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Effects of sublethal concentrations of bifenthrin on the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract

Bifenthrin is a broad-spectrum insecticide and acaricide that is widely used in China. We evaluated the effects of sublethal concentrations (LC_{10} and LC_{25}) of bifenthrin on the eggs and adult females of the two-spotted spider mite, *Tetranychus urticae*, in the laboratory at 26±1°C, 80% RH, and a 16 h: 8 h (L: D) photoperiod. The sublethal doses of bifenthrin decreased the intrinsic and finite rate of increase, net reproductive rate, survival rate, and reproductive value. The sublethal doses also increased the mean generation time, total pre-ovipositional period, and duration of the larval and nymphal stages. The intrinsic rate of increase dropped from 0.252/day in the control to 0.222 and 0.208/day in response to LC_{10} and LC_{25} treatments, respectively. Following LC_{10} and LC_{25} treatments, the net reproductive rate dropped from 60.65 offspring/individual in the control to 45.19 and 40.81, respectively. These laboratory results indicate that sublethal concentrations of bifenthrin may decrease the developmental rate of *T. urticae*, are unlikely to result in the resurgence of *T. urtciae* populations, and might therefore be useful in the integrated management of this pest.

Key Words: *Tetranychus urticae*, Bifenthrin, Sublethal concentration, Life table

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan and destructive pest of agricultural crops in China and elsewhere. Pesticides are widely used against *T. urticae*. Such pesticides include bifenthrin, which is a pyrethroid insecticide and acaricide that is widely used against many insect and mite pests of the agricultural crops and orchards. The current study concerns the effects of sublethal concentrations of bifenthrin on *T. urticae*.

When too little pesticide has been applied or when the pesticide has degraded, pests are likely to be exposed to sublethal concentrations. In some cases, sublethal concentrations of pesticides can contribute to pest management. For example, sublethal pesticide concentrations may increase pest developmental time and reduce adult longevity and fecundity (Wang *et al.* 2009; Song *et al.* 2013; He *et al.* 2013). In other cases, however, sublethal doses of insecticides can cause a resurgence of the pest population (Hall 1979; Liu *et al.* 1998). Therefore, an understanding of sublethal effects is fundamental to understanding the efficacy and risk of pesticide application (Desneux *et al.* 2007).

Pyrethroid insecticides like bifenthrin interfere with the insect nervous system, resulting in trembling or paralysis, which is usually followed by death. Because of their rapid action and excellent contact toxicity against a broad-spectrum of arthropod pests, pyrethroids are often used to control insects and spider mites (Herron *et al.* 2001; Zhang *et al.* 2012). The effects of lethal and

sublethal concentrations of some pyrethroid insecticides on various mite species have been investigated (Liu *et al.* 1998; Bowi *et al.* 2001; Zhang *et al.* 2012), and most studies have found that pyrethroid application induces resurgence of the pest population (Gerson & Cohen 1989; Dutcher 2007). In a laboratory study with the mite *T. cinnabarinus*, deltamethrin increased oviposition and cypermethrin increased population growth, suggesting that application of these pyrethroids probably contributed to the population resurgence observed in treated fields (Liu *et al.* 1998). In a field study, deltamethrin application increased *T. cinnabarinus* numbers (Gao *et al.* 1991). The results of other studies, however, did not indicate that the effects of sublethal pesticide concentrations would result in the resurgence of mite populations (Bowi *et al.* 2001; Zhang *et al.* 2012).

As noted earlier, the pyrethroid bifenthrin is widely used against many insect and mite pests including *T. urticae*. Because the effects of sublethal concentrations of bifenthrin on *T. urticae* are unknown, we conducted a laboratory study to determine the sublethal effects of bifenthrin on eggs and adult females of *T. urticae*. The results obtained will provide fundamental information for the management of this important pest. More specifically, the results will increase our understanding of whether bifenthrin application contributes to the resurgence of *T. urticae* populations.

