

Lethal and Sublethal Effects of Clothianidin on the Development and Reproduction of *Bemisia tabaci* (Hemiptera: Aleyrodidae) MED and MEAM1

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Abstract

The *Bemisia tabaci* (Gennadius) cryptic species complex includes important crop pests, and among them, the cryptic species Mediterranean (MED) and Middle East-Asia Minor 1 (MEAM1) cause substantial crop losses in China. The second-generation neonicotinoid clothianidin acts as an agonist of the nicotinic acetylcholine receptor in the insect nervous system and has both stomach and contact activity. In this study, the toxicity of clothianidin and five other insecticides to MED and MEAM1 was examined. The sublethal effects of clothianidin on the development and reproduction of MED and MEAM1 were also investigated. Among the six insecticides tested, clothianidin showed toxicities to both MED and MEAM1 adults with LC₅₀ values of 5.23 and 5.18 mg/liter, respectively. The sublethal effects of clothianidin were assessed by treating MED and MEAM1 adults with the LC₂₅ of 1.58 and 1.13 mg/liter, respectively. The LC₂₅ treatments accelerated the development of the F₁ generation but reduced survival and fecundity of both species. Our results indicate that clothianidin could be useful for the management of *B. tabaci* MED and MEAM1.

Key words: *Bemisia tabaci*, neonicotinoid, clothianidin, toxicity, sublethal effect

The tobacco whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a species complex comprising more than 35 morphologically indistinguishable cryptic species, several of which are globally invasive pests (De Barro et al. 2011, Boykin et al. 2012, Liu et al. 2012). These pests damage plants directly by feeding on the phloem and indirectly by transmitting viruses (Liu et al. 2007). In China, the most damaging cryptic species of this complex are *B. tabaci* Mediterranean (MED) and *B. tabaci* Middle East-Asia Minor 1 (MEAM1), which were formerly referred to as the Q and B 'biotypes,' respectively (De Barro et al. 2011, Pan et al. 2011). MEAM1 was first detected in China in 1995 and subsequently became the dominant whitefly species in most areas of the country (Chen 1997, Luo et al. 2002). MED was first detected in China in Yunnan Province in 2003 (Chu et al. 2006). Once introduced, MED rapidly spread across many provinces of China and has now replaced MEAM1 and other species in the *B. tabaci* complex (Pan et al. 2011).

Currently, chemical insecticides have been and still are important tools for controlling *B. tabaci* worldwide. The extensive application of insecticides (including organophosphates, insect growth regulators, and pyrethroids), however, has resulted in the development of

resistance in *B. tabaci* populations (Ahmad et al. 2002, Nauen et al. 2002, Basit et al. 2013). For this reason, large-scale employment of above insecticides is not a rational way for low insecticide residues in the process of agricultural production. Neonicotinoid insecticides, which are often used to control *B. tabaci*, act as agonists of nicotinic acetylcholine receptors and cause hyperexcitation and then death (Jeschke and Nauen 2008). Because they are effective against many insect pests, have favorable toxicological properties, and can be applied in a variety of ways, neonicotinoid insecticides have been widely used (Goulson 2013, Bass et al. 2015) but are particularly active effective against sucking insects like those in the *B. tabaci* complex (Nauen et al. 2008, Jeschke et al. 2011). In addition to killing pests directly, neonicotinoids have sublethal effects that impair insect behavior and physiology (Tan et al. 2012, Wang et al. 2016, Qu et al. 2017).

