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Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*

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Abstract Neonicotinoid insecticides are widely used for controlling sucking pests, and sublethal effects can be expected in beneficial arthropods like natural enemies. *Serangium japonicum* is an important predator in many agricultural systems in China, and a potential biological control agent against *Bemisia tabaci*. We evaluated the toxicity of imidacloprid to *S. japonicum* and its impact on the functional response to *B. tabaci* eggs. *S. japonicum* adults exposed through contact to dried residues of imidacloprid at the recommended field rate on cotton against *B. tabaci* (4 g active ingredient per 100 l, i.e. 40 ppm [part per million]), and reduced rates (25, 20, 15 and 10 ppm) for 24 h showed high mortality rates. The mortality induced by a lowest rate, 5 ppm, was not significantly different than the control group and thus it was considered as a sublethal rate. The lethal rate 50 and hazard quotient (HQ) were estimated to be 11.54 ppm and 3.47 respectively, indicating a risk for *S. japonicum* in treated fields (HQ > 2). When exposed to dried residues of imidacloprid at the sublethal rate (5 ppm) on cotton leaves, functional response of *S. japonicum* to *B. tabaci* eggs was affected with an increase in handling time and a reduction in peak consumption of eggs. Imidacloprid residues also disturbed predator voracity, the number of *B. tabaci* eggs consumed on treated leaves being significantly lower than on untreated leaves. All effects disappeared within a few hours after transfer to untreated cotton leaves. Imidacloprid systemically applied at the recommended field rate (for cotton) showed no toxicity to *S. japonicum*, nor affected the functional response of the predator. Sublethal effects of imidacloprid on *S. japonicum* observed in our study likely negatively affect *S. japonicum* development and reproductive capacity and may ultimately reduce predator population growth. These results hint at the importance of assessing potential effects of imidacloprid on *S. japonicum* for developing effective integrated pest management programs of *B. tabaci* in China.

Keywords Toxicity · Hazard quotient · Neonicotinoid · Risk assessment · Integrated pest management

Introduction

Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) is one of the most important agricultural pests worldwide (Oliveira et al. 2001; Baldin and Beneduzzi 2010), including China where, since the 1990s, it largely affects many major crops resulting in huge yield losses every year (Luo and Zhang 2000; Ren et al. 2001). Multiple studies have reported that natural enemies could act along with other mortality agents to inflict high levels of mortality in populations of *B. tabaci* in various crops (Naranjo and Ellsworth 2005; Stansly and Naranjo 2010). Biological control of whiteflies has proved to be successful, notably using generalist predators, parasitoids or fungal pathogens (Faria and Wraight 2001; Gerling et al. 2001; Ren et al.

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2004). Seventeen predator species has been already identified as preying largely on *B. tabaci* in Chinese agricultural systems, and the native coccinellid *Serangium japonicum* Chapin (Coleoptera: Coccinellidae) has shown promising potential for effective biological control of *B. tabaci* in various crops, notably on cotton (Ren et al. 2001, 2004). Larvae and adults of *S. japonicum* prey on eggs and all juvenile instars of *B. tabaci*, and its high daily consumption (730 prey per adult per day) could slow down or suppress *B. tabaci* population growth (Yao et al. 2004).

However, management of *B. tabaci* in China relies primarily on broad spectrum chemical insecticides in most cropping systems, which are noxious to beneficial arthropods (Desneux et al. 2007). Extensive use of insecticides has lead to the development of resistant strains of *B. tabaci* to most conventional insecticidal compounds (Palumbo et al. 2001; Ahmad et al. 2010), including relatively new classes of insecticides (Cahill et al. 1996; Wang et al. 2010; Zhang et al. 2011). Therefore, it is necessary to develop integrated pest management (IPM) programs that do not rely only on insecticides and that could provide sustainable control of *B. tabaci* in China. As IPM aims at reducing pest status to tolerable levels by using methods that are effective, economically sound and that mitigate the harmful effects of pesticides to natural enemies (Croft 1990), understanding risks posed by pesticides to natural enemies is of absolute importance (Desneux et al. 2006a, 2007). Natural enemies can be exposed to pesticides through direct exposure to spray droplets or residues on the crop foliage when foraging for hosts or prey (Jepson 1989), or through dietary exposure when feeding on pesticide-contaminated food (e.g. prey) (Ahmad et al. 2003; Huo et al. 2004; Torres and Ruberson 2004).

