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NEW APPROACHES TO CONTROL *LOXOSCELES INTERMEDIA* (ARANEAE: SICARIIDAE)

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Abstract The integrated pest management (IPM) concept to reduce *Loxosceles intermedia* populations in homes may rely upon many strategies. In this work, a range of new approaches have been studied with the aim of controlling these brown spiders, which include observing and evaluating the following: the effects of pyrethroids on *L. intermedia*, the efficacy of vacuum cleaners for the integrated control of the brown spider *L. intermedia*, the predatory behavior of the tropical house gecko (*Hemidactylus mabouia*) on L. intermedia under laboratory conditions, the effects of moderately heated airflow on L. intermedia and the chemical signals involved in the attraction and courtship behavior of *L. intermedia*.
Key Words Brown spiders, pyrethroids, vacuum cleaner, *Hemidactylus mabouia*, heated airflow, semiochemicals.

INTRODUCTION

Bites of recluse or brown spiders (genus *Loxosceles*) can cause necrotic lesions and systemic effects in humans throughout the world (Silva et al., 2004). This genus of spiders is widely distributed over the world's temperate, subtropical and tropical zones (Gertsch and Ennik, 1983). These spiders build irregular webs in dark places such as crevices, under rocks and debris, and in caves, and the species vary in their propensity toward mobility and synanthropic association. Despite the over reporting of accidents related to *Loxosceles* in certain localities such as the western American states (Vetter, 2008), thousands of bites really occurs every year in other regions. This is the case for Southern Brazil, particularly in the State of Parana where loxoscelism is considered a serious public health problem. The species responsible for the majority of the reported accidents is L. intermedia Melo-Leitão (Marques-da-Silva and Fischer, 2005; Marques-da-Silva et al., 2006). This species prefers indoor environments (Fischer and Vasconcellos-Neto, 2005), and wanders extensively, increasing its chances of human contact, with accidents being more frequent in the hottest months of the year (Marques-da-Silva et al., 2006). In this work we briefly describe the results of a broad study in which different methodologies were tested aiming at the control of *L. intermedia* population such as the effect of different pyrethroids on *L. intermedia*, the predatory behavior of the tropical house gecko *Hemidactylus mabouia* on *L. intermedia* under laboratory conditions, the efficacy of vacuum cleaners in the control of *L. intermedia*, the effects of heated airflow on *L. intermedia* and the chemical signals involved in the attraction and courtship behavior of *L. intermedia*.

MATERIALS AND METHODS

Field and Laboratory evaluation of the effect of pyrethroids on *L. intermedia*.

L. intermedia individuals were collected in homes in the urban area of Curitiba, State of Paraná, Brazil, during 2005 - 2007. The spiders were manually captured by a specialized team from the Centro de Pesquisa e Produção de Imunobiológicos, Instituto de Saúde do Paraná, SESA, and individually placed in 100 ml plastic containers. Larvae of *Tenebrio molitor* (Coleoptera) were supplied one week prior to the bioassays as food.

Except for deltamethrin (K-Othrine® CE 25), the evaluated insecticides were selected from a list of registered products from ANVISA, a Brazilian government agency, which included the indication for spider control. The products and Product label recommendations (PLR) were microencapsulated (ME) lambda-cyhalothrin (Demand® 2.5 CS; PLR = 0.023 mg/kg), lambda-cyhalothrin (Icon® five CE; PLR = 0.0075 mg/kg), cypermethrin (Cymperator® 25 CE; PLR = 0.006 mg/kg) and deltamethrin (K–Othrine® CE 25; PLR for insects = 0.006 mg/kg). Although not indicated for spider control, K-Othrine® was also evaluated because it is freely sold and commonly used in the city of Curitiba. Products were acquired from local retail stores.

Laboratory bioassays were based on Robertson and Preisler (1992) and on the World Health Organization guidelines for insect vector resistance monitoring (WHO, 1981; WHO, 1992). Qualitative filter paper sheets of 12.5 cm diameter (nominal weight 80 gm 2, 205 µm thickness and 0.5 % of ash) were individually impregnated with 2 ml of the given insecticide aqueous solutions at the desired concentrations and allowed to air dry for 24 hours on disposable Petri dishes.