In 1996, resistance to a neonicotinoid (imidacloprid) in *B. tabaci* was reported for the first time (Cahill et al. 1996). Since then, reports of neonicotinoid resistance have steadily increased (Bass et al. 2015). The development of resistance has caused many farmers to apply the insecticide at higher rates, which has increased the

threat to nontarget organisms (Fairbrother et al. 2014). In China, both MEAM1 and MED in many geographic regions have evolved high levels of resistance to several neonicotinoids (Luo et al. 2010, Wang et al. 2017a). Clothianidin is a second-generation neonicotinoid insecticide that binds to the nicotinic acetylcholine receptors of arthropods (Sallam et al. 2009) and is effective against a variety of insect pests (Magalhaes et al. 2010, Zhang et al. 2015, Rahman and Broughton 2016, Zhang et al. 2016). Both lethal and sublethal effects of clothianidin have been reported on several insect pests (Abbott et al. 2008, Kullik et al. 2011, Pecenka and Lundgren 2015). In the current research, there were five other tested insecticides to adults of *B. tabaci* MEAM1 and MED. Among them, Imidacloprid is a neonicotinoid belonging to group 4A (IRAC 2017 <http://www.irac-online.org/modes-of-action/>) and sulfoxaflor is a sulfoxamide of group 4C (IRAC 2017 <http://www.irac-online.org/modes-of-action/>), although both insecticides act on the nicotinic acetylcholine receptors, their chemical structures and mode of action are different (Sparks et al. 2013). Pyriproxyfen is an analog of juvenile hormone and suppressor of adult formation, which could inhibit eclosion of insect pests. Spirotriamat acts by inhibiting the synthesis of lipids, and buprofezin is an insect growth regulator and interfere with chitin biosynthesis. Moreover, we also assessed the effects of treating MEAM1 and MED adults with sublethal levels of clothianidin on development, fecundity, and oviposition period of the F_1 generation and of egg hatch in the F_2 generation.

Materials and Methods

Insects

An insecticide-susceptible strain of *B. tabaci* MED was originally collected from poinsettia in Beijing in 2008 and was subsequently reared on cotton plants (*Gossypium hirsutum* L. var. 'Shiyuan 321') in a growth chamber. An insecticide-susceptible strain of *B. tabaci* MEAM1 was originally collected from cabbage in Beijing in 2004 and was also subsequently reared on cotton plants (*Gossypium hirsutum* L. var. 'Shiyuan 321') in another growth chamber. The two strains were reared without exposure to any insecticide at $27 \pm 1^\circ\text{C}$, $60 \pm 10\%$ relative humidity (RH), and a photoperiod of 16:8 (L:D) h. All adults tested in bioassays were ≤ 7 d old, and both males and females were used at a ratio of 1:1.

Insecticides

The following commercial insecticides were used in bioassays: clothianidin, pyriproxyfen, spirotetramat, buprofezin, sulfoxaflor, and imidacloprid. Clothianidin (20% SC[I]) was provided by Hebei Veyong Biochemical Co., Ltd., Hebei, China. Pyriproxyfen (100 g/liter EC) was provided by Sumitomo Chemical Co., Ltd., Tokyo, Japan. Spirotetramat (240 g/L SC) was provided by Bayer Crop Science, Monheim, Germany. Sulfoxaflor (50% WG) was provided by Dow AgroSciences LLC, Indianapolis, USA. Imidacloprid (10% WP) was provided by Jiangsu Dongbao Chemical Industry Co., Ltd., Jiangdu, China.

Toxicities of the Six Insecticides to MED and MEAM1 Adults

The toxicities of the six insecticides to MED and MEAM1 adults were measured (Supp Table 1 [online only]) using the leaf-dipping bioassay method (Qu et al. 2017). Leaf discs (22 mm diameter) from cotton plants were dipped in the insecticide solution or in distilled water for 20 s. After the discs dried, each was placed in the bottom of a flat-bottom, 78-mm long glass tube containing

agar (2 ml of 15 g/liter) with the adaxial surface facing down. Adults of MED and MEAM1 were transferred into these tubes by inverting the tubes above the leaves on cotton plants reared in the glasshouse. This allowed the mixed adults to fly into the tube. After 20–30 adults had flown into a tube, the tube was sealed with a cotton plug. The tubes were maintained in a growth chamber at $27 \pm 1^\circ\text{C}$, $60 \pm 10\%$ RH, and a photoperiod of 16:8 (L:D) h. Mortality was recorded after 48 h and immobile adults were scored as dead. LC_{50} values for the six insecticides were calculated with a Probit statistical model and LeOra Software. 2002. LC_{25} values were also calculated for clothianidin using the PoloPlus software

