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Lufenuron kills deltamethrin-resistant *Blattella germanica* (Blattodea)

SECCACINI, Emilia A.*, JUAN, Laura W., VASSENA, Claudia V., ZERBA, Eduardo N., & ALZOGARAY, Raúl A.

Centro de Investigaciones de Plagas e Insecticidas (UNIDEF-CITEDEF-CONICET-CIPEIN).
Juan B. de La Salle 4397 (1603) Villa Martelli, Buenos Aires, Argentina.
*E-mail: eseccacini@gmail.com

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Lufenurón mata a *Blattella germanica* (Blattodea) resistente a la deltametrina

RESUMEN. Lufenurón es un inhibidor de la síntesis de quitina usado para controlar diversas plagas de insectos. La cucaracha alemana, *Blattella germanica*, es un insecto cosmopolita de importancia médica y económica. Existen numerosos reportes de resistencia a insecticidas en poblaciones de *B. germanica* alrededor del mundo. El objetivo de este trabajo fue evaluar el efecto de lufenurón en dos colonias de *B. germanica*, una susceptible a insecticidas (CIPEIN) y otra resistente a deltametrina (JUBA). Se expusieron poblaciones experimentales de ambas colonias a papeles de filtro tratados con 50 mg de lufenurón/m^2. Los controles fueron expuestos a papeles de filtro tratados con solvente solo. Al cabo de siete semanas, las dos poblaciones expuestas a lufenurón presentaron el mismo tamaño promedio (1,3 ± 0,7 individuos); mientras que los tamaños promedio de las poblaciones controles fueron 206,5 ± 39,5 (CIPEIN) y 136,5 ± 43,5 individuos (JUBA). Este drástico efecto sobre el tamaño poblacional sugiere que lufenurón podría ser usado como una alternativa para controlar cucarachas alemanas resistentes a deltametrina. A la vista de estos resultados, se debería explorar la toxicidad de lufenurón en otras poblaciones de *B. germanica* resistentes a piretroides u otros insecticidas.

PALABRAS CLAVE. Cucaracha alemana. Reguladores del crecimiento de los insectos. Resistencia a piretroides.

ABSTRACT. Lufenuron is a chitin synthesis inhibitor used for controlling several pest insects. The German cockroach, *Blattella germanica*, is a cosmopolitan insect of medical and economic importance. Resistance to insecticides has been worldwide reported in this species. The objective of this work was to evaluate the effect of lufenuron in two colonies of *B. germanica*, one susceptible to insecticides (CIPEIN) and another pyrethroid-resistant (JUBA). Experimental populations from each colony were exposed to filter papers treated with 50 mg of lufenuron/m^2. Controls were exposed to filter papers treated with solvent alone. After seven weeks, the average size of both lufenuron-treated populations was 1.33 ± 0.7 individuals, while the average size of control populations were 206.5 ± 39.5 (CIPEIN) and 136.5 ± 43.5 (JUBA) individuals. This drastic effect suggests that lufenuron could be used as an alternative for the control of pyrethroid-resistant German cockroaches. Future studies should explore the toxicity of lufenuron on other populations of *B. germanica* resistant to pyrethroids or other insecticides.

KEYWORDS. German cockroach. Insect growth regulators. Pyrethroid-resistance.

Chitin, a polymer of N-acetyl-D-glucosamine, is one of the main components of the insect cuticle (Cohen, 2010). Several benzoylphenylurea derivatives are chitin synthesis inhibitors (CSI) (Pener & Dhalialla, 2012), which disrupt the deposition of chitin in the newly synthetized cuticle and hence interfere with the process

of insect moulting. Insects treated with these substances die during or after the following moult.

Lufenuron is a benzoylphenylurea with good CSI activity on species of Diptera, Dyctioptera, Coleoptera, Lepidoptera, Isoptera, and Siphonaptera (Kaakeh et al., 1997; Rust, 2005; Saénz-de-Cabezón et al., 2006; Karimzadeh et al., 2007; Moya et al., 2010; Correia et al., 2013; Wang et al., 2014). It has also been reported as a fly chemosterilant (Casaña-Giner et al., 1999). Commercial products based on lufenuron are used to protect several crops such as corn, potatoes, cotton, sugar beet, citrus, grapes, and other ornamental species (FAO, 2008). Other products have been developed for controlling fleas on pets (Franc & Cardiegues, 1996).

The German cockroach, *Blattella germanica*, is a hemimetabolous insect belonging to the order Blattodea, familiy Blattellidae. It is an omnivorous species with worldwide distribution and is associated with any human presence that provides it a room with appropriate temperature, humidity and food (Cochran, 2003). *Blattella germanica* is very prolific and any place it colonizes quickly becomes an important source of dead bodies, faeces and empty oothecae that release unpleasant odours. These remains are also a threat to human health when deposited on food. This species is classified as a health pest because it is a mechanical vector for microbes (Graczyk et al., 2005; Mpuchane et al., 2006). Moreover, the particles of their faeces and exuviae are allergenic to some people and trigger asthma in others (Gao, 2012). Cockroaches are also unpleasant for most people and produce extreme reactions in entomophobia sufferers (Firoozfar et al., 2012).