Acute toxicity (lethal effect) and selectivity of pesticides has been examined in predators (Zhu et al. 1998; Qiu et al. 2000), including in predatory coccinellid beetles (Hao et al. 1990; Zhu et al. 1998; Wu et al. 2007; Urbaneja et al. 2008; Xi 2008; Cabral et al. 2011). However, in addition to their lethal effect, pesticides also can induce various sublethal effects. Such effects (either physiological or behavioral) are defined as effects on individuals that survive pesticide exposure and they can occur after an exposure to dose/concentration/rate that can be either sublethal or lethal (see Desneux et al. 2007 for a thorough review). Occurrence of such effects is likely given the multiple ways natural enemies could be exposed to pesticides. Sublethal effects can impair various key processes of natural enemies' efficacy against pests (Desneux et al. 2006c, 2007; Thornham et al. 2008; Suma et al. 2009; Evans Samuel et al. 2010; Arnó and Gabarra 2011; Saber 2011; Stara et al. 2011; Castro et al. 2012). Given the multiple possible behavioral effects, functional response of predators to prey could be impaired by pesticides (Wang and Shen 2002; Poletti et al. 2007). However, sublethal effects of insecticides on coccinellids, notably neonicotinoid insecticides (e.g. imidacloprid which is used on a large scale in China), are scarcely documented (Smith and Krischik 1999; Wang and Shen 2002; Papachristos and Milonas 2008; Eisenback et al. 2010) and no information is currently available on sublethal effects of insecticides on *S. japonicum*.

In this context, the aims of the present work were to provide a lethal and sublethal toxicity assessment of the neonicotinoid imidacloprid on *S. japonicum*, and to assess risks posed by imidacloprid use in crops. We studied the lethal toxicity of imidacloprid to *S. japonicum* through various exposure routes that aimed at representing various exposure scenarios (and because exposure methods could have an impact on results, Stark et al. 1995; Desneux et al. 2006a). We also studied the effects of imidacloprid on functional response of *S. japonicum* to *B. tabaci* eggs and on predator voracity as sublethal effects on these key predator traits (O'Neil 1997; Cabral et al. 2011; Madadi et al. 2011) could greatly reduce predator impact on *B. tabaci*. This study constitutes the basis for future guidelines for imidacloprid rational use, and it can help optimizing IPM programs involving use of both natural enemies and imidacloprid against *B. tabaci* in China.

Materials and methods

Insects

Adults of *B. tabaci* (biotype B) and adults of *S. japonicum* were collected from vegetable fields in Fuzhou city (Fujian Province, China) in 2008 and 2009, respectively. These insects (100 *S. japonicum* adults and 1,000 *B. tabaci* adults) were used to establish colonies in the laboratory. *B. tabaci* was reared on cotton plants (*Gossypium hirsutum*) and *S. japonicum* was reared on cotton plants infested with *B. tabaci* (all plants were free of any insecticides). All colonies were reared in growth chambers at 25 ± 1 °C, 65 ± 5 % RH, 14:10 L.D. and the coccinellids used in all experiments were 24–48 h old adult females (mated prior experiments).

Insecticide solutions

Imidacloprid-based commercial product, BIDAN 10WP (Shenyang Chemical Ltd., Shenyang, China), was diluted with deionized water. In toxicity bioassays, final concentrations tested were as follows: the recommended field rate on cotton against *B. tabaci* (4 g active ingredient per 100 l, i.e. 40 ppm [part per million]) and reduced rates (25, 20, 15, 10 and 5 ppm) to mimic lower rates that usually occur in fields during the days/weeks/months following initial insecticide application (owing to degradation of pesticides) (Desneux et al. 2005; Zhang et al. 2011).

Toxicity of imidacloprid residues on glass

Coccinellid adults were exposed to dry residues of imidacloprid on glass in tubes, using a worst-case exposure scenario (accordingly to Candolfi et al. 2000), using methods developed in previous studies (Zhu et al. 1998; Desneux et al. 2004a). Imidacloprid solutions were applied to the inner surface of glass tubes (length: 18 cm; diameter: 1.8 cm). To obtain a homogeneous deposit and total coverage of the internal surface of the tube, they were first fully filled (i.e. 45.8 ml) with the various solutions (or water as control), then the solutions were poured off and tubes were rotated until no more droplets were seen on the glass. The tubes were left on the bench to allow complete evaporation of solutions before introducing *S. japonicum* (ten adult females per tube). Tubes were then sealed with gauze and placed in growth chambers (25 ± 1 °C, 65 ± 5 % RH, 14:10 L.D.). Mortality was recorded after 24 h of exposure. Each treatment was replicated six times, with at least 60 coccinellids exposed per concentration tested. Individuals that did not react when pushed with a brush were considered dead.