2. Materials and methods

2.1 Mite and insecticide

Specimens of *T. urticae* were originally obtained from an apple orchard in Tai'an Shandong Province, China, in June 2009. The population was maintained on bean leaf discs (var. Bifeng) on moist sponges in Petri dishes (12-cm-diameter dishes) in an incubator at 26±1°C, 80% RH, and a photoperiod of 16 h: 8 h (L: D). Cotton strips placed around each leaf disc prevented mite escape. These incubator conditions were used for all experiments in this study.

The bifenthrin formulation used in this study was an emulsifiable concentrate (Bayer Cropscience China Co., Ltd., China) containing 100 g/kg of active ingredient.

2.2 Bioassay and determination of sublethal concentrations

Bioassays were conducted with eggs and adult females of *T. urticae* using the leaf dipping method (He *et al.*, 2011). Serial dilutions of bifenthrin were prepared with pure water. Six concentrations (including the water control) were used. Each bean leaf disc (2 cm in diameter), which contained either 30 recently deposited eggs or 30 24-h-old adult females, was dipped into a solution for 5 s and then quickly dried using small pieces of filter paper (the filter paper absorbed the excess acaricide solution attached to the surface of the mites and leaf disc). Each leaf disc was then placed on a sponge in a Petri dish, and the dishes were placed in the incubator. One Petri dish with one disc was regarded as a replicate, and four replicate dishes were used for each concentration. Mortality of adult females was assessed after 24 h; female mites that could not crawl and were non-functional when touched with a camel hair brush were scored as dead. For the eggs, mortality was assessed daily starting with the eclosion of the first protonymph (about 6 d after treatment) and continuing for five successive days. When more than 90% eggs in the controls had eclosed except those that had died because of physiological causes, eggs that had not developed into larvae were scored as dead. Egg mortality was determined by subtracting the number of protonymphs from the total number of eggs.

Mortality data for adult females and eggs were corrected using the Abbott's formula (Abbott 1925), and the LC_{10} and LC_{25} values and their 95% fiducial limits and slope $\pm$ SE were calculated from probit analysis using Polo Plus Version 1.0 software (LeOra Software, Berkeley, CA,USA). According to the bioassay results, the LC_{10} and LC_{25} values were calculated from the regression equation and selected as the sublethal doses for the subsequent experiments.

2.3 Sublethal exposure of *T. urticae* eggs to bifenthrin

Adult females were placed on bean leaf discs (about 30 females per 2-cm-diameter disc), which were placed on sponges in Petri dishes (3.5-cm-diameter) in an incubator. After 24 h, the adults were removed, and eggs were removed until 30 remained on each disc. The discs with the eggs were then dipped into the water control or bifenthrin at LC_{10} or LC_{25} concentrations for 5 s. The discs were placed on sponges in Petri dishes and were incubated as described in section 2.2. Each of the three treatments was represented by 10 replicate Petri dishes. The development of eggs was observed daily. When the eggs had developed into larvae, 200 of the surviving larvae from the combined replicate per treatment were transferred onto a new, untreated leaf disc (one larva per disc) and incubated as before. The development of larvae was documented daily. When a nymph developed into the late second stationary phase, one male mite from the stock colony was introduced for mating and then removed after 2 days. The discs were examined with a dissecting microscope, and the number of eggs deposited was determined daily. After the number of eggs was recorded, a soft brush was used to transfer each female to a new leaf disc. This was repeated until the mites were dead. Dead or escaped females were excluded from the analysis.

2.4 Sublethal exposure of *T. urticae* adult females to bifenthrin

About 300 adult females (24 h old) from the laboratory colony were transferred to 10 fresh bean leaf discs (30 mites/disc), each of which was placed on a sponge in a Petri dish (3.5-cm-diameter disc). Once the mites began to feed (after about 30 min on the discs), the discs with mites were dipped into the water control or bifenthrin at LC_{10} or LC_{25} concentrations for 5 s, dried, and then transferred to the incubator as described earlier. After 24 h, each female mite was carefully transferred to a new, fresh bean leaf disc and was reared under the same condition. During the experiment, the leaf discs were kept moist and were changed when necessary. For each of the three treatments (LC_{10}, LC_{25}, and control), more than 100 adult females were investigated, and each female was considered as one replicate. The longevity of each adult female was recorded. The number of eggs deposited per female was also recorded daily until the females died.