Sublethal Effects of Clothianidin on MED and MEAM1

After MED and MEAM1 adults were exposed to sublethal concentrations (LC_{25} values) of clothianidin for 48 h as described in the previous section, the following parameters were assessed for the F_1 generation as the experimental unit, respectively: developmental time and survival rate of the eggs, nymphal instars and pseudopupae stages; fecundity; and oviposition duration. The hatchability of eggs produced by the F_1 generation was also determined. In brief, 20 insect-free host plants in total were randomly placed in four separate insect-proof cages equally (two cages for experimental MED and MEAM1, and others for the controls). For each cryptic species (MED or MEAM1), *B. tabaci* adults ($n = 100$) that previously treated with clothianidin (LC_{25}) cotton leaves were then introduced into the experimental cage for egg laying. One hundred untreated *B. tabaci* adults were added into the control cage. After 12 h for oviposition, the plants were removed from the cages, and two leaves in each plant were randomly selected and marked. The eggs on the nonselected leaves were removed with the aid of a microscope, and 20 eggs were retained on each selected leaf. The location of each remaining egg was marked on the abaxial surface of the selected leaf, and the drawings allowed us to track each egg until adult emergence. Each combination of species (MEAM1 or MED) and treatment (\pm clothianidin) was represented by 10 leaves. Ten chosen leaves were employed for each treated group in either MEAM1 or MED, and for the control group as well, respectively. Each plant was then placed in a separate growth chambers at $27 \pm 1^\circ\text{C}$, $60 \pm 10\%$ RH, and a photoperiod of 16: 8 (L:D) h. When pseudopupae were observed on the leaf, the whole plant was transferred to a separate cage with an insect-proof net. The numbers of nymphs and adults on each plant were recorded every day. Each newly emerged adult was moved to a new leaf, which was cut from the stem to take notes of the fecundity until the death of all individuals. The percentage of eggs that hatched was also determined.

Statistical Analysis

All data were checked for normality using nonparametric Kolmogorov–Smirnov tests ($P < 0.05$). Data for the first assay, which was used to determine LC_{50} and RF values for the six insecticides, were statistically analyzed by LeOra Software. 2002. For the second assay, data were compared between the control (CK) and LC_{25} treatment; this was done separately for MEAM1 and MED. Data showing a normal distribution (survival rate, oviposition duration, fecundity, and hatchability) was compared using Student's *t*-test ($P < 0.05$). Data that were not normally distributed (developmental duration) were compared using the nonparametric Mann–Whitney *U*-test ($P < 0.05$). SPSS software (2001) was used for all statistical analyses.

Results

Lethal Effects of Six Insecticides on MED and MEAM1 Adults

The LC_{50} values of six insecticides against MED and MEAM1 adults are listed in Table 1. Mortality was <5% in the control groups. LC_{50} values for clothianidin were shown in both MED and MEAM1 (5.18 mg/liter and 5.23 mg/liter of LC_{50} for MEAM1 and MED, respectively). As the counterparts, LC_{50} values for imidacloprid (6.58 and 10.86 mg/liter of LC_{50} , respectively), sulfoxaflor (10.74 and 11.49 mg/liter of LC_{50} , respectively), buprofezin (223.73 and 736.54 mg/liter of LC_{50} , respectively), pyriproxyfen (315.45 and 846.52 mg/liter of LC_{50} , respectively) and spirotetramat (681.88 and 1064.93 mg/liter of LC_{50} , respectively) were also shown in both MED and MEAM1. Moreover, the LC_{90} values for the six tested insecticides were calculated and shown in Table 1.

