Effects of Buprofezin and Imidacloprid on the Functional Response of *Eretmocerus mundus* Mercet

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Abstract

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Eretmocerus mundus Mercet is one of the key natural enemies of *Bemisia tabaci* (Gennadius). In this study, the sublethal effects of LC_{25} of imidacloprid and field-recommended concentration of buprofezin on the functional response of *E. mundus* to different densities of second instar *B. tabaci* nymphs were evaluated. The results revealed a type III functional response in the control and imidacloprid treatment. The type III functional response was altered into a type II by buprofezin. Although imidacloprid did not alter the type of functional response of *E. mundus* compared to the control, it negatively affected the handling time and maximum attack rate of the parasitoid. Therefore, the use of this insecticide should be evaluated carefully in IPM programs.

Keywords: *Bemisia tabaci*; insecticide; parasitoid; sublethal effects

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is an important pest of cotton, vegetables, and ornamentals worldwide (Sethi *et al.* 2008). Plant damage is caused by the transmission of plant viruses, direct feeding, plant physiological disorders, honeydew production, and associated fungal growth (Al-Mazra'Awi & Ateyyat 2009). In Khouzestan province (SW of Iran), this pest causes damage to several plant species, particularly cucumber and other cucurbitaceous plants (Zandi Sohani *et al.* 2007). At present, the control of *B. tabaci* in Iran relies mainly on insecticide applications. Because of the extensive use of insecticides, this pest has developed resistance to almost all major chemicals available on the market (Heidari 2004).

An alternative in the management of *B. tabaci* consists in releases of biological control agents such as the parasitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae), which is considered as one of the key natural enemies of *B. tabaci* worldwide (Gonzalez-Zamora *et al.* 2004; Urbaneja & Stansly 2004).

Evaluation of the impact of insecticides on natural enemies is important when considering their utility in integrated pest management (IPM) programs. Insecticides may cause the death of the natural enemies (lethal effects) or change several other features of their physiology and behaviour without killing the individuals (sublethal effects) (Desneux *et al.* 2007; Řezáč *et al.* 2010).

The parasitoid functional response is regarded as a main factor in host-parasitoid dynamics and an important determinant of stability of the system (Oaten & Murdoch 1975; Fernandez-Arhex & Corley 2003). Holling (1959, 1966) proposed three types of functional responses. In type I, the response curve increases linearly to a plateau. In type II, the response curve rises in a negatively accelerating manner to a plateau. In type III, the response curve rises in a sigmoid manner to a plateau. Studies on the effects of pesticides on the functional response of natural enemies are scarce (Perera 1982; Claver *et al.* 2003; Poletti *et al.* 2007; Rafiee Dastjerdi *et al.* 2009; Abedi *et al.* 2012). The chloronicotinyl insecticide, imidacloprid, and the insect growth regulator (IGR), buprofezin, are two relatively new insecticides for controlling whiteflies in Iran (Talebi-

Jahromi 2007). The present study was aimed at evaluating the sublethal effects of these insecticides on the functional response of *E. mundus* to *B. tabaci*. Such information would support the selection of safe insecticides to protect beneficial arthropods and thereby improve the IPM.

MATERIAL AND METHODS

Insects. Adults of *B. tabaci* used in all experiments were captured from cucumber fields, in Ahvaz, Iran in October 2009. The colony was kept in a muslin-walled cage (120 × 60 × 60 cm) on cucumber plants (cultivar Superdominus). Rearing conditions were 16–25°C, 40–50% relative humidity (RH), and a photoperiod of 14 : 10 h (light : dark). Adults of *E. mundus* were collected from cucumber plants in a greenhouse in Ahvaz in October 2010. Parasitoids were reared thereafter in the laboratory under conditions identical to those for whiteflies, on cucumber plants infested with *B. tabaci* nymphs. Each week new cucumber plants harbouring nymphal stages of sweet potato whitefly were added to the cages to maintain the *E. mundus* population.

Insecticides. The insecticides used in this study were buprofezin 400 g/l SC (Applaud® 40 SC; Syngenta, Basel, Switzerland) and imidacloprid 350 g/l SC (Confidor® 35 SC; Gyah, Teheran, Iran). Tween 20 at a concentration of 500 ppm was used as a non-ionic surfactant in this experiment.

