity indicated an absence of hormesis, which was one of the important sublethal effects of insecticides. Defined as the dose–response relationship characterizing by reversal of the response between low and high stress doses (Jager et al. 2013, Guedes and Cutler 2014), hormesis has been demonstrated in several species and pesticides, such as the higher fecundity in *Myzus persicae* Sulzer (Hemiptera: Aphididae) exposed to 0.6 mg/liter of imidacloprid (Ayyanath et al. 2013) and the outbreaks of *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae) induced by pyrethroid in Cordeiro et al. (2013). Without the hormesis in F0 generation here, we inferred that insecticide-induced outbreak will not happen in *A. gossypii* adults directly exposed to LC_{25} flupyradifurone.

Since all effects of toxicants on insect populations can be taken into account by the means of demographic toxicological study (Stark and Banks 2003), life table analysis was used on the research of F1 generation population. Our results revealed that the duration of the fourth instar and the preadult of F1 aphids treated with LC_{25} flupyradifurone was significantly prolonged, i.e., the pesticide exposure on F0 generation delayed the development rate of their nymphs, may be because the nymphs devote more energy into detoxification and survive at the cost of development and reproduction (Hannig et al. 2009, Vilca Mallqui et al. 2014). The delayed development rates were similar to the previous studies on various insects, such as the delayed development rate from fourth instar larval to pupa in *Plutella xylostella* L. (Lepidoptera: Plutellidae) treated with LC_{25} of chlorantraniliprole (Guo et al. 2013), and the increased development time of each stage in *Rhopalosiphum padi* L. (Hemiptera: Aphididae) treated by the sublethal concentrations of beta-cypermethrin or indoxacarb (Zuo et al. 2016) APRP showed no significant change, while there was a significant increase in TPRP with sublethal

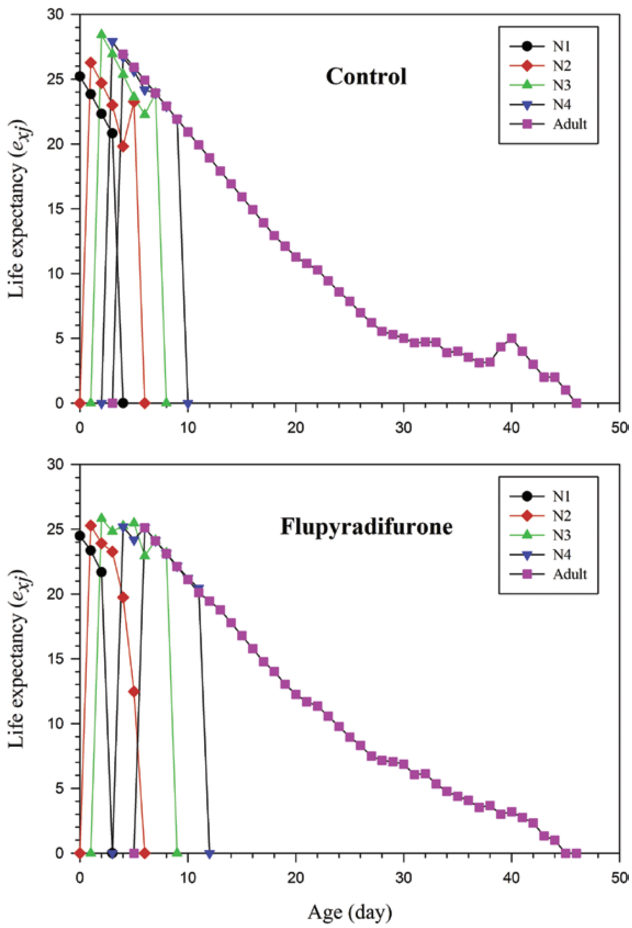

Fig. 3. Age-stage specific life expectancy (e_{xj}) for F1 generation *A. gossypii* from initial adults exposed to sublethal flupyradifurone concentration

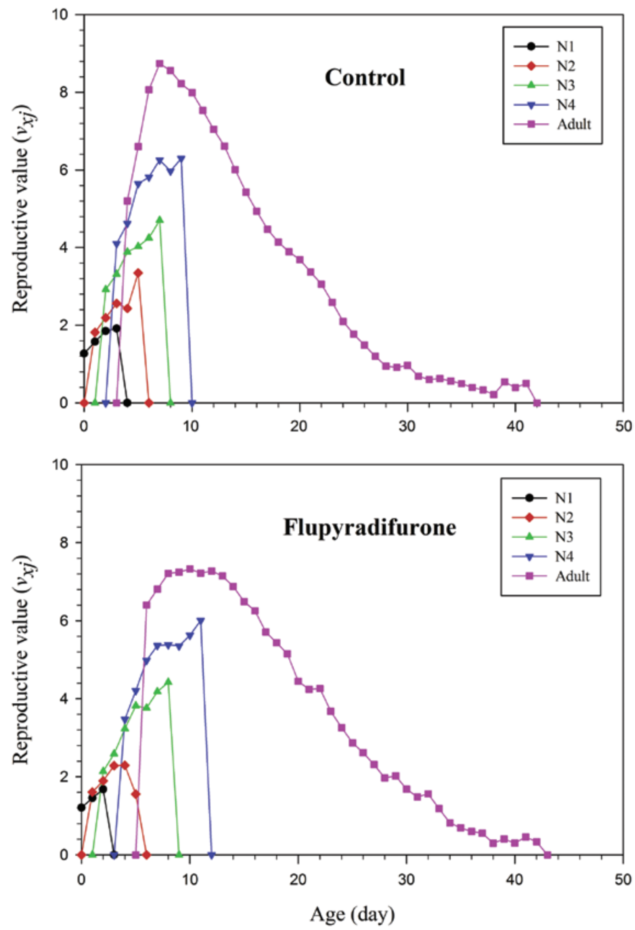

Fig. 4. Age-stage reproductive value (v_{xj}) for F1 generation *A. gossypii* from initial adults exposed to sublethal flupyradifurone concentration.

