

Effects of imidacloprid on *Harmonia axyridis* (Coleoptera: Coccinellidae) larval biology and locomotory behavior

CHARLES VINCENT¹, ANDRÉ FERRAN², LUDOVIC GUIGE², JACQUES GAMBIER² and JACQUES BRUN³

¹Centre de Recherche et de Développement en Horticulture, Agriculture et Agro-Alimentaire Canada, 430 Boul. Gouin, Saint-Jean-sur-Richelieu, QC Canada J3B 3E6; e-mail: vincentch@em.agr.ca

²INRA-Laboratoire de Biologie des Invertébrés, 37 boul. du Cap, 06600 Antibes, France

³INRA-Laboratoire de Biologie des Invertébrés, 1382 Route de Biot, 06560 Valbonne, France

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Abstract. The effects of imidacloprid on 1-day-old third instars of *Harmonia axyridis* were assessed by topical treatment and contact with treated glass plates in laboratory bioassays. When 5 µl of imidacloprid solutions were applied topically, the LD₅₀ was 0.085 g/l per insect after 24 h. Contact with imidacloprid-treated plates had little effect on the number of third instars that became adults. Average duration of larval development was not significantly affected by duration of contact and imidacloprid concentrations. There were no significant differences in maximal larval weight, weight gain and day at maximum larval weight. There were significant differences in average weight gain per day (from third instar to prepupa) after treatments with different imidacloprid concentrations. A temporary knockdown effect was observed with higher concentrations and longer durations of contact with treated plates. Compared with untreated third instars, contact with imidacloprid-treated plates caused an increase in time spent (in seconds) on the glass plates resulting from an increase in number of stops (per second) and angular speed (degrees per second) and a decrease in linear speed, excluding stops (mm/second). The changes in locomotory behavior (i.e., duration of stay on untreated plate, number of stops and angular speed) lasted up to 24 h after contact with imidacloprid-treated plates.

INTRODUCTION

While searching for prey, coccinellid predators show complex changes in behavior (Ferran & Dixon, 1993). They exhibit extensive searches (relatively fast and linear movements) between prey patches and intensive search (sinuous slow tracks interrupted by numerous stops) within patches (Banks, 1957). Most studies on search behavior have been done in insecticide-free situations (e.g. Ferran & Dixon, 1993; Ferran et al., 1994). However, coccinellid predators are likely to be exposed to insecticide residues in commercial agricultural ecosystems. Sub-lethal effects of neurotoxic insecticides may affect the behavior, and ultimately the performance, of natural enemies (Haynes, 1988; Croft, 1990). Walking velocity of an insect, the proportion of pesticide transferred from the plant to the insect during walking, and its area of contact with the leaf surface influence mortality levels following exposure to insecticide residues (Jepson et al., 1990). For instance, adult *Coccinella septempunctata* (L.) walked and groomed significantly more in deltamethrin-treated fields than in unsprayed winter wheat fields (Wiles & Jepson, 1994).

Imidacloprid (1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolodininime), is a chloronicotinyl analogue that acts on postsynaptic acetylcholine receptors in the insect nervous system (Abbink, 1991). Imidacloprid has insecticidal properties on a wide range of arthropods (Elbert et al., 1991; Pflüger & Schmuck, 1991). Bullock and Pelosi (1993) found that imidacloprid exerted adequate control on 10 selected insect and mite species.

Little is known about the effects of imidacloprid on natural enemies and what is known is conflicting. Pflüger & Schmuck (1991) stated that because of its predominantly systemic action, imidacloprid has limited effects on natural enemies, including coccinellid predators. Mizell & Sconyers (1992) assessed the LD₅₀ of several predatory insect species, including last instars and adults of coccinellids such as *Hippodamia convergens* (Guérin-Méneville), and *Olla v-nigrum* (Uhler). They concluded that, used as foliar sprays, imidacloprid should have detrimental impact on several natural enemy species. Kaakeh et al. (1996) estimated the LD₅₀ of imidacloprid on adult *H. convergens* by topical treatments as 1.8 µg/g and found that LT₅₀ was 5 h, a shorter time than carbaryl (8 h), chlorpyrifos (10 h), diazinon (10 h), propoxur (10 h) and fipronil (13 h).