Functional response bioassay. Young adult parasitoids (< 24 h old) were exposed to the field-recommended concentration of buprofezin and the LC$_{25}$ of imidacloprid (2 and 0.001 ml/l water, respectively). In this study, the field-recommended concentration of buprofezin was used to evaluate the sublethal effects of this insecticide on the parasitoid functional response, as the results of the toxicity bioassays indicated that this concentration caused little mortality to *E. mundus* adults. Cucumber leaves were dipped for 10 s into insecticide solutions diluted to the required concentration or into distilled water as a control. The treated leaves were allowed to dry for 1 hour. The experimental cages consisted of round plastic containers (52 mm high, 40 mm in diameter) with four lateral screened holes (10 mm in diameter) in order to facilitate ventilation. Leaf discs (40 mm in diameter) from treated leaves were placed with their adaxial side on a thin layer of 1% agar (2–3 mm). 40–50 pairs of young adult parasitoids (< 24 h old) were exposed to treated leaves for 48 hours. A thin strip of honey was smeared on the underside of each

container lid as a food source. The cages were kept in an incubator (25 ± 1°C, 70 ± 5% RH, and a photoperiod was set at 14 : 10 h (light : dark). After 48 h, six randomly selected alive females were transferred individually to clip cages (40 mm in diameter) on fresh, undipped leaves with different densities (2, 4, 8, 16, 32, and 64) of second instar *B. tabaci* nymphs. To obtain second instar whitefly nymphs used as host in the present study, groups of approximately 20–30 adult whiteflies were confined to the lower surface of cucumber leaves using clip cages (20 mm in diameter). The whiteflies were allowed to oviposit for 24 h and subsequently removed. The plants with whitefly eggs were incubated under conditions of 25 ± 1°C, 70 ± 5% RH, and a photoperiod of 14 : 10 h (light:dark) for ca. 11 days to allow the second nymphal instar to develop. Wasps were removed after 24 h and plants harbouring parasitised nymphs were incubated under conditions as described above. Number of parasitised whitefly nymphs was recorded 10 days after removal of wasps with the aid of a stereoscopic microscope. Ten replicates per treatment were used in each host density.

Data analysis. A two-step approach recommended by Juliano (2001) was used to determine the type of functional response and estimate parameters. In the first step, the curve shape or type of functional response (type II or III) was determined by a logistic regression analysis of the proportion of host parasitised (Na/N_0) as a function of host density (N_0) (PROC CATMOD, SAS Institute 2003). The logistic model is as follows:

$$\frac{Na}{N_0} = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)} \quad (1)$$

where: *Na* – number of host parasitised; N_0 – host density; P_0, P_1, P_2, P_3 – intercept, linear, quadratic, and cubic parameters, respectively, estimated using the method of maximum likelihood

If the linear parameter (P_1) is negative, a type II functional response is evident, whereas a positive linear parameter indicates a type III functional response (Juliano 2001).

In the second step, the functional response parameters were estimated by fitting a non-linear least squares regression to the number of hosts parasitised versus the number of hosts available (PROC NLIN, SAS Institute 2003). As the host was not replaced during the experiment, Rogers (1972) random parasitoid equation (Eq. 2) was used to obtain functional response parameters. The handling time and attack

Table 1. Maximum likelihood estimates from logistic regression of the proportion of *B. tabaci* nymphs parasitized by *E. mundus* as a function of initial host density

Treatment	Parameter	Estimate	Standard error (SE)	Chi-squared value	*P*-value
Control	P_0 (constant)	0.9827	0.4117	5.70	0.0170
	P_1 (linear)	0.0116	0.0612	0.04	0.8491
	P_2 (quadratic)	−0.00315	0.00229	1.89	0.1691
	P_3 (cubic)	−0.000044	0.000022	3.76	0.0524
Buprofezin	P_0 (constant)	1.7013	0.4408	14.89	0.0001
	P_1 (linear)	−0.1435	0.0648	4.90	0.0268
	P_2 (quadratic)	0.00593	0.00242	6.00	0.0143
	P_3 (cubic)	−0.00006	0.000024	6.99	0.0082
Imidacloprid	P_0 (constant)	−0.2108	0.3729	0.32	0.5718
	P_1 (linear)	0.0989	0.0569	3.02	0.0822
	P_2 (quadratic)	−0.00533	0.00216	6.09	0.0136
	P_3 (cubic)	0.000054	0.000021	6.47	0.0110

rate for a type III functional response were estimated with replacing Equation (3) (Juliano & Williams 1987) in Equation (2):

$$N_{par} = N[1 - \exp(-(a\,T_t/1 + aT_h\,N))] \qquad (2)$$

$$a = (d + bN)/(1 + cN) \qquad (3)$$

where: N_{par} – number of parasitised host; N – initial host density; a – attack rate; T_t – total time available for searching during the experiment (in this case $T = 24$ h); T_h – handling time; b, c, d – constant