flupyradifurone treatment. Besides, the fecundity was significantly decreased by the application of this insecticide. Such results have been reported on *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae) treated with LC_{30} buprofezin (Ali et al. 2017) and *Scolothrips longicornis* Priesner (Thysanoptera: Thripidae) exposed to low lethal concentration of abamectin (Pakyari and Enkegaard 2015). The prolonged development period and the decreased fecundity of F1 aphids exposed to flupyradifurone indicated that there will be no induction of the resurgence in offspring when parental aphids treated with this insecticide. Besides, we also obtained that *A. gossypii* is susceptible to flupyradifurone and especially strongly influenced in their immature stage, the similar illustration has also been reported on *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) treated with chlorantraniliprole (Nawaz et al. 2017).

Long-term life table parameters were strongly influenced in treated F1 generation on account of transgenerational effects, i.e., the effects owing to the insecticides exposure on parental aphids, their values of r, λ, and R_0 were significantly lower compared to the control, whereas the T value was higher with the treatment. As described in many previous works (Rahmani and Bandani 2013, Moscardini et al. 2015), these influenced parameters indicated that the growth of population was suppressed by application of flupyradifurone on sublethal concentration. Similar phenomena were observed in another lab population of *A. gossypii* treated with LC_{25} sulfoxaflor (Chen et al. 2016), as well as the *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae) in Dong et al. (2017) treated with LC_{30} cyantraniliprole. Besides, Zhao et al. (2016) illustrated a dose–response relationship of the similar sublethal effects in different concentrations of benzothiazole. However, sometimes the different insecticides in different concentrations resulted in opposite endpoints, such as the decreased T value of *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) exposed to imidacloprid (Lashkari et al. 2007), the increased R_0 of *A. gossypii* exposed to nitenpyram (Wang et al. 2016b), and the increased R_0 and decreased T values of *Tetranychus urticae* Koch (Acari: Tetranychidae) treated with spinetoram (Wang et al. 2016a).

The other parameters such as s_{xj}, l_x, and v_{xj} also illustrated the adverse effects of flupyradifurone on *A. gossypii* population growth. Overlaps among stages in two s_{xj} curves were generated due to different development rates among individuals, and there existed an obvious decrease in the s_{xj} curve of the treatment group in the stages N2 and N3. If the life table data were analyzed by using the traditional female age-specific life table (Lewis 1942, Leslie 1945, Birch 1948, Carey 1993), the stage overlaps would not have been observed (Hu et al. 2010). The shorter life expectancy in the LC_{25}-treated group showed no hormesis effect due to insecticide treatment. The v_{xj} was also remarkably reduced owing to the insecticide application. Additionally, the decrease in m_x and $l_x m_x$ curves of treatment group indicated that the fecundity of F1 is disturbed by this insecticide (Tang et al. 2015). Since l_x is a simplified version of s_{xj}, the l_x curve of the insecticide-treated group could only show that flupyradifurone inhibited the survival rate in the earlier period during the entire life. However, the higher survival rate after age 33 d in the flupyradifurone-treated aphids showed a possible hidden hormesis as mentioned in Chen et al. (2016). Because the population fitness is affected by many factors (the developmental rate, survival rate, fecundity, reproductive age, etc.), any single factor may not reveal the overall effect of pesticide treatment. Our results showed that the life table analysis can offer the most comprehensive analysis on the pesticide effect on a population.

Since we already discussed whether sublethal effect is impaired or stimulated, it depends on different insecticides, applied concentrations, and insect species. Qu et al. (2015) well demonstrated that the low concentration of 0.2 mg/liter imidacloprid suppressed the population growth of *Aphis glycines* Matsumura (Hemiptera: Aphididae) while the relative lower doses (0.05 mg/liter) stimulated the reproduction and showed hormesis effect. Therefore, flupyradifurone applied here suppressed the growth of *A. gossypii* population, but induced hormesis on *D. citri* exposed to LC_{25} flupyradifurone, i.e., the same pesticide and relative concentration (Chen et al. 2017a). More additional studies are needed to evaluate precisely on flupyradifurone-induced sublethal effects on *A. gossypii*, to give appropriate guidance on the future application of this insecticide.

As the insecticide resistance management (IRM) programs highlight the rotation among insecticides MOA groups of insecticides (Herron and Wilson 2017), the discovery of alternatives inside the same MOA group is important. Sublethal and transgenerational effects of the novel broad-spectrum butenolide insecticide, flupyradifurone, on *A. gossypii* were studied here. And the results suggest that the sublethal concentration of flupyradifurone can delay the development and suppress the population growth of the offspring, indicating flupyradifurone possesses a great potential on the control of *A. gossypii* although further researches are needed for a thorough understanding of the sublethal effects.

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