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Locomotor activity, knockdown, and recovery in first instar nymphs of *Blattella germanica* (Dictyoptera: Blattellidae) treated with imidacloprid

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Abstract

Locomotor activity, knockdown, and recovery were studied in first instar nymphs of *Blattella germanica* treated with a neonicotinoid insecticide, imidacloprid. No effects on locomotor activity were observed in insects placed on filter paper impregnated with 3, 30, or 300 µg/cm² of imidacloprid. The Knockdown Dose 50% (KD50) for imidacloprid was 63.0 and 317.3 ng/insect at 18 and 28°C, respectively, leading to a temperature coefficient of –5.0. The variation of KD50 values as a function of time was also studied, with and without simultaneous application of piperonyl butoxide (PBO). Recovery from the knockdown was observed in insects treated with imidacloprid alone, whereas no recovery was observed when insects were simultaneously treated with PBO. These results suggest that imidacloprid is metabolized by mixed-function microsomal oxidases.

Keywords: German cockroach, temperature coefficient, neonicotinoid, piperonyl butoxide.

Introduction

Hyperactivity, temporary knockdown, and a negative temperature coefficient are usually observed in insects treated with pyrethroid insecticides (Naumann, 1990). Hyperactivity, an increase in the locomotor activity, was

reported as the first symptom of poisoning by pyrethroids in a number of insects (Gammon, 1978; Miller & Adams, 1982; Benoit *et al.*, 1985; Toth & Sparks, 1990; Alzogaray *et al.*, 1997). Knockdown is a part of the pyrethroid action (Naumann, 1990): it is rapid in onset, sometimes very long lasting, and not necessarily associated with lethal action. As a trend, DDT and the pyrethroid insecticides show a negative temperature coefficient: the toxicity increases as temperature decreases (Naumann, 1990). However, there are exceptions to this trend, depending on factors such as insecticide, species, instar, and the range of temperatures studied (Sparks *et al.*; 1983; Toth & Sparks, 1988; Alzogaray *et al.*, 1998).

Imidacloprid was the first active ingredient of the chemical class of nicotinoid insecticides to reach the market (Thyssen & Machemer, 1999). It binds to the nicotinic acetylcholine receptor located on the postsynaptic membranes and results in postsynaptic blockage in the nervous system of the insects. Because of its novel mode of action, imidacloprid has become an important option in the control of conventional insecticide-resistant insect strains (Elbert *et al.*, 1998). It is a molecule of high selectivity, being very toxic to insects but not to mammals or plants (Thyssen & Machemer, 1999).

A temporary knockdown effect was reported in insects treated with imidacloprid (Tharp *et al.*, 2000; Vincent *et al.*, 2000; Barry *et al.*, 2004). Synergism studies with piperonyl butoxide (PBO) suggested that mixed-function microsomal oxidase-mediated detoxification was responsible for resistance to imidacloprid in insects (Wen & Scott, 1997; Zhao *et al.*, 2000).

The aim of this work was to study the toxicity of imidacloprid on the German cockroach, *Blattella germanica*. In particular, we studied its effect on locomotor activity, knockdown at two temperatures (18 and 28°C), and recovery in first instar nymphs treated with and without PBO that is an inhibitor of mixed-function microsomal oxidases.

We choose the first instar nymphs of *B. germanica* as a model for the present work, following a standardized method to evaluate insecticide toxicity in this insect developed by Taiariol *et al.* (2001). Koehler *et al.* (1993) showed that adults and young nymphs of *B. germanica* have about the same susceptibility to insecticides.

Material and methods

Biological material

First instar nymphs of *B. germanica* were obtained from a colony maintained in our laboratory at 28°C. The experimental work was done on nymphs 2-7 days old that had been fed with rat pellets.

Chemicals

Imidacloprid (technical grade) and PBO were a gift from Chemotecnica SA (Argentina). Acetone was from Merck (Darmstadt, Germany).

Bioassays

The locomotor activity was recorded using an image analyzer (Videomex V, Columbus, OH) provided with a video camera and connected to a personal computer (a detailed description of the recording equipment can be found in Alzogaray and Zerba, 2001). The test arena was a rectangular filter paper (8 x 9 cm). Insect movements were limited by a glass ring (5 cm in diameter). The filter papers were impregnated with 0.5 mL of acetonic solutions of imidacloprid or acetone alone (control). Groups of three nymphs were put in the test arena and their locomotor activity was recorded for 30 min. Locomotor activity was expressed in units of pixels/area, according to Alzogaray *et al.* (1997). Data were analyzed using ANOVA.

To estimate the values of Knockdown Dose 50% (KD50), 0.2 µL of an acetone solution of imidacloprid was topically applied on the ventral side of the body of each insect. Control groups were topically treated with acetone alone. A minimum of 4 concentrations of each compound and 10 nymphs at each concentration were used to estimate the KD50 at 18 and 28 ± 1°C in a controlled temperature chamber. The effect evaluated was knockdown. Each experiment was replicated at least 3 times. Data from the different replicates were pooled when the confidence limits of the respective KD50 overlapped.

The KD50 values were calculated using the probit method. In all cases, differences between values were considered significant ($P < 0.05$) if the respective 95% confidence limits did not overlap. Temperature coefficients were calculated by dividing the larger KD50 values by the smaller KD50 (Toth & Sparks, 1990). Temperature coefficients were designated as negative if the KD50 at the higher temperature were larger than the respective KD50 at the lower temperature.

To evaluate the effect of PBO on recovery, imidacloprid solutions were prepared using PBO 10% in acetone as solvent.