Toxicity of imidacloprid through systemic treatment

We assessed acute toxicity of imidacloprid to *S. japonicum* adults when feeding on *B. tabaci* eggs deposed on cotton leaf treated with imidacloprid through systemic application (as it would occur in case of imidacloprid-dressed cotton seeds or direct soil applications). The petioles of cut cotton leaves infested with *B. tabaci* eggs were immersed in an imidacloprid solution at field rate (40 ppm) or in water as control. After 24 h at 25 ± 1 °C in a ventilated hood, leaves were placed with their adaxial surface downwards onto agar in a Petri dish (diameter: 11 cm). Ten *S. japonicum* adults were transferred onto the leaf-discs. Each Petri dish was then covered with a perforated lid and placed in growth chambers (25 ± 1 °C, 65 ± 5 % RH, 14:10 L.D.). Each treatment was replicated six times (total of 120 coccinellids tested). Mortality was recorded after 24 h of exposure using the mortality criterion described above.

Impact of imidacloprid on functional response of *S. japonicum* to *B. tabaci* eggs

The assessment of effects of imidacloprid on the functional response of *S. japonicum* to *B. tabaci* eggs was done by testing different exposure scenarios: (i) *B. tabaci* eggs were provided on cotton leaves treated systematically with imidacloprid at field rate (40 ppm) (described in "Toxicity of imidacloprid through systemic treatment" section). In this case *S. japonicum* is not exposed to the insecticide directly. (ii) *B. tabaci* eggs were treated with imidacloprid at 5 ppm (reduced rate and actually a sublethal rate for *S. japonicum* adults, see "Results" section) by egg-dip method, based on the method developed by Horowitz et al. (2002). Briefly, cotton leaves infested with 1 day-old eggs, were dipped for 5 s in the 5 ppm imidacloprid solution and then leaves were allowed to dry. In this situation *S. japonicum* was exposed to both imidacloprid residues on cotton leaves and *B. tabaci* eggs that had been treated with imidacloprid. Because imidacloprid is mainly used as foliar spray application in China, assessing side effects induced by exposure to cotton leaves and prey treated through the egg-dip method is highly relevant (realistic). (iii) *S. japonicum* adults were first exposed to imidacloprid residues (5 ppm) on glass for 24 h (method described in "Toxicity of imidacloprid residues on glass" section) and *B. tabaci* eggs were sprayed with water. This situation mimics what may happen when a predator forages in a treated field in which it experiences a discontinuous exposure to the insecticide (alternation between pesticide-treated and refuge areas, Desneux et al. 2005). (iv) *S. japonicum* and *B. tabaci* eggs were not treated with imidacloprid (control group). These scenarios are referred hereafter as "systemic", "egg-dip", "discontinuous" and "control" respectively.

We followed the method developed by Wang and Shen (2002) to estimate the functional response of the predator to *B. tabaci* eggs under these different exposure scenarios. We placed the cotton leaves infested with different *B. tabaci* egg densities (100, 200, 300, 400, 500, 600, 800, 1000 and 1200 eggs per leaf) on agar within Petri dishes (diameter: 11 cm). Within each Petri dish, we introduced one *S. japonicum* adult female that was previously starved for 24 h. Each Petri dish was then covered with a perforated lid and placed in growth chambers (25 ± 1 °C, 65 ± 5 % RH, 14:10 L.D.). The number of *B. tabaci* eggs consumed was counted 24 h after predators had been introduced into Petri dishes. Each prey density treatment was replicated ten times.

Impact of imidacloprid on predator voracity

Cotton leaves infested with 1000 *B. tabaci* eggs were exposed to the 5 ppm imidacloprid solution (i.e. sublethal rate, see "Results" section) using the egg-dip method (see above) or in water as control and leaves were placed onto agar within a 11 cm diameter Petri dish after they dried. The density of *B. tabaci* (1000 eggs) was chosen because it was higher than maximal daily consumption of eggs by *S. japonicum* (see results on functional response). One

S. japonicum adult (previously starved for 24 h and not previously exposed to imidacloprid) was introduced per Petri dish and dishes were placed in growth chambers (25 ± 1 °C, 65 ± 5 % RH, 14:10 L.D.). After 24 h, we counted the number of *B. tabaci* eggs consumed and the surviving coccinellids were transferred onto untreated cotton leaves bearing 1000 untreated *B. tabaci* eggs. The number of eggs consumed was then counted after 8, 16 and 24 h to assess recovery of potential effects of imidacloprid on *S. japonicum* voracity. Each treatment was replicated ten times.