The coefficient of determination was calculated as $r^2 = 1 - $ (residual sum of squares/corrected total sum of squares) (Allahyari *et al.* 2004; Farrokhi *et al.* 2010).

An additional 3 × 6 Two-way analysis of variance (ANOVA) was applied to study the possible effects of insecticide, host density, and their interaction on the number of parasitized *B. tabaci* nymphs by *E. mundus*.

RESULTS

The logistic regression analysis showed that parasitoids in both control and those exposed to imidacloprid exhibited a type III functional response by a positive P_1 parameter and a negative P_2 parameter of Eq. (1) (Figure 1 and Table 1). However, type II functional response was expressed in parasitoids exposed to buprofezin as the P_1 parameter of Eq. (1) was negative and P_2 was positive (Figure 1 and Table 1).

The attack rates and handling times are shown in Table 2. The data for the functional response of buprofezin-exposed parasitoids were described by the random parasitoid equation (model 2) (Rogers 1972). For parasitoids in the control and imidacloprid treatment, Eq. (3) was substituted in Eq. (2) and the

Table 2. Parameters (± SE) estimated by random parasitoid equation indicating functional response of *E. mundus* to different treatments

Treatment	a (h⁻¹)	b ($a = bN$)	T_h (h)	T/T_h	r^2
Control	–	0.00128 ± 0.000248 (0.000788 – 0.00178)	0.2765 ± 0.0507 (0.1750 – 0.3780)	86.80	0.92
Buprofezin	0.0762 ± 0.0284 (0.0193 – 0.1332)	–	0.2195 ± 0.092 (0.0341 – 0.4049)	103.34	0.90
Imidacloprid	–	0.0109 ± 0.00387 (0.00318 – 0.0187)	1.7591 ± 0.0899 (1.5791 – 1.9391)	13.64	0.92

The values in parentheses are 95% confidence intervals; a – attack rate; b – constant; T_h – handling time; T/T_h, maximum attack rate
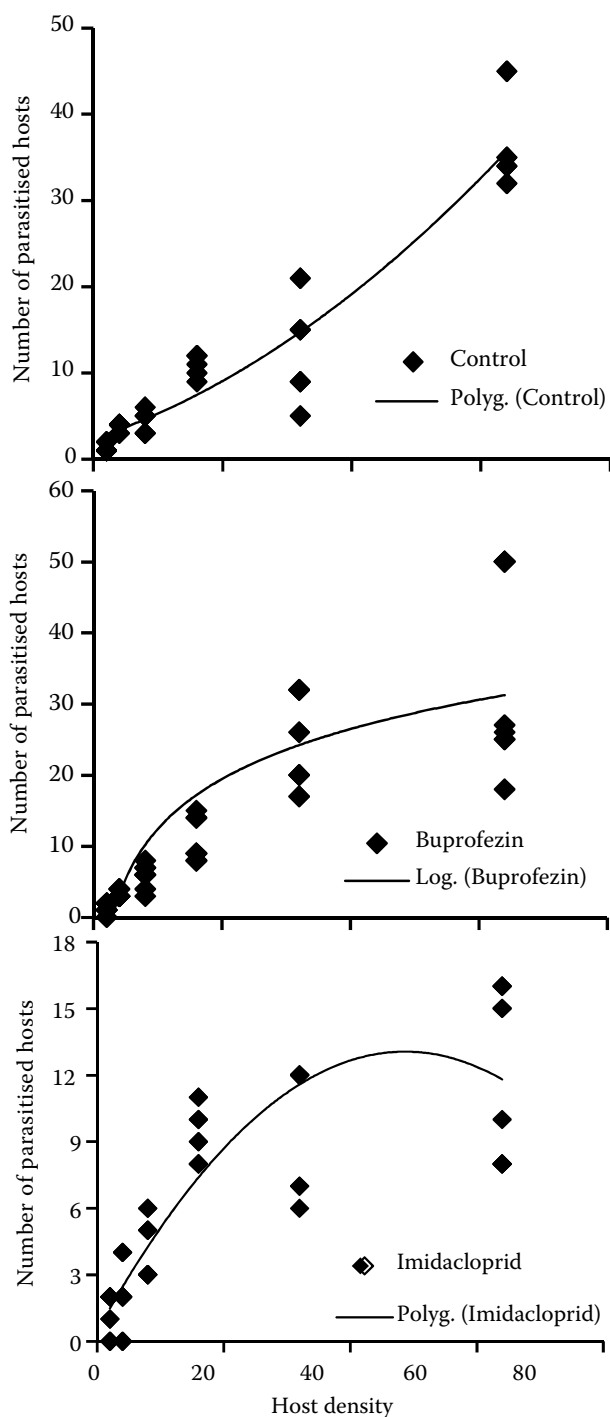
Figure 1. Functional response of *E. mundus* to different densities of *B. tabaci* nymphs, at different treatments (symbols: observed; line: predicted by model). Overlapping values are shown as a single dot