With reports of resistance to forty-two active ingredients from different populations around the world, *B. germanica* occupies the seventh position in the "top 20" world ranking of species in which insecticide resistance has been reported (Whalon et al., 2008). When considering only urban pests, it ranks second after *Musca domestica*.

In Argentina, insecticide resistance in local populations of *B. germanica* was first described at the beginning of the twenty-first century (Taiariol, 2001). More recently, our laboratory detected pyrethroid resistance in German cockroaches from several Argentine cities (unpublished results). The aim of this work was to evaluate the efficacy of lufenuron in controlling an experimental population of *B. germanica* resistant to deltamethrin under laboratory conditions.

Two colonies of *B. germanica* were used in this study. The CIPEIN colony has been kept in the absence of insecticides and under laboratory conditions for over twenty years. The JUBA population, resistant to pyrethroids, was obtained from individuals collected in March 2013 in the city of Junín (province of Buenos Aires, Argentina). Both colonies were kept at 25-30 °C, 50-70% RH, and under a photoperiod of 12:12 h L:D. Rabbit pellet feed and water were provided *ad libitum.*

We used technical grade lufenuron (98%) (Shangyu Agrotrust Biotech Ltda.; Shaoxing, China), acetone (Sintorgan, Buenos Aires, Argentina), and silicone Dow Corning 556 cosmetic grade fluid (Auburn, MI).

Deltamethrin was applied as residues on glass. We used round glass containers (11 cm in diameter, 6 cm tall), with a flat base of 95 cm^2. Using a pipette, 1.5 ml of a deltamethrin solution in acetone was applied on the base of the container and distributed homogeneously over the entire surface. The following concentrations of deltamethrin were applied in different containers: 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, and 10.0 mg/m^2. Acetone alone was used as a control. The solvent was left to evaporate during 60 min and then ten first instar nymphs of the CIPEIN colony were deposited into each container where they remained for 60 minutes. The nymphs were then moved to plastic containers with water and food and kept until the following day under the same conditions as they were reared. Mortality was recorded twenty-four hours after the end of the treatment. Three independent replicas were carried out for each treatment. Values of lethal concentration 50% (LC50) and lethal concentration 95% (LC95) were calculated with the results using Polo Plus software (LeOra Software, 2002). To assess toxicity in the field individuals (JUBA population), a discriminating dose of 100 mg/m^2 was used, approximately equivalent to LC95 x 20. This discriminating dose was applied on the bottom of the glass containers as previously explained. Three independent replicas were carried out for each colony.

Lufenuron was applied as residues on #1 filter paper (Whatman International, Maidstone, United Kingdom). The insecticide was dissolved in a 1:1 mixture of acetone and silicone (0.9 mg/ml). Two squares of filter paper, one large (20 x 20 cm) and one small (8 x 17 cm), were impregnated with 50 mg of lufenuron/m^2. This concentration was chosen according to preliminary tests. The solvent was allowed to evaporate for 24 hours after which the large square was placed on the bottom of an acrylic box (20 x 20 x 20 cm). The smaller square was folded and deposited on the larger square for the cockroaches to use as a refuge. A thin layer of solid vaseline was applied on the upper internal margin of the acrylic box to prevent the cockroaches escaping. Twenty-two cockroaches were placed inside the box (ten small nymphs, ten large nymphs, one male individual and one female individual with an ootheca). Finally, the box was covered with a metallic mesh held in place by an acrylic frame and placed inside a chamber under the same environmental conditions as they were reared in. Filter papers impregnated with acetone:silicone (1:1) alone were used as controls. The treatment with lufenuron was repeated three times for each colony, and the treatment with acetone:silicone (1:1) alone was repeated twice. The number of insects in each box was registered weekly during a period of 49 days. Results were analysed with Student's t-test.