Introduced into France from China in 1982, the polyphagous predator *Harmonia axyridis* (Pallas)(Coleoptera: Coccinellidae) has been mass-reared on *Ephestia kuhniella* Zeller (Lepidoptera: Pyralidae) eggs and is now commercially available for the control of the aphids *Macrosiphum rosae* (Ferran et al., 1996) and *Phorodon humuli* (Trouvé et al., 1996). *H. axyridis* was also introduced in the United States and is now found throughout North America (Chapin & Brou, 1991; Tedders & Schaefer, 1994; Coderre et al., 1995; Nalepa et al., 1996).

There is no published information on sublethal effects of imidacloprid on coccinellid predators. This study reports on the effects of imidacloprid on larvae of *H. axyridis*. They were conducted to evaluate the LD₅₀ of imidacloprid on larvae, to determine the effects of contact with imidacloprid-treated plates on larval biology, and to

evaluate proximate and delayed effects of contact with imidacloprid-treated plates on locomotory behavior of *H. axyridis* larvae.

MATERIALS AND METHODS

The experiments were conducted with one-day old *H. axyridis* third instars (mean weight 7.2 ± 1.4 mg, $n = 40$) that were reared at 23°C, a 16L : 8D photoperiod and 70–80% RH on *E. kuehniella* eggs that were bought from Biotop (Valbonne, France). *E. kuehniella* eggs were killed either by UV light treatment or by freezing. Although the behavioral effects observed could be somewhat different from those expected with the preferred hosts, i.e. aphids, *E. kuehniella* eggs allowed standardization of diet quality across experiments. Insecticide solutions were sprayed at a pressure of 0.9 atmosphere for ca. 15 sec on 20 by 20 cm glass plates in a modified Potter tower (Burgeron, 1956; Brun, 1985, 1988). The sprays, that were uniformly distributed on the glass plates, weighed an average of 0.5 mg/cm² (0.475–0.625) after drying. Control plates were sprayed with distilled water. Sprayed glass plates were allowed to dry for at least 60 min before the experiments.

Experiment 1. Effect of imidacloprid applied topically on larval mortality. Larvae were kept at 5°C for ca. 20 min. to slow their movements. Each larva was then treated on its dorsum with 5 µl of solution with a Hamilton® micro-syringe. The solutions were 0.3, 0.15 (i.e. the recommended field rate: ACTA, 1996), 0.015 or 0.0015 g/l of imidacloprid (i.e. Confidor® 200SL, Bayer SA, Puteaux, France) or water (control). There were ca. 20 third instars per treatment and three replicates per treatment. Ten minutes after treatment, larvae were individually reared in petri dishes at 20°C a 16L : 8D photoperiod and 70–80% RH. In experiments 1, 2, 3 and 6 the larvae were reared individually after treatment and provided with *E. kuehniella* eggs ad libitum. Larval mortalities were recorded 24 and 48 h post-treatment.

Experiment 2. Effect of dose and duration of contact with imidacloprid-treated plates on larval developmental time from third instar to adult. The glass plates were treated with the following solutions: 0.3, 0.15, 0.015, 0.0015 g/l of imidacloprid or water (control). Twenty larvae were fed with *E. kuehniella* eggs and maintained at 10°C to minimize cannibalism. Larvae were confined for 1, 5, 25 or 125 min on imidacloprid-treated plates under a 10-cm plastic petri dish lid, whose inner vertical walls were coated with fluon (a dispersion of polytetrafluorethylene) to prevent larvae from escaping and avoiding contact with the treated glass plate. The upper wall of the petri lid (6 cm in diameter) was pierced and covered with a fine mesh material to allow escape of insecticide fumes. The larvae were then transferred with a paintbrush into a petri dish and were reared. Larval mortality and time required to reach the adult stage were recorded daily. Three replicates of the 20 concentration-contact time combinations were conducted.