2.5 Data analysis

Raw data on the survivorship, longevity, and daily fecundity of *T. urticae* individuals were analyzed according to the age-stage, two-sex life table (Chi & Liu 1985; Chi 1988) using the computer program TWOSEX-MSChart (Chi 2012). The effects of sublethal concentrations of bifenthrin on survival and development of immature *T. urtciae*, adult longevity, and fecundity were assessed by analyses of variance (ANOVAs) with SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). When an ANOVA was significant ($P < 0.05$), means were compared by LSD ($P < 0.05$). Plots for survival, fecundity, life expectancy, and reproductive value were prepared with SigmaPlot 10.0 (Systat Software Inc., Point Richmond, CA, USA).

3. Results

3.1. Toxicity of bifenthrin to *T. urticae* eggs and adult females

The LC_{50} was 18.61 mg/L for eggs and 75.25 mg/L for adult females (Table 1). The LC_{10} and LC_{25} for eggs was 5.47 and 9.77 mg/L, respectively, and the LC_{10} and LC_{25} for adult females was 20.98 mg/L and 38.41 mg/L, respectively (Table 1).

TABLE 1. Toxicity of bifenthrin to *T. urticae* eggs and adult females. Values in parentheses are 95% CL.

Stage	Slope±SE	LC_{10} (mg/L)	LC_{25} (mg/L)	LC_{50} (mg/L)
Egg	2.41±0.16	5.47 (4.31–6.61)	9.77 (8.25–11.25)	18.61 (16.53–20.84)
Adult female	2.31±0.25	20.98 (6.38–34.04)	38.41(18.92–55.39)	75.25 (51.65–116.69)