Sublethal Effects of Clothianidin on MED and MEAM1

LC_{25} concentrations (MED and MEAM1 adults with the LC_{25} of 1.58 and 1.13 mg/liter) were used to assess the sublethal effects of the clothianidin on MED and MEAM1 development and reproduction. Exposure of adults to the sublethal concentration of

clothianidin significantly affected developmental time, survival rate, and fecundity of the F_1 generation. For MED, the LC_{25} treatment significantly reduced developmental time from egg to first-instar nymph ($U = 17319.5$, $Z = -2.311$, $P = 0.021$), and from egg to adult ($U = 5218.0$, $Z = -7.079$, $P < 0.0001$) (Table 2). The LC_{25} treatment significantly reduced the survival of MED first- and second-instar nymphs, third-instar nymphs, and pseudopupae ($t = 2.664$, $df = 18$, $P = 0.016$; $t = 2.268$, $df = 18$, $P = 0.036$; $t = 2.862$, $df = 18$, $P = 0.010$; and $t = 3.848$, $df = 18$, $P = 0.001$, respectively) (Table 3). The LC_{25} treatment significantly reduced MED fecundity ($t = -3.253$, $df = 18$, $P = 0.004$), while the differences of oviposition duration ($t = -1.346$, $df = 18$, $P = 0.195$) and egg hatching rate ($t = -1.224$, $df = 18$, $P = 0.237$) are not significant (Fig. 1). For MEAM1, the LC_{25} treatment significantly reduced the developmental time from egg to first-instar nymph ($U = 15252.5$, $Z = -4.226$, $P < 0.0001$) and from egg to adult ($U = 5833.5$, $Z = -5.115$, $P < 0.0001$) (Table 2). The LC_{25} treatment also significantly reduced the survival of MEAM1 first-instar nymphs, third-instar nymphs, and pseudopupae ($t = 3.433$, $df = 18$, $P = 0.003$; $t = 2.913$, $df = 18$, $P = 0.009$; and $t = 3.217$, $df = 18$, $P = 0.005$, respectively) (Table 3). Survival of second-instar MEAM1 nymphs was not significantly affected by the LC_{25} treatment ($t = -0.289$, $df = 18$, $P = 0.776$) (Table 3). The LC_{25} treatment significantly reduced MEAM1 fecundity ($t = 2.982$,

Table 1. Median lethal concentration (LC_{50}) of the six different insecticides on *B. tabaci* MEAM1 and MED

Insecticide	Cryptic species	Number ^a	Slope \pm SE	LC_{50} (mg/liter)	LC_{50} (95% FL) (mg/liter) ^b	LC_{90} (mg/liter)	LC_{90} (95% FL) (mg/liter) ^c	χ^2 (df)	P-value
Clothianidin	MEAM1	672	1.02 \pm 0.10	5.18	3.99–8.68	93.22	58.58–182.37	3.91 (4)	0.4610
	MED	689	1.30 \pm 0.11	5.23	3.74–7.04	50.88	30.67–116.05	5.35 (4)	0.3318
Imidacloprid	MEAM1	704	1.06 \pm 0.11	6.58	4.92–8.33	107.53	72.39–188.21	2.80 (4)	0.6330
	MED	695	1.17 \pm 0.10	10.86	8.76–13.24	136.75	93.25–231.11	2.92 (4)	0.6009
Sulfoxaflor	MEAM1	700	1.20 \pm 0.10	10.74	8.71–13.02	126.15	87.467–207.53	3.35 (4)	0.5465
	MED	714	1.14 \pm 0.10	11.49	9.29–14.00	151.44	102.27–259.48	2.92 (4)	0.5968
Buprofezin	MEAM1	703	1.03 \pm 0.11	223.73	152.85–297.13	3943.85	2716.51–6721.74	1.76 (4)	0.7961
	MED	712	1.44 \pm 0.12	736.54	600.82–878.86	5726.63	4416.87–8020.99	2.08 (4)	0.7363
Pyriproxyfen	MEAM1	676	1.28 \pm 0.11	315.45	244.70–389.09	3140.83	2338.29–4657.81	2.32 (4)	0.6542
	MED	699	1.57 \pm 0.12	846.52	707.91–994.02	5553.72	4364.27–7552.97	1.51 (4)	0.8423
Spirotetramat	MEAM1	695	1.22 \pm 0.11	681.88	529.40–841.76	7603.89	5543.84–11640.20	2.03 (4)	0.7121
	MED	716	1.78 \pm 0.12	1064.93	919.200–1223.810	5603.83	4522.78–7315.79	2.42 (4)	0.5647

^aNumber of adults tested.