Experiment 3. Effect of imidacloprid on development as measured by larval weight and developmental time from third instar to the prepupa. Forty larvae were confined for 125 min under a 10 cm petri dish coated with fluon (as described in Experiment 2) on glass plates treated with: 0.3, 0.15, 0.015 or 0.0015 g/l of imidacloprid or water (control). They were then reared up to the prepupal stage. Developmental data were recorded daily until maximal weight was reached.

Four developmental parameters were assessed, i.e. (1) average maximum larval weight (mg); (2) day at average maximum larval weight; (3) average weight gain (mg); and (4)

average weight gain per day (mg/day) (i.e., from treatment day to maximum larval weight day).

Experiment 4. Direct effects: locomotory behavior of third instars walking on imidacloprid-treated plates. After being starved for 3 h, 53 larvae were confined individually for 1 min on treated (0.3 g/l imidacloprid) plates under a 2.5 cm diameter petri dish lid coated with fluon. Control larvae ($n = 54$, starved for 3 h) were confined 1 min on water-treated plates, and then locomotory behavior was video recorded. To study the behavioral parameters as a function of time, the 5 min video recording was divided into 15 successive path lengths of 20 s each. Behavioral parameters (described subsequently) were then plotted against time.

Experiment 5. Proximate effects: knockdown effect and locomotory behavior of third instars that recovered from contact with imidacloprid-treated plates. Larvae were confined (in groups of ca. 20) with a petri dish lid coated with fluon on glass plates treated with 0.3 g/l imidacloprid for 5, 30, 60, 90 and 120 min. Thirty-nine larvae were placed on control (water) glass plates for 10 min and 36 larvae on imidacloprid treated plates for each duration of contact. Immediately after treatment, larvae that exhibited a normal posture (i.e., standing) were counted and selected for locomotory studies. The coccinellids were individually placed on untreated glass plates, and confined 1 min under a 2.5 cm diameter petri lid coated with fluon. By removing the lid, they were allowed to walk freely on a glass plate and video recorded for 5 min.

Experiment 6. Delayed effects (24 h): locomotory behavior of imidacloprid-exposed third instars walking on untreated glass plates. Larvae were confined (in groups of ca. 50) for 10 and 60 min on imidacloprid-treated plates (i.e. 0.3 g/l imidacloprid) under a petri dish lid coated with fluon. Control larvae ($n = 50$) were confined for 60 min on water-treated plates. Each group was then reared in plastic containers. Twenty-four hours later, all larvae exhibited a normal posture (i.e., standing) before the experiment. They were starved for 1 h before the experiment and confined individually on untreated glass plates for 1 min under a petri dish lid coated with fluon. They were then allowed to walk freely on a glass plate by removing the lid. Locomotory behavior was video recorded.

Behavioral parameters and video recording. For experiments 4, 5, and 6 individual larvae were filmed for up to 5 min with a video camera (Ferran et al., 1994). Their locomotory behavior was quantified by assessing: (1) duration on the glass plates (seconds); (2) number of stops (per second); (3) linear speed, excluding stops (millimeters per second) and (4) angular speed (degrees per second). Upon video playback, these parameters were assessed and computed with custom software (Ferran et al., 1994).

Statistical analyses. In experiment 1, Probit analysis was performed with POLO-PC software (LeOra Software, 1994) and the results interpreted according to Robertson & Preisler (1992). In experiment 2, a two-way analysis of variance of the number of adults obtained by rearing treated larvae was done with STAT ITCF (version 5) (ITCF, 1991). In experiment 3, developmental parameters were tested by a one-way ANOVA (software SuperANOVA; Abacus Concepts, 1991). The behavioral parameters were first calculated with a custom software (Ferran et al. 1994). The results of experiments 4 and 6 were analyzed by ANOVA with STAT ITCF. Because few individuals were available for experiment 5, a Kruskal-Wallis non-parametric test was used with STAT ITCF.