3.2 Effects of exposing *T. urticae* eggs to sublethal concentrations of bifenthrin

The pre-oviposition period and the developmental time of eggs and nymphs were significantly increased when eggs were exposed to LC_{10} and LC_{25} of bifenthrin (Table 2). The development of larvae was not significantly affected by the LC_{10} treatment but was significantly prolonged by the LC_{25} treatment. The development of protonymphs and deutonymphs was prolonged by both sublethal concentrations of bifenthrin. However, the length of female adult period and the fecundity were both significantly reduced by the LC_{10} and LC_{25} treatments of bifenthrin (Table 2).
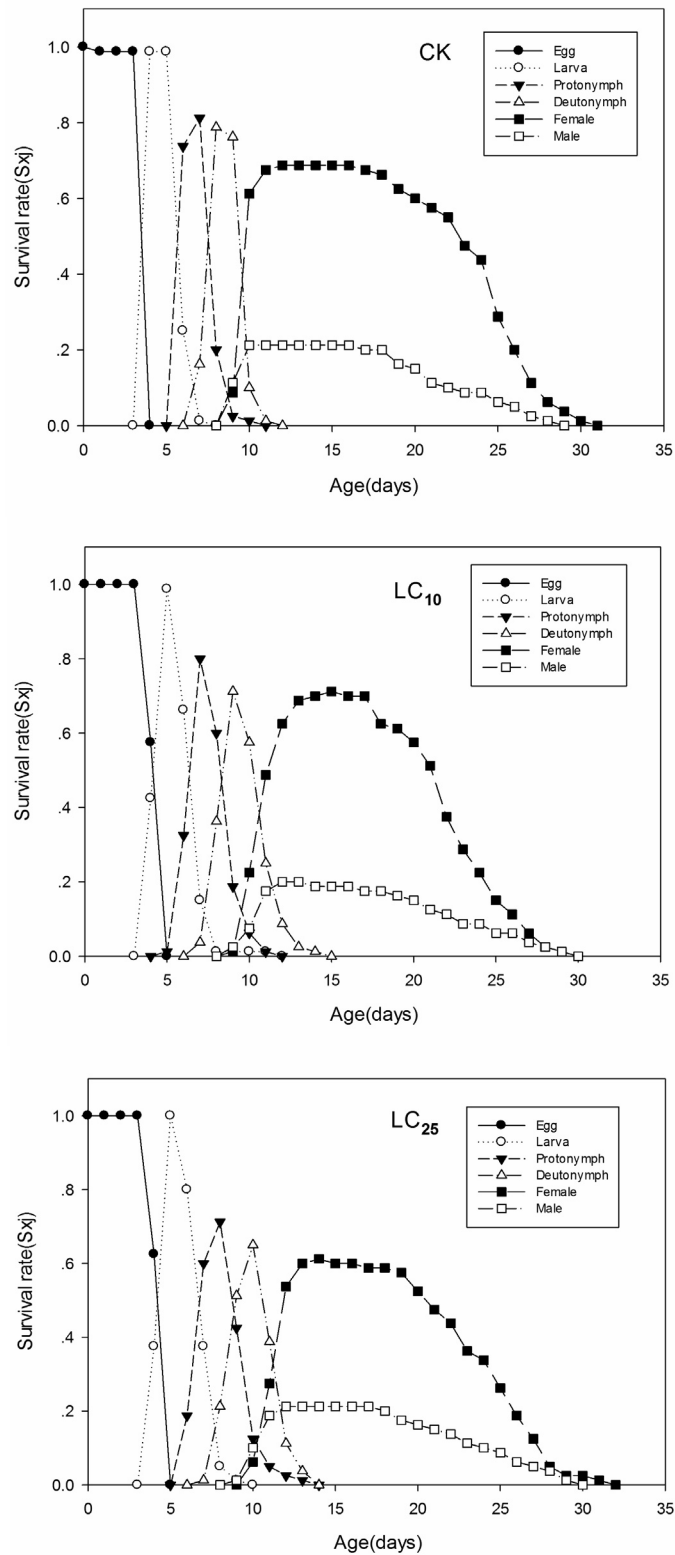
TABLE 2. Effects of exposing *T. urticae* eggs to sublethal concentrations of bifenthrin on the duration of egg, larval, nymph, and adult female stages and on female fecundity. Values are means ± SD. Means in a row followed by different letters are significantly different (*P*<0.05). CK is the water control.