^bConcentration of insecticide killing 50% of adults and its 95% fiducial limits.

^cConcentration of insecticide killing 90% of adults and its 95% fiducial limits.

Table 2. Sublethal effects of clothianidin on developmental time in specific stages of the F_1 generation of *B. tabaci* MED and MEAM1

Developmental time from egg to the indicated stage (d)	Control	LC_{25} treatment	U(Z)	P-value
MED				
First instar	6.85 \pm 0.05	6.68 \pm 0.04*	17319.5(-2.311)	0.021
Second instar	10.31 \pm 0.08	10.30 \pm 0.08	16773.5(-0.195)	0.845
Third instar	12.77 \pm 0.11	12.34 \pm 0.09	12377.0(-1.897)	0.058
Pseudopupae	16.14 \pm 0.11	16.15 \pm 0.10	11775.0(-0.300)	0.764
Adult	20.77 \pm 0.12	19.51 \pm 0.10*	5218.0(-7.079)	<0.0001
MEAM1				
First instar	6.65 \pm 0.05	6.38 \pm 0.05*	15252.5(-4.226)	<0.0001
Second instar	9.82 \pm 0.08	9.69 \pm 0.09	15875.5(-0.844)	0.398
Third instar	12.89 \pm 0.09	12.86 \pm 0.08	13814.0(-0.208)	0.835
Pseudopupae	15.82 \pm 0.10	15.83 \pm 0.10	10377.0(-0.414)	0.679
Adult	19.41 \pm 0.10	18.62 \pm 0.11*	5833.5(-5.115)	<0.0001

Values are means \pm SE. Statistical comparisons are between whiteflies not treated with clothianidin (Control) and those treated with the LC_{25} of clothianidin.

*The means in a row are significantly different at $P < 0.05$ according to the nonparametric Mann-Whitney U-test.

Table 3. Sublethal effects of clothianidin on survival in specific stages of the F₁ generation of *B. tabaci* MED and MEAM1

Survival (%)	Control	LC ₂₅ treatment	<i>t</i> (df)	<i>P</i> -value
MED				
From first instar to second instar	97.50 ± 1.12	90.87 ± 2.23*	2.664 (18)	0.016
From second instar to third instar	93.37 ± 1.29	86.68 ± 2.65*	2.268 (18)	0.036
From third instar to pseudopupae	95.63 ± 1.05	89.29 ± 1.95*	2.862 (18)	0.010
From pseudopupae to adults	95.38 ± 1.17	87.04 ± 1.83*	3.848 (18)	0.001
MEAM1				
From first instar to second instar	96.10 ± 0.72	87.83 ± 2.40*	3.433 (18)	0.003
From second instar to third instar	91.24 ± 1.06	91.97 ± 2.29	-0.289 (18)	0.776
From third instar to pseudopupae	95.49 ± 1.42	83.81 ± 3.75*	2.913 (18)	0.009
From pseudopupae to adults	94.11 ± 1.52	86.25 ± 1.91*	3.217 (18)	0.005

Values are means ± SE. Statistical comparisons are between whiteflies not treated with clothianidin (Control) and those treated with the LC₂₅ of clothianidin.

*The means in a row are significantly different at $P < 0.05$ according to the Student's *t*-test.