RESULTS

Experiment 1. Twenty-four h after treatment, larval mortality was 0, 4.6, 13.1, 53.3 and 68% respectively in the treatments with 0 (=control), 0.0015, 0.015, 0.15 and 0.3 g/l imidacloprid. The same concentrations caused respectively 6.8, 4.6, 14.7, 78.3 and 73.0% cumulative mortality after 48 h. The LD₅₀ of imidacloprid applied topically was 0.085 g/l (95% confidence interval: 0.049–0.152) and 0.082 g/l (95% confidence interval: 0.039–0.141) after 24 and 48 h respectively. The slopes were 1.103±0.129 and 1.517±0.233 for 24 and 48 h respectively: as their calculated t-ratios were respectively 8.5 and 6.5 (p < 0.05), the regression models were significant.

Experiment 2. There were no significant differences in the number of third instars that became adult among imidacloprid concentrations (F = 0.70, df = 4, p = 0.067) and duration of contact with the plates (F = 0.62, df = 3, p = 0.060) (Table 1). The mean number of third instars that became adults ranged from 13.0 to 17.3 in the treated

TABLE 1. Average number of adults and average duration of development (day), obtained after rearing third instar *H. axyridis* (n = 20) subjected to different concentrations and contact time on imidacloprid-treated glass plates

Imidacloprid concentration (g/l)	Duration of contact with imidacloprid-treated plate (min)			
	1	5	25	125
No. of adults (Mean ± SEM)				
0 (control)	14.0 ± 4.4	16.3 ± 0.6	17.0 ± 2.7	15.3 ± 2.1
0.0015	16.7 ± 2.1	17.3 ± 0.6	16.0 ± 3.6	16.0 ± 4.4
0.015	15.0 ± 1.0	14.7 ± 2.1	17.3 ± 1.2	15.7 ± 2.3
0.15	14.7 ± 1.2	17.3 ± 0.6	15.3 ± 2.5	16.7 ± 0.6
0.3	16.3 ± 1.5	14.7 ± 4.5	15.7 ± 0.6	13.0 ± 2.0
Duration of development (day)(Mean ± SEM)				
0 (control)	14.7 ± 1.0	14.8 ± 0.9	13.9 ± 0.9	14.8 ± 1.8
0.0015	14.6 ± 1.3	14.3 ± 1.1	14.6 ± 1.3	14.9 ± 1.4
0.015	15.2 ± 1.5	14.8 ± 1.2	15.0 ± 1.2	14.6 ± 1.0
0.15	14.6 ± 1.4	14.2 ± 0.9	14.5 ± 1.0	14.4 ± 1.0
0.3	14.9 ± 1.3	14.8 ± 1.4	14.8 ± 1.7	15.3 ± 1.6

group, and from 14.0 to 17.0 in the control group, regardless of the duration of contact. The average duration (in days) of development from 3rd instar to the adult stage was not significantly different between different durations of contact (F = 1.08, df = 3, p = 0.36) (Table 1), but was significantly different between imidacloprid concentrations (F = 3.43, df = 4, p = 0.01). The calculated averages ranged from 14.4 to 14.9 days, but the latter significance level may be biologically irrelevant because the measurements were made daily.

Contact with imidacloprid-treated plates did not affect the mortality and duration of larval development. However, we observed a temporary knockdown effect (i.e., larvae immobile, lying on their side), typical for higher

concentrations and duration of contact with imidacloprid-treated plates.