Statistical analysis

Datasets were first tested for normality and homogeneity of variance using Kolmogorov–Smirnov D test and Cochran's test respectively, and transformed if necessary. We compared mean mortality among treatments using ANOVA followed by a Tukey's post hoc test for multiple comparisons, and the lethal rate 50 (LR_{50}) was determined using a log-probit model (Finney 1971). In addition, we estimated the hazard quotient (HQ) value which is calculated by dividing crop-specific application rates by the LR_{50} derived from worst-case-scenario laboratory study (exposure to residues on glass) (Candolfi et al. 2001) and which gives an indication of the risk when testing commercial pesticide products.

The functional response model proposed by Holling (1959) was used to describe the variation in the number of prey consumed by the predator, estimating $N\alpha$ by:

$$N\alpha = \frac{\alpha TN}{1 + \alpha ThN}$$

where α is the attack coefficient, T is the duration of the experiment ($T = 1$ day in the present study), Th is the handling time by the predator during the processes of prey identification, capture, attack and consumption, and N is prey (egg) density. The parameters of the equations were estimated by nonlinear regression. Nonlinear regression and ANOVA were conducted using SAS Version 8.0 (SAS Institute Inc., Cary, USA). The peak consumption in each treatment was estimated using Th reciprocal.

Results

Toxicity on glass and through systemic exposure, LR_{50} and HQ

Dried residues of imidacloprid induced significantly mortality to *S. japonicum* when exposed by contact on glass ($F_{6,41} = 147.59$, $P < 0.001$; Fig. 1). Linear regression of the dose–mortality relationship ($Y = -3.85 + 3.62\ X$) was fitted to the observed data (i.e. no significant deviation between the observed and the expected data; $\chi^2 = 2.93$, df = 4, $P = 0.569$) and the LR_{50} was considered as valid (*inf lim* < LR_{50} < *sup lim*: *10.08* < 11.54 ppm < *12.91*). Mortality in the control group was lower than 2 %. The recommended field rate of imidacloprid on cotton in China (40 ppm) was higher than the LR_{50} and HQ was equal to 3.47. At the recommended field rate (40 ppm) all individuals died within the 24 h exposure period. Mortality rates of more than 40 % in exposed individuals were recorded for all reduced rates except at 5 ppm. Mortality induced by 5 ppm imidacloprid was not significantly different than control mortality and therefore this rate could be designated as a sublethal rate of imidacloprid for *S. japonicum* (Desneux et al. 2007) and we refer to it as such hereafter.

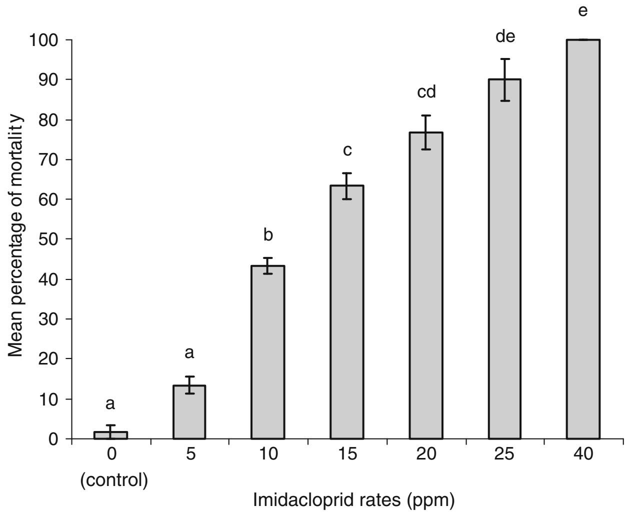

Fig. 1 Mean percentage (±SEM) of mortality after exposure to dried residues of imidacloprid on glass. *Histograms* bearing *different letters* are significantly different ($P < 0.05$, ANOVA followed by Tukey's post hoc test)

Imidacloprid applied systemically to cotton leaves at the field rate (40 ppm) did not induce significant mortality when *S. japonicum* adults fed on *B. tabaci* eggs on these leaves ($P > 0.05$).