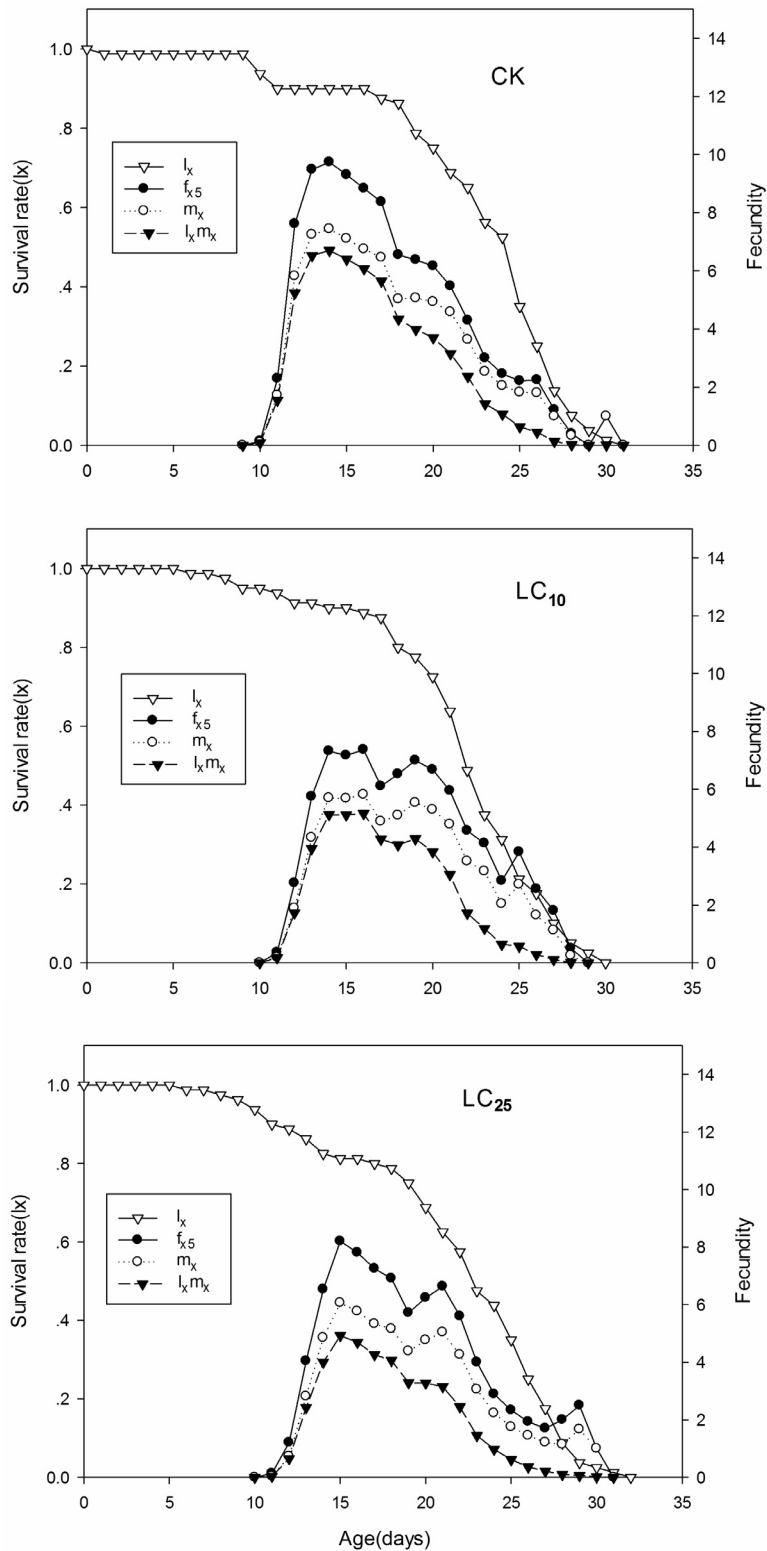| Stage	CK	LC_{10}	LC_{25}
Egg duration (d)	4.00±0.00 b	4.58±0.5 a	4.62±0.49 a
Larval duration (d)	2.27±0.47 b	2.23±0.53 b	2.62±0.63 a
Protonymph duration (d)	1.77±0.53 b	2.00±0.55 a	2.06±0.47 a
Deutonymph duration (d)	1.92±0.43 b	2.25±0.52 a	2.15±0.58 a
Pre-oviposition period (d)	1.18±0.48 b	1.46±0.69 a	1.40±0.57a
Adult duration (female)	14.22±3.63 a	11.99±3.56 b	12.62±3.83 b
Fecundity (eggs/female/day)	7.89±0.50 a	6.62±0.55 b	6.85±0.53 b

Exposing *T. urticae* eggs to sublethal concentrations of bifenthrin also affected *T. urticae* population parameters. The intrinsic rate of increase (*r*), the finite rate of increase, and the net reproduction rate (R_0) were significantly reduced by the LC_{10} and LC_{25} treatments, while the mean generation time (*T*) was significantly increased by the LC_{10} and LC_{25} treatments (Table 3).

TABLE 3. *T. urticae* population parameters as affected by exposing eggs to sublethal concentrations of bifenthrin. Values are means ± SE. Means in a row followed by different letters are significantly different (*P*<0.05). CK is the water control.

Population parameter	CK	LC_{10}	LC_{25}
Intrinsic rate of increase (*r*) (d^{-1})	0.252±0.0055 a	0.222±0.0055 b	0.208±0.006 b
Finite rate of increase (λ) (d^{-1})	1.286±0.007 a	1.249±0.007 b	1.232±0.007 c
Net reproductive rate (R_0) (offspring/individual)	60.65±5.32 a	45.19±4.12 b	40.81±4.06 c
Mean generation time *T* (d)	16.29±0.12 b	17.15±0.17 a	17.79±0.20 a

FIGURE 1. Age-specific survival rate (S_{xj}) of *T. urticae* after eggs were exposed to sublethal concentrations of bifenthrin.