Experiment 3. There were no significant differences among maximal weight (F = 1.23, df = 4, p = 0.30) and average weight gain (F = 1.20, df = 4, p = 0.31) in treated larvae (Table 2). No significant differences in the day of maximal weight were detected by the ANOVA (F = 1.49, df = 4, p = 0.21) among imidacloprid concentrations. The

TABLE 2. Growth of *H. axyridis* third instars subjected to a 125-min contact with imidacloprid-treated glass plate (n = 40)

Imidacloprid concentration (g/l)	Mean ± SEM			
	Maximum larval weight (mg)	Weight gain (mg)	Day at maximum larval weight	Weight gain per day ^a (mg/day)
0 (control)	34.9 ± 1.3	27.7 ± 1.2	4.0 ± 0.2	7.1 ± 0.4
0.0015	34.2 ± 1.6	26.4 ± 1.6	4.1 ± 0.3	6.3 ± 0.5
0.015	32.1 ± 1.8	24.5 ± 1.8	4.3 ± 0.3	5.3 ± 0.4
0.15	36.1 ± 1.4	28.4 ± 1.3	4.5 ± 0.2	6.3 ± 0.4
0.3	32.4 ± 1.5	25.2 ± 1.4	4.7 ± 0.2	5.5 ± 0.3

^aAverage weight gain per day; calculated up to the day when maximum larval weight was recorded.

day at which maximum larval weight was reached was earlier in the control (d 4.0), and increased consistently with imidacloprid concentration, reaching the highest value (d 4.7) for imidacloprid concentration 0.3 g/l. Again, there were significant differences among average weight gain per day (F = 2.86, df = 4, p = 0.03), the highest value being maximal for the control group (7.1 mg/day) and minimal (5.5 mg/day) for the 0.3 g/l imidacloprid treatment. Among individuals treated with imidacloprid, the difference between the maximum (6.3) and minimum (5.3) weight gain per day was equal to the periodicity of weight measurement, i.e. 1 day. Compared to untreated individuals, imidacloprid slightly affects weight gain per day in treated individuals.

Experiment 4. The larvae stayed significantly longer on treated (59.8 s) than on control (37.7 s) plates (Table 3). The longer stay on treated plates could be explained by the significantly greater number of stops (1.6 more stops per second), significantly lower linear speed (0.3 mm per second), and significant greater angular speed (12.4 degrees per second faster).

For all path lengths, the total stay on the glass plates was consistently lower for treated than untreated larvae (Fig. 1A). Considering the number of stops, linear speed and angular speed, three periods could be distinguished (Figs 1B, C, D): period 1 (i.e., successive path lengths 1 to 6), where treated larvae generally showed a higher number of stops, a higher angular speed and a lower linear speed than untreated larvae (i.e., a search pattern more akin to intensive searches in treated than untreated individuals); period 2 (i.e., successive path lengths 7 to 10) where angular speed, number of stops and linear speed were approximately equal in treated and untreated groups (i.e., a search pattern more akin to extensive

TABLE 3. Locomotory behavior of *H. axyridis* third instar larvae walking on imidacloprid-treated (0.3 g/l) plates

Behavioral parameter (mean ±SEM)	Control (n=54)	Imidacloprid -treated plate (n= 53)	F ^a (p) ^b
Stay on glass plate (sec)	37.7 ± 11.2	59.8 ± 14.2	2.39(0.002)
Number of stops (per sec)	6.2 ± 0.7	7.8 ± 0.7	3.13(0.002)
Linear speed, excluding stops (mm per sec)	9.8 ± 0.2	9.5 ± 0.1	3.10(0.003)
Angular speed (degrees per sec)	48.7 ± 4.9	61.4 ± 5.3	3.48(0.009)

^a value of the computed statistic F, from Anova.

^b probability associated with calculated F

searches in treated individuals); and period 3 (i.e., successive path lengths 11 to 15) where the behavior was the same as described in period 1.

Experiment 5. Preliminary observations (not quantified) in experiment 2 of temporary knockdown effects at higher imidacloprid concentration and stay on plates were confirmed in experiment 5 at a concentration of 0.3 g/l imidacloprid. There were 33.3, 58.3, 86.1, 97.2 and 88.9% (n=36 individuals per duration) of larvae knocked down respectively after exposure for 5, 30, 60, 90 and 120 min to imidacloprid-treated plates and 10.8% (n=39 larvae) in the control.