Functional response

In all imidacloprid treatments, *S. japonicum* adults showed similar patterns of functional response when compared with the control, as the number of prey consumed per predator increased quickly as the prey density offered increased, leveling off with additional prey (above 800 eggs or 450 eggs depending of treatments) (Fig. 2). We fitted the type II functional response equation to the dataset and estimated the parameters α and Th for the various treatments (Table 1). *S. japonicum* showed significantly

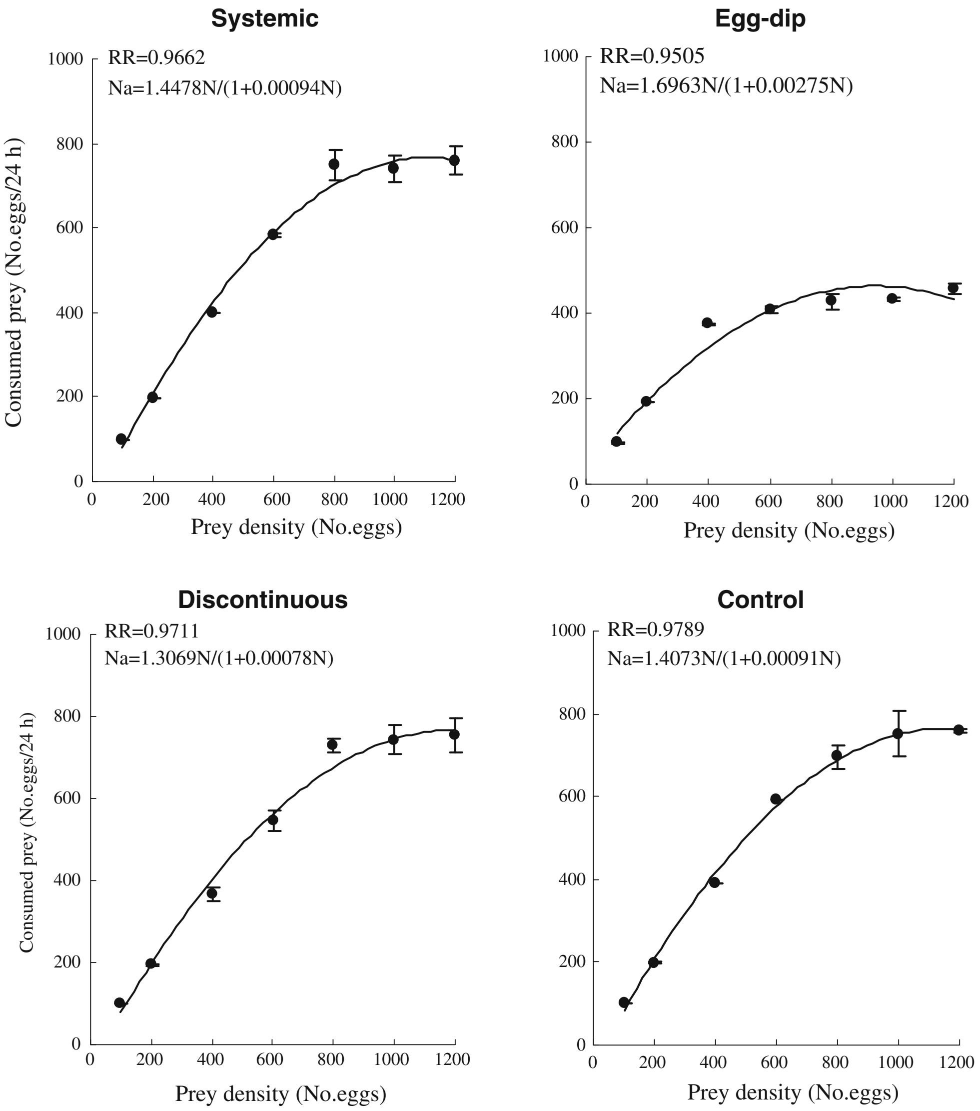

Fig. 2 Effect of imidacloprid on functional response of *S. japonicum* adults on *B. tabaci* eggs. Exposure scenarios: *systemic B. tabaci* eggs treated with imidacloprid by systemic application (40 ppm), *egg-dip B. tabaci* eggs treated with imidacloprid by egg-dip method (5 ppm), *discontinuous S. japonicum* adults were pretreated with imidacloprid by residual application for 24 h (5 ppm), and *control S. japonicum* and *B. tabaci* eggs were not treated with imidacloprid. *Dots* represent means and *vertical lines* their respective standard errors, *continuous lines* represent nonlinear models fitted for each treatment

longer prey *Th* when it was exposed to both imidacloprid residues on leaves and imidacloprid-contaminated prey (egg-dip method). In contrast a similar *Th* was observed among systemic application treatment, when *S. japonicum* was exposed to dried residues of imidacloprid on glass at the sublethal rate (5 ppm) before the trial and the control group. *S. japonicum* exhibited similar attack coefficient (α) for all treatments (Table 1).