A previous assay was carried out with 12 concentrations of each insecticide, using four repetitions of three spiders per concentration, plus the respective controls (spiders placed on filter paper with no insecticide added). Due to possible intraspecific predation, the spiders were individualized in 12 cm diameter transparent plastic containers with the paper sheet on the bottom. A 2.5 cm diameter opening was made in the top of the containers to allow the introduction of the spiders. Mortality was assessed at 2, 4, 12, and 24 h after the contact of the spiders with the products, and seven concentrations were finally established for each product as causing between 1 and 99% mortality. Bioassays to determine the median lethal concentrations (LC50) were conducted in the same way, with the mortality rate being assessed 24 h after introducing the spiders into the containers. Spiders were considered dead when they no longer reacted to being touched with a rubber-tipped shaft. Knock-down was evidenced by the occurrence of spasmodic leg motions when touched.

For the field assays, two adjacent and uninhabited houses located at the Centro de Pesquisa e Produção de Imunobiológicos in Piraquara city, State of Paraná, were used. The houses have similar constructed area and a shared attic. They were free from insecticide treatments, contained conventional furniture and were cleaned weekly by physical methods only, without use of any sanitary product, until the week prior to application of the insecticide. Most debris were removed from the attic months before the experiment, as well as carrying out a general vacuuming. The experimental house had all gaps and crevices found in its structure, as well as the spaces behind door frames, cracks, etc., sealed with grout, whereas the control house was left in its original condition. The best evaluated insecticide in the laboratory assay was used for the experimental house treatment. A pest control company was asked to carry out the application, supervised by the research team. The pesticide application was carried out at the recommended label dose, using a backpack sprayer (maximum working pressure 6 kgf/cm2, nozzle - JD12P). The product was sprayed to a height of 50 cm above the ground on the internal and external walls, and out to 40 cm from room's walls corners, all the way up to the ceiling. A sprayed area of 40 cm was also done around door's thresholds, windows and framed pictures. In the attic, the entire upper surface of the ceiling, as well as the rafters, was sprayed. The total area sprayed and the amount of pesticide used were estimated to check whether the application corresponded to the recommended volume per square meter. No further cleaning activity was carried out in the houses and inspections for spiders were carried out 24 h, 48 h, 7 days, 14 days, 30 days and 60 days after the product application. Only dead spiders were collected; live individuals found were not removed from the location. For each spider it was recorded its sex or developmental stage as male, female, young (up to the third stage) or sub-adult (between the third stage and adult). Dead individuals were collected using tweezers, and no cleaning activity was carried out in the house during the two months experiment, to avoid removal of dead individuals during the interval between inspections.

The total area sprayed with ME-lambda-cyhalothrin in the experimental house was 560 m2, and the amount of pesticide solution applied was 17 liter, resulting in a estimated treatment of 22.8 mg (AI)/m2, which was below the product label recommendation for spider control (30 mg [AI]/m2) but close to the treatment recommended for insects (20 mg [AI]/m2). Dead and alive spiders were found up to the final inspection 60 days after treatment. Spiders found dead in the houses displayed body posture similar to that of spiders exposed to pyrethroids in the laboratory.

Data Analysis

GW-Basic software was used for Probit analysis (Finney, 1971), to calculate the LC50, the X2 test and the best performing pesticide.

RESULTS AND DISCUSSION

Laboratory and field evaluation of the effect of pyrethroids on *L. intermedia*.

In this work we evaluated the effect of four different products based on pyrethroids: microencapsulated (ME) lambda-cyhalothrin (Demand® 2.5 CS), lambda-cyhalothrin (Icon® 5 CE), cypermethrin (Cymperator® 25 CE) and deltamethrin (K–Othrine® CE 25). The use of filter paper impregnated with pesticide, instead of the common evaluation of the direct application on the back of the spiders (e.g. Wingo, 1964, Hite et al., 1966, Gladney and Dawkins, 1972), permits a more realistic evaluation, since it represents a better approach to the residual treatments commonly used for control operations, due to the preference displayed by these spiders for cracks, crevices and dark locations that are seldom disturbed (Fischer and Vasconcellos-Neto, 2005).