As the percentages of larvae knocked down were high (i.e. > 86%) in treatments ≥ 60 min, experiments on locomotory behavior were performed only with larvae from the control, and from shorter treatments. Among the treatments there were significant differences in the duration of stay on plates, the number of stops, and the angular speed (Table 4). For the stay on plates, number of stops, and angular speed, the differences were markedly higher in the 30-min treatment than in the control and the 5-min treatment. There were no significant differences in linear speed among treatments. For the 30 min treatment, larvae stayed longer on treated plates because of an increase in number of stops and an increase in angular speed.

Experiment 6. Locomotory behavior of larvae was affected as observed in experiment 5, even 24 h after contact with imidacloprid-treated plates (Table 5). Compared with control larvae, duration of stay on untreated plates was significantly higher in larvae treated for both 10 and 60 min. This greater duration resulted from a significant increase in the number of stops, a significant increase in angular speed and a significant decrease in linear speed. All behavioral parameters had similar values for larvae exposed to treated plates for 10 and 60 min.

DISCUSSION

In integrated pest management programs, *H. axyridis* will likely be exposed to insecticide residues on crops. In field situations, exposure of coccinellid predators to pesticides, including imidacloprid, may occur as a result of ingestion of treated prey, direct exposure to sprays or by

contact with treated surfaces. Our study focused on the latter two mechanisms, with particular reference to larval biology and locomotory behavior.

Imidacloprid had a lethal effect when applied topically (Experiment 1), as found for *H. convergens* adults by Kaakeh et al. (1996). Contact with imidacloprid-treated plates did not affect larval mortality (Experiment 2), or larval growth (Experiment 3). However, there were significant differences in average weight gain per day (Experiment 3). Because there is a linear relationship between the number of aphids consumed and larval weight (Ferran & Larroque, 1977), larval weight parameters suggest that the predatory capacity of larvae will be unaffected by contact with imidacloprid-treated surfaces. However, larvae that contacted imidacloprid residues showed a knockdown effect (Experiment 5). Knockdown effects, also observed in other coccinellid predators, e.g.

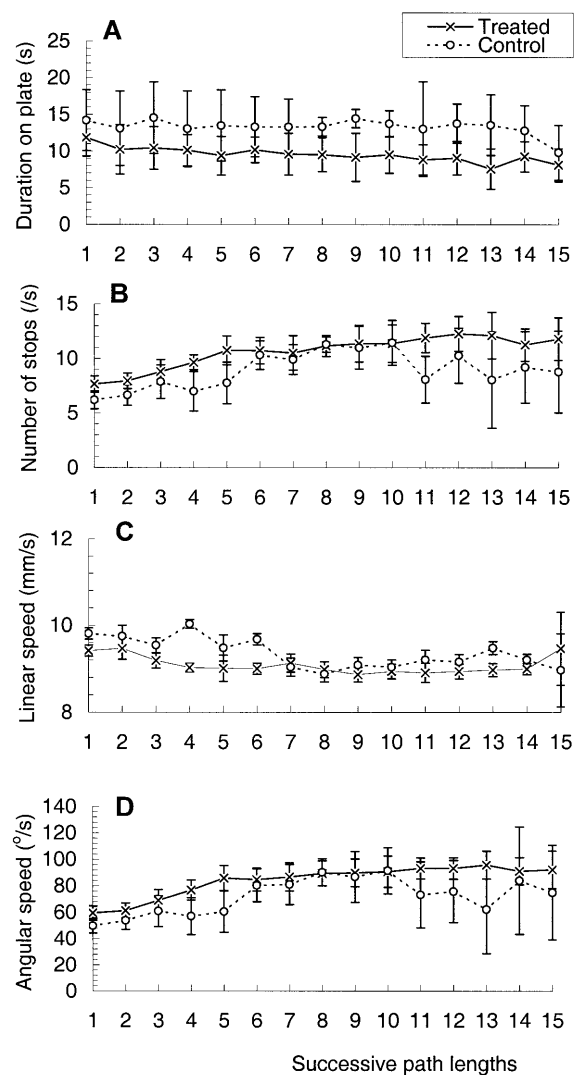


Fig. 1. Locomotory behavior of *H. axyridis* third instars walking on untreated (control) and imidacloprid-treated glass plates. The 5 min video recording was divided into 15 successive path lengths of 20 seconds each. Vertical bars are standard error of means.