The peak consumption estimated for *S. japonicum* was reduced by approximately 60 % in the “egg-dip” treatment when compared to control group. In contrast, it was not affected in case of “systemic” and “discontinuous” treatments (Fig. 3).

Predator voracity

When applied through the egg-dip method for 24 h, the sublethal rate (5 ppm) of imidacloprid significantly affected *S. japonicum* voracity on *B. tabaci* eggs (Table 2). The number of *B. tabaci* eggs consumed on treated leaves was significantly lower than that on untreated ones ($F_{1,18} = 171.48$, $P < 0.001$). However, after transfer to untreated leaves, *S. japonicum* voracity (consumption of eggs) rapidly returned to control levels (Table 2, all $P > 0.05$).

Discussion

Insecticides are mainly used as foliar spray applications in Chinese fields. Therefore natural enemies likely experience exposure to insecticide residues when foraging on treated plants/leaves after treatments and it may cause various direct and indirect effects. We demonstrated that residues of imidacloprid on leaves, when applied at the sublethal rate of 5 ppm (according to the definition of sublethal dose

Table 1 Estimates of functional response parameters of *S. japonicum* on *B. tabaci* eggs for the various exposure scenarios tested

Treatment	α (95 % CL)[a]	*Th* (95 % CL)[b]
Control	0.910 < 1.407 < 1.905	0.0003 < 0.0006 < 0.0009
Systemic: systemic application of imidacloprid at field rate (40 ppm)	0.794 < 1.448 < 2.102	0.0003 < 0.0007 < 0.0010
Egg-dip: leaves and prey treated with a sublethal rate of imidacloprid (5 ppm) via egg-dip method	0.849 < 1.696 < 2.543	0.0012 < 0.0016 < 0.0021*
Discontinuous: predators were previously exposed to sublethal rate of imidacloprid (5 ppm) on glass for 24 h	0.763 < 1.307 < 1.851	0.0002 < 0.0006 < 0.0010

* Significant difference from control at 5 % level when 95 % confidence intervals of estimates did not overlap

[a] Attack coefficient

[b] Handling time

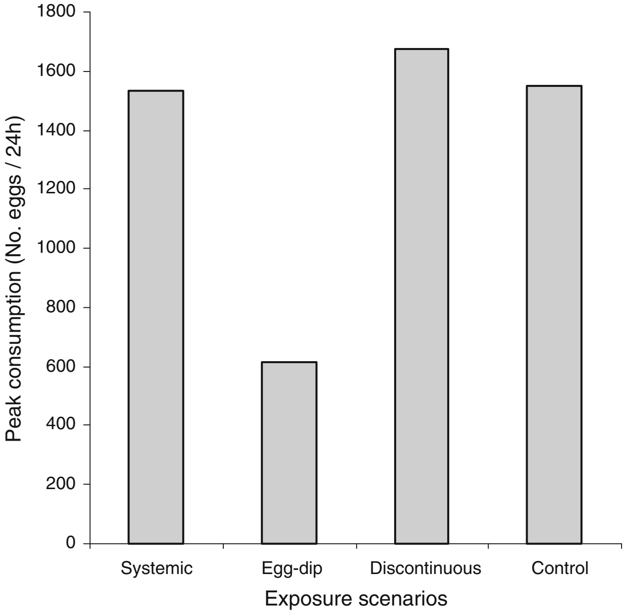

Fig. 3 Effect of imidacloprid on peak consumption (estimated using *Th* reciprocal) of *S. japonicum* female adults on *B. tabaci* eggs (no. eggs/24 h). Exposure scenarios: *systemic B. tabaci* eggs treated with imidacloprid by systemic application (40 ppm), *egg-dip B. tabaci* eggs treated with imidacloprid by egg-dip method (5 ppm), *discontinuous S. japonicum* female adults were pretreated with imidacloprid by residual application for 24 h (5 ppm), and *control S. japonicum* and *B. tabaci* eggs were not treated with imidacloprid

by Desneux et al. 2007) using the egg-dip method, affected the functional response of *S. japonicum* adults, markedly we observed an increase in *Th*, and a reduced peak in *B. tabaci* eggs potentially preyed (Table 1; Fig. 3). The sublethal rate of imidacloprid also impaired predator voracity with 41 % reduction in predation on *B. tabaci* eggs (Table 2). In all cases tested, predators recovered rapidly after the end of the exposure period and sublethal effects disappeared within a few hours following end of exposure. Consistently, exposure for 24 h via contact with dried residues of imidacloprid on glass (at sublethal rate), prior to assessment of *S. japonicum* functional response on imidacloprid-free cotton leaves, did not show any effects ("discontinuous" treatment, Table 1). Recovery after exposure to sublethal or low lethal rates of insecticides has previously been reported for other natural enemies exposed to pesticides (Desneux et al. 2004a, c, 2007), although it is not always found (Desneux et al. 2004b; Moser and Obrycki 2009).