data set was fitted to it. Results of non-linear least squares regression indicated that parameters *c* and *d* were not significantly different from zero; therefore, we eliminated them from the model and a reduced model ($a = bN$) was used. Estimated *b* value for control and imidacloprid-exposed parasitoids were

0.00128 ± 0.000248 and 0.0109 ± 0.00387, respectively (Table 2).

The results of Two-way analysis of variance (ANOVA) indicated that *E. mundus* exhibited different searching behaviour in three treatments (df = 2, 162; *F* = 24.80; *P* < 0.0001) at different host densities (df = 5, 162; *F* = 129.07; *P* < 0.0001) with a significant interaction of treatment and initial host density on the number of parasitized hosts (df = 10, 162; *F* = 13.67; *P* < 0.0001).

DISCUSSION

Studies on the effects of pesticides on functional response can contribute to the success of IPM programs and to the release of the natural enemies (Abedi *et al.* 2012). The results of this study indicated that there was a type III functional response of *E. mundus* in response to host density in imidacloprid treatment as well as the control. The type III of functional response was altered into the type II by buprofezin. We are unaware of any other studies reporting the effects of insecticides on the functional response of *E. mundus*. Heidari (2004) studied the effect of buprofezin on the functional response of *Encarsia formosa* (Gahan) to *Trialeurodes vaporariorum* Westwood and reported a functional response of type II which is consistent with our results. A recent study on the sublethal effects of imidacloprid and buprofezin on functional response of the sweet potato whitefly parasitoid, *Encarsia inaron,* showed a type II response for insecticide treatments and the control (Sohrabi 2012).

The results of the effects of the insecticides on functional response parameters showed that although imidacloprid did not alter the type of functional response, it negatively affected the handling time and maximum attack rate of *E. mundus* compared to the control. This effect might be due to the repellent effect of imidacloprid on the parasitoid as reported in the previous studies (Richter *et al.* 2003; Richter 2006). Similarly, Poletti *et al.* (2007) reported that imidacloprid did not change the type of functional response of predatory phytoseiid mites *Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks), but it caused a conspicuous increase in handling time, and a decrease in the attack rate of the predators. However, as our results with buprofezin showed, sublethal effects of pesticides on host detection and oviposition may not always be unfavourable to parasitoids (Desneux *et al.* 2007). Chlorpyrifos at LD$_{20}$ caused an increase (5.1-fold) in host searching by *Leptopilina heterotoma*, a parasitoid of *Drosophila*

larvae that probes substrate with its ovipositor (Rafalimanana *et al.* 2002). Furthermore, the authors reported that treated females found host larvae and oviposited into them by 46% faster than did control females. In addition, hormoligosis or hormesis defined as elevated performance of individuals following exposure to chemicals or other stresses has been reported in a wide range of organisms including insects (Luckey 1968; Stebbing 1982). In agreement with our findings, Heidari (2004) reported no adverse effects of buprofezin on functional response parameters of *E. formosa*.

From the results reported in the present study, it can be verified that the insecticides can affect the functional response of parasitoid and thereby the success of biological control programs. In general, between insecticides tested, imidacloprid had higher adverse effect on functional response parameters of *E. mundus*. However, further experiments under semi-field and field conditions are needed to support the present laboratory study.

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