TABLE 4. Locomotory behavior of *Harmonia axyridis* third instars walking on untreated glass plates. Only individuals that recovered after 5 and 30 min contact with an imidacloprid-treated (0.3 g/l) plate.

Behavioral parameter (mean ±SEM)	Imidacloprid-treated plate			H ^a (p) ^b
	Control (n = 35)	5 min (n = 24)	30 min (n = 15)	
Stay on glass plate (sec)	43.6 ± 6.6	42.6 ± 7.6	63.3 ± 9.9	9.52(0.01)
Number of stops (per sec)	9.5 ± 0.7	9.0 ± 0.9	11.5 ± 1.7	6.03(0.005)
Linear speed, excluding stops (mm per sec)	9.4 ± 0.2	9.4 ± 0.3	9.1 ± 0.2	1.58(0.46)
Angular speed (degrees per sec)	80.8 ± 7.1	85.8 ± 10.5	114.8 ± 5.0	22.7(0.001)

^a value of the computed statistic H, from the Kruskal-Wallis test.

^b probability associated with calculated H.

Coleomegilla maculata Timberlake (Roger et al., 1994, 1995), may affect predatory performance.

Contact with imidacloprid caused effects on the locomotory behavior of *H. axyridis* larvae (Experiments 4, 5 and 6). Compared with the control larvae, imidacloprid caused an increase in the number of stops (per second) and in angular speed (degrees per second). Consequently, larvae stayed longer on the treated plates. The changes in locomotory behavior occurred rapidly (Experiment 5), and probably preceded the knockdown effect. Although larvae apparently rapidly recovered from the knockdown state, changes in locomotory behavior persisted for at least 24 h after contact with imidacloprid-treated plates (Experiment 6).

In an integrated pest management context, imidacloprid could be detrimental to *H. axyridis* predatory activities in three ways. First, larvae could be killed by direct sprays of imidacloprid on plants. Second, contact with imidacloprid residues may cause the larvae to fall off the plant, by knockdown effect. Although the fate of fallen larvae is unknown, the probability of mortality could be enhanced due to adverse conditions (e.g., remoteness of optimal

TABLE 5. Delayed effect (24 h) of contact with imidacloprid-treated (0.3 g/l) glass plates on *H. axyridis* larval locomotory behavior.

Behavioral parameter (mean ±SEM)	Imidacloprid-treated plate			F ^a (p) ^b
	Control (n = 50)	10 min (n = 46)	60 min (n = 44)	
Stay on untreated plate (sec)	71.3 ± 76.0	109.4 ± 75.6	103.9 ± 80.8	4.51(0.012)
Number of stops (per sec)	5.4 ± 2.2	6.9 ± 1.4	6.6 ± 1.6	13.6(0.000)
Linear speed, excluding stops (mm/sec)	9.9 ± 1.4	9.3 ± 0.5	9.3 ± 0.5	7.9(0.000)
Angular speed (degrees per sec)	56.0 ± 25.2	75.0 ± 22.1	73.7 ± 24.4	12.5(0.000)

^a value of the computed statistic F, from Anova.

^b probability associated with calculated F.

prey patches, soil predators, energy requirements to climb back on the plant). Third, locomotory behavior changes due to contact with imidacloprid residues for more than 5 min would increase the duration of contact with the treated plant and the probability of knockdown effect. In the context of agroecosystems, it is possible that imidacloprid also exerts negative effects over a wide range of natural enemies and additional research is needed.

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