Lethal effect

There was a good fit between the observed dose–mortality relationship and the linear regression, so our experimental conditions gave reliable estimates of LR_{50}. The ratio between the field application rate and the LR_{50}, i.e. HQ, gives an indication of the risk. For use of imidacloprid in cotton, the HQ value is higher than the trigger value of 2 (Candolfi et al. 2001) which indicates a risk for *S. japonicum*. This result is in concordance with previous laboratory studies showing that spray formulations of imidacloprid were highly toxic to various predatory mite species (*Galendromus occidentalis*, *Neoseiulus fallacis* and *Amblyseius andersoni*) (James 2003), and studies reporting high toxicity of imidacloprid to the parasitoid *Anagyrus pseudococci* (Krischik et al. 2007) and to the predatory coccinellid *Hippodamia undecimnotata* (Papachristos and Milonas 2008). However, previous studies on toxicity of imidacloprid also showed some selectivity of imidacloprid toward predators (i.e. more toxic to pests than to predators, Qiu et al. 2000; Tang et al. 2007). In addition, in field conditions, planting imidacloprid-dressed soybean seeds did not affect coccinellid and predatory bug populations (Seagraves and Lundgren 2012). However, because HQ was higher than 2 in our study, additional assessments of potential sublethal effects of imidacloprid on predator voracity and functional response to *B. tabaci* were required (Candolfi et al. 2001). For such assays, we used almost exclusively the sublethal rate of 5 ppm. In our study, systemic application imidacloprid at the field rate (40 ppm) was safe for *S. japonicum* adults and it may mean that

Table 2 Mean consumption (SEM) of *B. tabaci* eggs by *S. japonicum* on imidacloprid-treated and untreated leaves

	Consumption—24 h exposure period	Consumption—24 h recovery period			
		0–8 h	8–16 h	16–24 h	Total (24 h)
Control	753.00 ± 14.46a	282.20 ± 27.19a	143.40 ± 16.87a	184.00 ± 16.71a	609.60 ± 12.60a
Imidacloprid	445.67 ± 19.73b	268.20 ± 25.30a	151.60 ± 14.98a	207.20 ± 12.78a	627.00 ± 24.66a
Statistics	$F_{1,18} = 171.48$ $P < 0.001$	$F_{1,18} = 0.142$ $P = 0.711$	$F_{1,18} = 0.132$ $P = 0.721$	$F_{1,18} = 1.216$ $P = 0.285$	$F_{1,18} = 0.395$ $P = 0.538$

Consumption during the 24 h imidacloprid-exposure period (egg-dip method, 5 ppm) and during the 24 h recovering period is reported. Data followed by different letters within the same column are significantly different (ANOVA)

imidacloprid-dressed seeds are safer for coccinellid predators than foliar applications (consistent with Ohnesorg et al. 2010; Seagraves and Lundgren 2012).

Sublethal effects on *S. japonicum* predation activity

Imidacloprid specifically targets the nicotinic acetylcholine receptors of insects (Palumbo et al. 2001) and therefore behavioral effects in predators are likely because, for example, sophisticated nervous activity is required in the process of prey detection by predators (Desneux et al. 2007). Previous studies already reported side effects of imidacloprid on predatory traits. Sublethal doses of imidacloprid significantly increased *Th*, decreased searching efficiency and consumption rate, and reduced the peak consumption of the coccinellid *Harmonia axyridis* on its aphid prey (Wang and Shen 2002) and of the predatory mite *Neoseiulus californicus* on spider mite eggs (Poletti et al. 2007). Adverse effects of imidacloprid on motor activity in insects have been also reported (Lambin et al. 2001), notably in predatory beetles (Smith and Krischik 1999; Eisenback et al. 2010). In our study, imidacloprid residues were harmful to *S. japonicum* adults, and the adverse effects on predator voracity and its functional response to *B. tabaci* eggs may be linked to multiple nervous disruptions following contact exposure with the residues. Other insecticides have been shown to adversely affect functional response of natural enemies because of sublethal effects on searching efficiency and attacking coefficient (α in our equation) (Gu et al. 1991; Claver et al. 2003). In our experiments, reduction in food intake may have been linked to haphazard movement and increased restlessness in predators (Claver et al. 2003; Tran et al. 2004) and disturbances in coccinellids feeding behaviors that consume imidacloprid-contaminated prey may also indicate antifeedant effects (Desneux et al. 2007). Such effects have previously been reported for imidacloprid at sublethal doses on Hemiptera (Nauen et al. 1998) and honey bees (Ramirez-Romero et al. 2008; Han et al. 2010). Specific behavioral and/or physiological assays are required to identify more precisely which traits are disturbed but our results clearly demonstrated that sublethal effects occur in individuals foraging on cotton plants previously treated by foliar applications of imidacloprid.