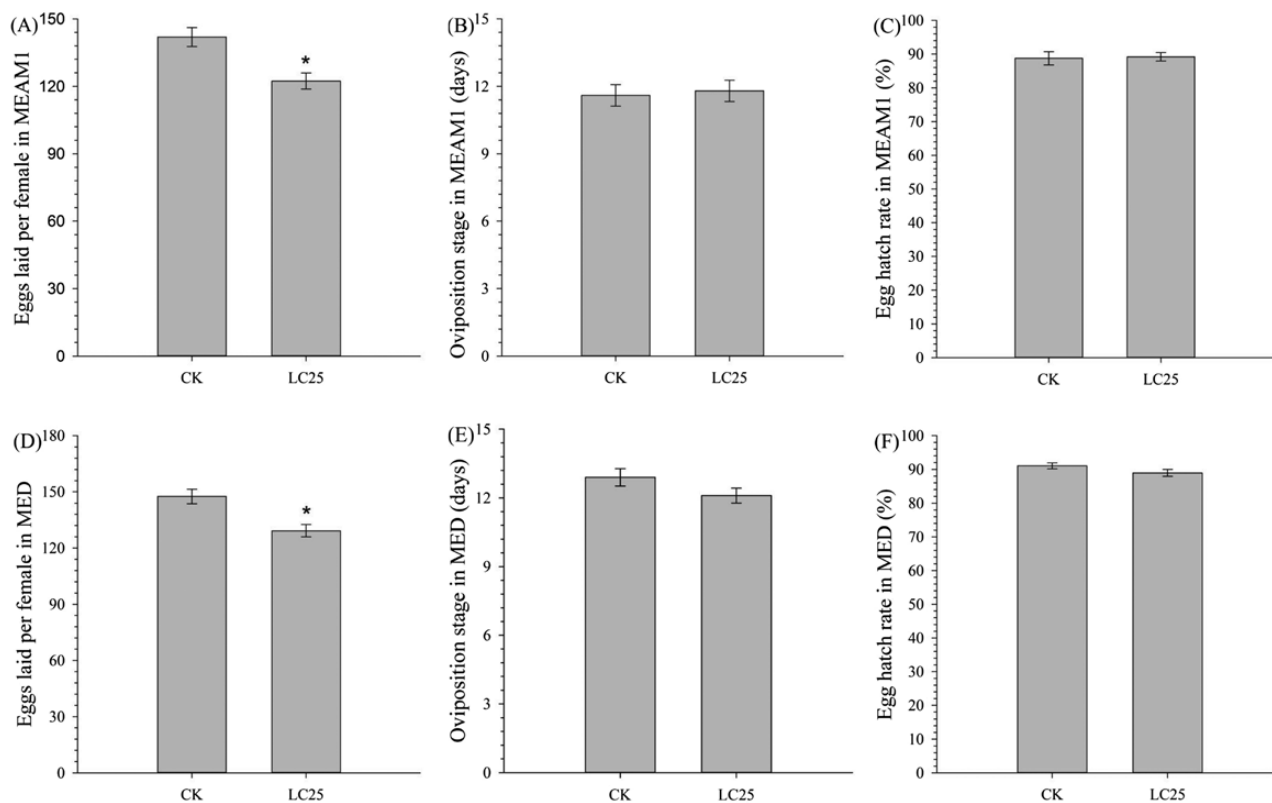


Fig. 1. Sublethal effects of the LC₂₅ of clothianidin on the fecundity, oviposition duration, and egg hatching rate of *B. tabaci* MEAM1 (A, B, and C) and *B. tabaci* MED (D, E, and F). The LC₂₅ for MEAM1 and MED is 1.13 and 1.58 mg/liter, respectively. *A significant difference between the nontreated control (CK) and the LC₂₅ treatment ($P < 0.05$).

df = 18, $P = 0.008$), while the differences of oviposition duration ($t = -0.245$, df = 18, $P = 0.809$) and egg hatching rate ($t = -0.06$, df = 18, $P = 0.953$) are not significant (Fig. 1).