FIGURE 2. Age-specific survival rate (l_x), female age-specific fecundity (f_{x5}), age-specific fecundity of the total population (m_x), and age-specific maternity ($l_x m_x$) of *T. urticae* after eggs had been exposed to sublethal concentrations of bifenthrin.

The age-stage specific survival rates (S_{xj}) (Fig. 1) can be used to indicate the probability that an egg will survive to age x and develop to stage j after treatment with water (CK) or with bifenthrin at LC_{10} or LC_{25}. Obvious overlap phenomenon was found in these curves for the sake of the difference of various developmental rates among individuals (Fig. 1). Protonymph and deutonymph stages lasted longer if the mites originated from eggs that had been treated with the LC_{10} or LC_{25} rather than with water. The maximal survival rate for deutonymphs was lower with the LC_{10} or LC_{25} treatment than with the control. Male adults survived 1 day longer when treated with the sublethal concentrations of bifenthrin rather than with water (Fig. 1).

The age-specific survival rate (l_x) indicates the probability that an egg will survive to age x, and the curve of the age-specific survival rate is a simplified form of the curves of age-stage survival rate, regardless of the different developmental stage. Relative to the l_x curve of the control, the l_x curves of the LC_{10} and LC_{25} treatments declined significantly 20 days after eggs had been treated. The highest peaks in f_{x5} and m_x were higher in the control than in the LC_{10} and LC_{25} treatments. The $l_x m_x$ value changed depending on l_x and m_x, and the maximum $l_x m_x$ values were on day 14, 16, and 15 for the control, LC_{10}, and LC_{25} treatments, respectively (Fig. 2).

3.3 Effects of exposing *T. urticae* adults to sublethal concentrations of bifenthrin.

Treatment of adult females with sublethal concentrations of bifenthrin significantly reduced the number of eggs laid per female and significantly increased the length of the pre-oviposition period (Table 4). Treatment of adults with sublethal concentrations of bifenthrin also tended to increase adult longevity but the effect was not significant.

TABLE 4. Effects of treating *T. urticae* adults with sublethal concentrations of bifenthrin on adult fecundity and longevity. Values are means ± SD. Means in a row followed by different letters are significantly different ($P<0.05$).

Treatment	Preoviposition period (d)	Number of eggs/ female	Eggs/female/day	Longevity (d)
CK	2.24±0.43 b	38.35±11.29 a	4.17±1.17 a	11.81±2.74 a
LC_{10}	3.26±0.75 a	30.68±11.40 b	3.43±1.03 b	12.55±3.45 a
LC_{25}	3.64±1.08 a	29.44±14.19 b	3.34±1.01 b	12.56±3.55 a

4. Discussion

Chemical control remains important for the management of *T. urticae* and other mite and insect pests in agricultural fields in China. Many reports have indicated, however, that the application of an insecticide or acaricide at sublethal concentrations may cause pest numbers to increase rather than decline (Gerson & Cohen 1989; Morse & Zareh 1991; Zeng & Wang 2010). The stimulation of populations caused by sublethal concentrations of pesticides is incompletely understood but may result from a suppression of natural enemies (Dutcher 2007; Raupp *et al.* 2010; Abedi *et al.* 2014), a stimulation of reproduction, an increase in egg hatching, and an enhancement of pre-imaginal survivorship (Zeng & Wang 2010). Pyrethroid application may also result in increases in mite populations (Gerson & Cohen 1989; Chen & Chen 1990; Holland *et al.* 1993). In some studies, mites treated with pyrethroids deposited more eggs than untreated mites (Hall 1979; Penman *et al.* 1988; Liu *et al.* 1998). The pyrethroid permethrin increased the age-specific fecundity (m_x) and the net reproductive rate (R_0) of the mite *Panonychus citri* (Jones & parrella 1984). Such phenomenon

occurred as well with the cotton aphid, *Aphis gossypii*, treated with deltamethrin (Nandihalli *et al.* 1992).