Exposure routes and implications for risk assessment

Imidacloprid has known good root-systemic characteristics and is quite toxic through both contact and ingestion by insects. These properties allow imidacloprid to be applied in various ways such as foliar sprays, seed treatment and soil application (Palumbo et al. 2001). As pointed out previously, insecticides are mainly used as foliar spray applications in Chinese crops. Therefore natural enemies likely experience high exposure through direct contact with insecticide residues when foraging on treated plants. However, this exposure may be discontinuous because natural enemies likely alternate foraging in treated areas and untreated refuge areas as previously reported in other agro-ecosystems (Cilgi and Jepson 1992; Koch and Weisser 2001; Desneux et al. 2005).

In the specific case of predators, a major route of exposure to pesticides is through ingestion of contaminated prey. Studies already reported adverse effects on coccinellid predators when fed on pesticide-contaminated prey (i.e. prey were previously treated) (Wang and Shen 2002; Ahmad et al. 2003; Huo et al. 2004; Urbaneja et al. 2008). However, in our present study, we did not find direct mortality nor sublethal effect on *S. japonicum* when imidacloprid was applied systemically at recommended field rate. This is unexpected because imidacloprid is toxic to *B. tabaci* eggs when placed on systemically treated plants (He et al. 2011; He and Wu unpublished data), i.e. the eggs did receive imidacloprid (through water uptake by the pedicel of *B. tabaci* eggs from the treated plants, Buckner et al. 2002; Li et al. 2009) and they contained imidacloprid in such exposure situation. It may mean that the imidacloprid dose present in *B. tabaci* eggs through this type of exposure is not toxic for *S. japonicum* when the predator consumes such contaminated prey.

More broadly, imidacloprid may be more toxic to *S. japonicum* through contact with residues than when ingesting imidacloprid-contaminated prey as reported in previous studies involving coccinellids and imidacloprid (Ohnesorg et al. 2010; Seagraves and Lundgren 2012). However, we think that this conclusion should be tempered though because (i) *B. tabaci* nymphs may contain higher concentrations of imidacloprid when starting feeding (after eggs hatch) on imidacloprid-treated plants, and (ii) coccinellids did not feed on plant materials during our assays (but it could occur in the field as they can fed on imidacloprid-contaminated nectar or pollen). Taken as a whole, our experiments can be seen as realistic scenarios as they mimic what would happen at the initial phase of infestation of the plants by the pest. However, it is not totally realistic if we consider that eggs may hatch after few days and then young nymphs do fed on imidacloprid-contaminated sap. In addition, exposure to imidacloprid may be problematic in case of other major predators like hemipterans (Desneux et al. 2006b; Ragsdale et al. 2011), which are known to consume plant sap. Further studies should be conducted on such predators if they prove to be of interest against *B. tabaci* on cotton in China.

Our study shows that aside from direct high residual-contact lethal toxicity, imidacloprid residues on cotton leaves occurring after foliar application can also cause adverse effects on functional response of *S. japonicum* on *B. tabaci* eggs. As imidacloprid applied systemically did not induce adverse effects on *S. japonicum*, there might be an opportunity to optimize imidacloprid application methods in cotton fields in China (to minimize potential negative effects on natural enemies). In addition, further longer-term studies are necessary to investigate the possible effects on development of larvae and fecundity of *S. japonicum* adults (e.g. see Biondi et al. 2012) when exposed to imidacloprid and/when consuming imidacloprid-contaminated prey. A reduction in the consumption capacity of *S. japonicum* may affect its reproductive capacity, inhibiting its population growth and consequently affect its performance in pest management programs (Stark and Banks 2003; Desneux et al. 2007).

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