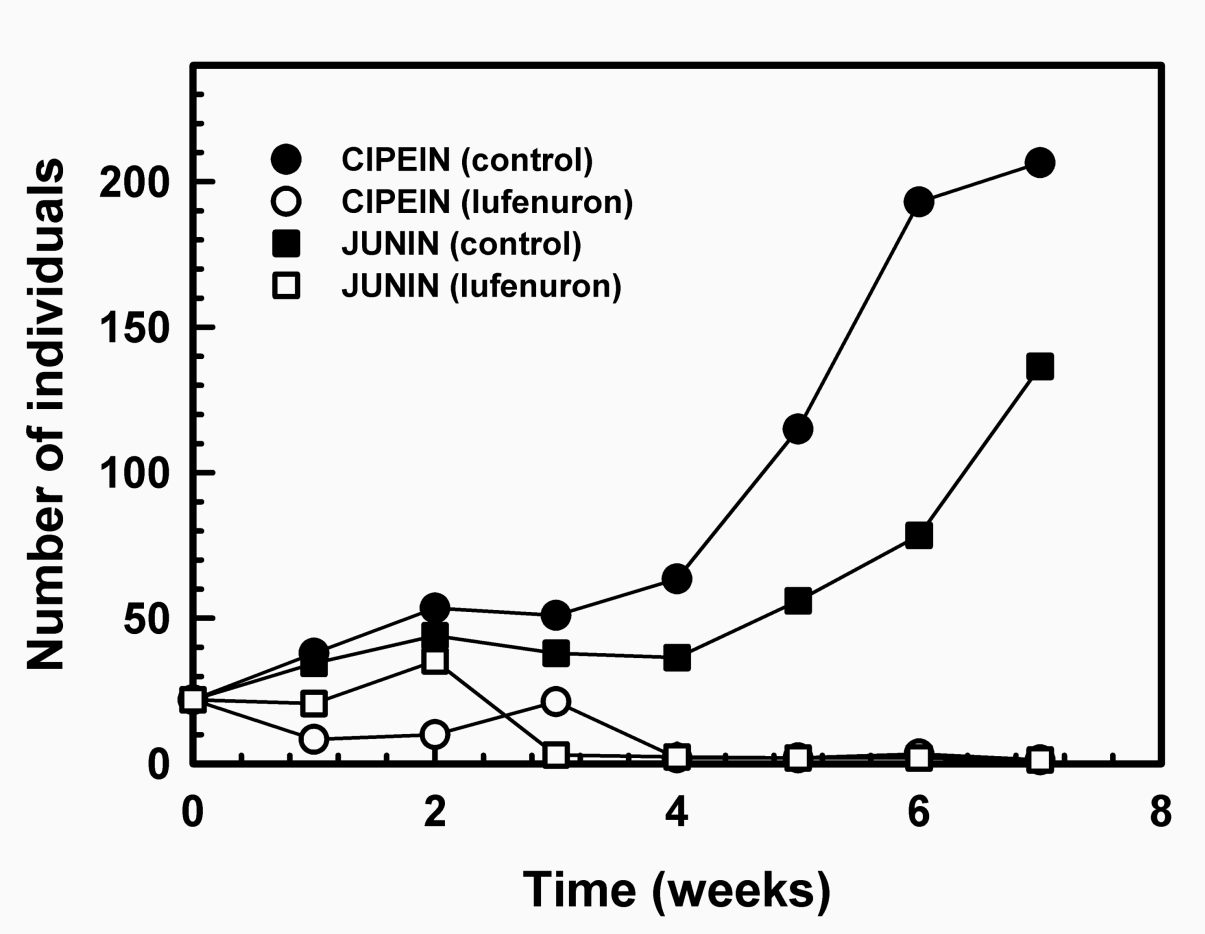

Fig. 1. Effect of permanent exposure to lufenuron on susceptible and deltamethrin-resistant *Blatella germanica*.

The values of LC50 and LC95 (CI 95%) for deltamethrin in the CIPEIN colony were 1.15 (0.90-1.56) and 5.01 (3.18-11.08) mg/m^2, respectively. The application of a discriminating dose (100 mg/m^2) produced an average of 100 and 0% mortality in the CIPEIN and JUBA colonies, respectively.

Exposure to lufenuron had a drastic effect on the size of both colonies (Fig. 1). After 49 days, the experimental populations of the control boxes reached an average size of 206.5 ± 39.5 (CIPEIN) and 136.5 ± 43.5 (JUBA). On the other hand, the average size in the boxes with the insecticide treatment was 1.3 ± 0.7 individuals for both colonies. In both cases, the size of each treated group was significantly smaller than their corresponding control (CIPEIN: t = 6.966, df 3, P = 0.006; JUBA: t = 4.167, df 3, P = 0.025).

Lufenuron is a CSI with good insecticidal activity in *B. germanica* (Schenker & Moyses, 1994; Mosson et al., 1995; Kaakeh et al., 1997). However, this is the first time the effect of this substance is studied on a deltamethrin-resistant colony. The insecticide produced a drastic effect on the growth of the *B. germanica* population and was as effective on the resistant individuals as on individuals from a deltamethrin-susceptible colony. This result could be attributed to both the effect of lufenuron as a moult-disrupting CSI and also to its activity as a reproductive inhibitor of *B. germanica* (Mosson et al., 1995). The dose applied in the present study (50 mg/m^2) is similar to the doses that produced positive results in other assays carried out on *B. germanica* individuals susceptible to insecticides. For example, lufenuron inhibited moulting and decreased oothecal production in doses between 10 and 50 mg/m^2 applied on masonite or plywood (Kaakeh et al., 1997).

The result of the present study suggests that lufenuron could be used as an alternative for controlling pyrethroid-resistant *B. germanica*. Its contact activity and adequate residual activity are desirable traits for being used in the control of these cockroaches (Kaakeh et al., 1997). As they have also shown to be effective orally (Mosson et al., 1995), it would be interesting to investigate their application in food baits. Future studies should explore whether this effectivity is manifested in other populations of *B. germanica* resistant to pyrethroids or other insecticides.

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