Although pyrethroids often cause resurgence of mite populations, the current results indicated that treatment of eggs with LC_{10} and LC_{25} concentrations of bifenthrin reduced the intrinsic rate of increase (r), the finite rate of increase (λ), and the net reproduction rate (R_0) while it increased the preovipositional period of *T. urticae* and prolonged the developmental time from egg to adult (this present study). These effects of bifenthrin were shown to be concentration-dependent. Our results indicate that sublethal doses of bifenthrin are likely to inhibit rather than enhance *T. urticae* population growth.

That sublethal concentrations of bifenthrin are likely to inhibit *T. urticae* population growth agrees with several other studies with pyrethroids. When treated with the LC_{25} concentration of fenpropathrin, adult females of *T. viennensis* Zacher exhibited reduced fecundity and longevity, although the LC_{10} treatment increased r_m from 0.237 to 0.259 (Li *et al.* 2006). On leaf discs treated with a sublethal concentration of bifenthrin, the fecundity of *Bemisia tabaci* B adults was reduced (He *et al.* 2013). Our results are also in accordance with the results obtained with another synthetic pyrethroid, esfenvalerate. The mites *P. ulmi* and *T. urticae* preferred esfenvalerate-free surfaces to treated surfaces; in addition, oviposition was negatively correlated with the concentration of esfenvalerate residues on surfaces (Bowi *et al.* 2001). Very recently, pyrethroid cypermethrin were proved to have acute toxicity on larval and adult stages of *Habrobracon hebetor* and negatively affected most of its life table parameters (Abedi *et al.* 2014).

That the effect of sublethal concentrations of pyrethroids can be complex is also demonstrated by a study with fenpropathrin. Zhang *et al.* (2012) reported that the response of a *T. urticae* population to LC_{10} and LC_{20} concentrations of fenpropathrin depended on the stage treated, i.e., the mite population was suppressed when eggs were treated but enhanced when adults were treated. As noted by Holland *et al.* (1993), the effects of sublethal concentrations of insecticides on pests probably depend on the insecticide and its concentration, the pest species, and the pest stage (Liu *et al.* 2012).

Sublethal concentrations of pesticides may also affect the F1 and F2 generations of the treated specimens. Sublethal concentrations of fenpropathrin increased the intrinsic rate of increase and net reproductive rate of *P. citri* in both the F1 and F2 generation (He *et al.* 2009). Treatment of the soybean aphid, *Aphis glycines*, with a sublethal concentration of beta-cypermethrin decreased the r_m and λ values but did not significantly affect adult longevity or fecundity of the F1 generation; population growth of the F2 generation, however, was enhanced by the prior treatment with beta-cypermethrin (Gao *et al.* 2008). Pakyari *et al.* (2013) reported that sublethal concentrations (LC_{10}, LC_{20}, and LC_{30}) of fenpropathrin could shorten the female life span of *Scolothrips longicornis* significantly, accompanied with large reductions in oviposition period and fecundity; their offspring also had significantly reduced longevity, oviposition period, and fecundity, although not to the same extent as experienced by their mothers. The results revealed that sublethal concentrations of pesticides can have long-term effects. This is in accord with other research showing that the exposure to a pesticide can lead to hereditable malfunctions and malformations (Adamski *et al.* 2009; He *et al.* 2011; Piiroinen *et al.* 2014).

Studies on the sublethal effects on the pests mainly aimed to find the negative, non-lethal impacts of pesticide on various life history parameters that might influence the population dynamics (Stark & Banks 2003). Given that sublethal concentrations of pesticides can have long-term effects, additional research is needed to determine how *T. urticae* life table parameters are affected for one or two generations following the exposure to sublethal levels of bifenthrin. In addition, future research should determine the mechanism by which sublethal concentrations of bifenthrin depress *T. urticae* population growth.

Acknowledgements

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