In laboratory tests, the most active pesticides in descending order were: microencapsulated lambda-cyhalothrin (LC50=0.023% v/v), non-microencapsulated lambda-cyhalothrin (LC50=0.047 % v/v), deltamethrin (LC50 = 0.26% v/v), cypermethrin (LC50=1.38% v/v) (Navarro-Silva et al., 2010). Microencapsulated lambda-cyhalothrin was chosen for application in two contiguous houses. The mean volume applied was 22.8 (AI)/m2. Dead spiders were found during all the inspections up to 60 days after the initial application. In total, 297 dead spiders were collected; 65.7% in the attic shared by the two homes, 10.8% inside the house that had most cracks and crevices sealed and 23.6% in the control house.

Vacuum Cleaners For The Integrated Control of *L. intermedia*.

Vetter et al. (2008) stated that "vacuuming spiders can be an effective control technique because their soft bodies usually do not survive this process"; however, those authors did not cite other experiments. The present study tested the efficacy of one type of vacuum cleaner (for professional and domestic use) in the control of *L. intermedia* populations. Vacuuming using standard accessories or a paper tube resulted in the death of all female (n=60), male (n=60), young (n=60) and just-hatched (n=60) *L. intermedia*, and all egg sacs (n=5) were destroyed. The removal of the plastic plate present at the bottom of the vacuuming tube inside the machine allowed some spiders to survive the vacuuming process. When kept inside a vacuum bag full of dust and debris, adult females (n=10) survived for 10 days; however, significant mortality was observed among male (n=10) and young individuals (n=10). Vacuum cleaners, such as the one used in the present investigation, are promising tools for integrated management of *L. intermedia* and other spiders in domestic environments (Ramires et al., 2007).

Hemidactylus mabouia* Predation on *L. intermedia.

Hemidactylus mabouia (Gekkonidae) lizards are synanthropic predators of arthropods. In this work, we evaluated the predatory behavior of the tropical house gecko *H. mabouia* on *L. intermedia* under laboratory conditions. Twelve geckos were observed, and all of them fed on brown spiders (n=123 observations). The attack consisted of a fast run followed by one bite on the spider's abdomen or legs. The geckos did not attack *L. intermedia* anterior body parts, probably due to the fangs present in this region. Two *Hemidactylus* individuals were killed by *L. intermedia* bites: during a predatory encounter, and by an induced bite on a restrained lizard. The observations summarized in this study show that *H. mabouia* could be used in the biological control of *Loxosceles* populations in human dwellings (Ramires and Fraguas, 2004). However, additional field studies are necessary to quantify the impact of *H. mabouia* predation on urban populations of *L. intermedia* and other species of the same genus.

Effect of Moderately Heated Airflow on *L. intermedia*.

Heat has been used to suppress pests since the beginnings of pest control. However, indoor application of heat as a pest control method is still rare nowadays, due to the high costs involved on the heating of houses or rooms by pest control companies as well as the risks to homeowners when manipulating high temperature or microwave equipment. In this work we showed that heated air blown at moderately high temperatures (from 60°C) and low speeds (ca. 5.8 m/s), causes very fast (in the order of seconds or less) knockdown and mortality on medically-important brown spiders, *L. intermedia*. We demonstrated that the heated airflow affects the structure of *L. intermedia* muscles, causing irreversible locomotory difficulties and death. Morphological analysis with light microscopy of spiders exposed to heated airflow did not show any obvious difference between experimental and control groups. However, ultrastructural studies with transmission electron microscopy showed clear evidence of damage to the muscular tissue.

Chemical signals involved in the attraction and courtship behavior of *L. Intermedia*

In this work we identified the compounds present in the cuticle of the brown spider *L. intermedia* as well the compounds that were released by these spiders when placed in a glass aeration chamber. Male and female produce the same cuticular compounds varying only in their ratio. We identified two female-produced compounds either in the extracts obtained through the washing of the spider bodies with solvent, as well in the extracts prepared by the aeration of the spiders.

Laboratory bioassays revealed that the cuticular compounds are likely to be involved in the species recognition while the female-produced compounds are involved in long range male attraction as well in short range sex recognition.

CONCLUSIONS

This study of the different approaches that have been adopted to control the *L. intermedia* population has led to an accumulation of strategic knowledge. It is hoped that the use of this knowledge by the public authorities can bring about a sharp reduction of the brown spider population, where there is a high rate of envenomation. As well as greaty improving the quality of life of the people, this will be beneficial to the government by reducing the costs incurred in treating the victims of brown spider attacks.

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