Results

Table 1 shows the locomotor activity recorded in insects exposed to filter paper treated with 3, 30, or 300 µg/cm^2. No significant effects of imidacloprid on the locomotor activity were observed at the concentrations used (ANOVA, $P > 0.05$). The three concentrations assayed did not produce any observable effect on the insects after 30 min or 24 h from the beginning of the exposure. When the insects were exposed to concentrations greater than 300 µg/cm^2, they showed poisoning symptoms (mainly incoordination) a few minutes after the beginning of the exposure. For this reason, 300 µg/cm^2 was the maximum concentration tested in this locomotor activity tests.

The KD50 values of imidacloprid at 18 and 28°C were 63.0 and 317.3 ng/insect, respectively (Table 2). The difference between these two values was significant (no overlapping of the CL 95%, $P < 0.05$), and the temperature coefficient was - 5.0.

Table 1. Locomotor activity of first instar nymphs of *B. germanica* exposed to different concentrations of imidacloprid.

Imidacloprid, µg/cm²	Locomotor activity, pixels/area ± SE
0	4,413 ± 2,228a
3	4,909 ± 1,924a
30	3,400 ± 952a
300	3,707 ± 2,228a

Values followed by the same letter are not significantly different (ANOVA, $P > 0.05$).

Table 2. KD50 values of imidacloprid at two temperatures on first instar nymphs of *B. germanica*.

KD50 at 18°C, ng/insect	KD50 at 28°C, ng/insect	Temperature Coefficient[1]
63.0a (45.6 - 87.1)[2]	317.3b (248.8 - 433.0)[2]	- 5.0

[1] Temperature Coefficient = Higher KD50 / Lower KD50; the negative sign means that the KD50 at 18°C was lower than the KD50 at 28°C.

[2] Confidence limits (CL) 95%.

Values followed by the same letter are not significantly different (based on non-overlapping of CL 95%, $P > 0.05$).

Fig. 1. KD50 for imidacloprid on first instar nymphs of *B. germanica* with and without piperonyl butoxide. Vertical lines are CL 95%. In each pair, bars with the same letter are not significantly different (based on non-overlapping of CL 95%, $P > 0.05$).

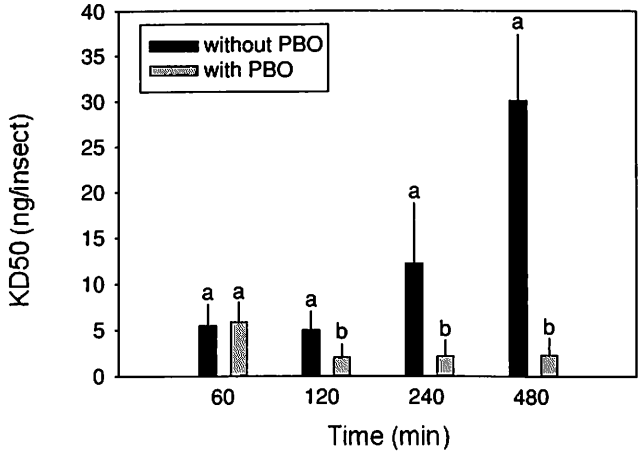

Fig. 1 shows the KD50 values for imidacloprid with and without PBO at different times. At times longer than 120 min, the KD50 values for the imidacloprid plus PBO treated insects were significantly lower than the values for the insects treated with imidacloprid alone (no overlapping of the CL 95%, $P < 0.05$).

Discussion

A characteristic of DDT and pyrethroids is their negative temperature coefficient of toxicity in insects (Naumann, 1990). However, the sign and magnitude of the Temperature Coefficient for the same molecule can change with different temperature ranges (Sparks *et al.*, 1983), methods of insecticide application, and species (Toth and Sparks, 1988).

The effect of temperature on the toxicity of imidacloprid has been poorly studied. Richman *et al.* (1999) investigated the toxicity of imidacloprid at 20, 26 and 35°C on cat flea. These authors found that this insecticide was most toxic to adult fleas at 35°C and to larvae at 20°C. In the same work, it was found that PBO synergized imidacloprid at 26 but not at 35°C on adult fleas; in larvae, PBO synergized imidacloprid at 20 but not at 26°C. Here we found that imidacloprid shows a negative temperature coefficient on first instar nymphs of *B. germanica* in the range 18-28°C.

The possible cause for these results are differences in penetration (Blum & Kearns, 1956) or in metabolic rates (Ruigt, 1985) observed at different temperatures. In the case of pyrethroids, depolarization of nerve terminals appears to be negatively correlated with temperature (Salgado *et al.*, 1983). It is possible that temperature affects in a similar way the binding of imidacloprid to the nicotinic acetylcholine receptor.

Penetration, metabolic degradation, and interaction of the insecticides with their sites of action are also known to influence the knockdown effect produced by pyrethroids (Ruigt, 1985). However, it is not clear if knockdown and kill are different manifestations of the same underlying mechanism. The degradation in the site of action was proposed to explain the rapid recovery in *Musca domestica* exposed to pyrethrins (Sawicki, 1962). This hypothesis was supported by the lack of housefly recovery in the presence of PBO. Similar results were reported in adults of the blood-sucking bug *Triatoma infestans* treated with both the pyrethroid deltamethrin and PBO (Casabé *et al.*, 1988). Our results also suggest that imidacloprid is metabolized by mixed-function microsomal oxidases in first instar nymphs of *B. germanica*.

Imidacloprid shares some toxicological properties with pyrethroid insecticides since it shows a negative Temperature Coefficient and produces a temporary knockdown that is blocked by PBO. However, imidacloprid failed to produce hyperactivity, a symptom of poisoning broadly observed in insects exposed to pyrethroids. This difference will probably be explained when more information about the effect of both imidacloprid and pyrethroids on the sensory nervous system becomes available.

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