Discussion

Our results indicated that among the six insecticides, clothianidin is relatively useful for controlling both MEAM1 and MED adults. As a recommendation for the field application, clothianidin, imidacloprid, and sulfoxaflor could be useful for controlling the adults of whitefly in the field. Specifically, combined with the LC₉₀ values of clothianidin for both MED and MEAM1, 100 mg/liter could be the recommended doses for field. Moreover, the finding that buprofezin, pyriproxyfen, and spirotetramat were ineffective in controlling

MEAM1 and MED adults was consistent with Xie et al. (2014). Also, it has been reported that buprofezin, pyriproxyfen, and spirotetramat showed high efficiency for eggs and nymphs of whiteflies (Xie et al. 2014, Peng et al. 2017). In summary, buprofezin, pyriproxyfen, and spirotetramat could be applied in the control of eggs and nymphs of whiteflies, and clothianidin with the concentration of 100 mg/liter would be promising for killing adults *B. tabaci* MED and MEAM1 in the field, and the evaluation of field work would be conducted in the next step. Previous studies have demonstrated that the effect of sublethal insecticide treatment on the time required for arthropod development depends on the insecticide and arthropod species. In some cases, the sublethal treatments extend the developmental times and decrease the survival of each stage (Chen et al. 2016, Dong et al. 2017, Zhou et al. 2017). In two studies with

B. tabaci, sublethal concentrations of insecticides decreased egg laying duration, fecundity, and egg hatching (Wang et al. 2016, 2017b). In our study, the treatment of MEAM1 and MED adults with the LC₂₅ of clothianidin significantly reduced the survival rate of most developmental stages. In other studies with *B. tabaci*, sublethal concentrations of imidacloprid significantly decreased the duration of egg and nymph stages (He et al. 2011), and sublethal concentrations of dinotefuran significantly reduced fecundity (Qu et al. 2017).

On the contrary, sublethal concentrations of insecticides could also accelerate the development and increase the fecundity in arthropods (Yu et al. 2010, Han et al. 2011). In the current study, the LC₂₅ of clothianidin reduced rather than increased the time required for development. The effects of accelerating development have been documented many times. Qu et al. (2015) reported that juveniles of the soybean aphid *Aphis glycines* developed faster when exposed to sublethal concentrations of imidacloprid. Cordeiro et al. (2013) reported that low concentrations of sulfoxaflor increased the fecundity of *Oligonychus ilicis*. Similarly, sublethal concentrations of insecticides increased reproduction of *Serangium japonicum*, *Laodelphax striatellus*, and *Myzus persicae* (Sulzer) (Yao et al. 2015, Xu et al. 2016, Zeng et al. 2016).

In addition to affecting insect development and reproduction, sublethal concentrations of insecticides could also affect insect behavior (Desneux et al. 2007). The use of electrical penetration graphing has demonstrated that sublethal concentrations of insecticides affect the feeding behavior of hemipterans, such as *B. tabaci*, *Myzus persicae*, and *Sitobion avenae* (Cui et al. 2012, Civolani et al. 2014, Zeng et al. 2016). In another example, a sublethal concentration of the novel neonicotinoid insecticide cycloxaprid reduced phloem ingestion by *Aphis gossypii* (Yuan et al. 2016). In the case of *B. tabaci*, He et al. (2011, 2013) found that sublethal concentrations of imidacloprid and bifenthrin reduced phloem feeding. The effects of sublethal concentrations of clothianidin on feeding by MEAM1 and MED remain to be studied.

In conclusion, the results of this research indicate that the LC₅₀ values against MEAM1 and MED adults were lower for clothianidin than for five other commercial insecticides. Although sublethal concentrations of clothianidin reduced developmental time, they also reduced survival and fecundity of both MEAM1 and MED. The effects of sublethal concentrations of clothianidin on feeding and other behaviors of MEAM1 and MED should be determined. The results indicate that clothianidin could be useful for the control of MEAM1 and MED.

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Supplementary Data

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

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