# Field Evaluation of a New Strategy to Control *Lutzomyia longipalpis*, Based on Simultaneous Application of an Adulticide–Larvicide Mixture

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# FIELD EVALUATION OF A NEW STRATEGY TO CONTROL *LUTZOMYIA LONGIPALPIS*, BASED ON SIMULTANEOUS APPLICATION OF AN ADULTICIDE–LARVICIDE MIXTURE

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ABSTRACT. *Leishmania infantum* (syn. *chagasi*) is the etiologic agent of visceral leishmaniasis in Argentina, and the phlebotomine fly *Lutzomyia longipalpis* is its main vector. The objective of this study was to evaluate the effectiveness of Dragon Max®, an emulsifiable concentrate formulation containing the pyrethroid permethrin and the larvicide pyriproxyfen, for *Lu. longipalpis* control under field conditions. The work was conducted in the city of Posadas (Misiones province, Argentina). Comparisons were performed between treated and untreated peridomiciles with poultry, which met previously determined criteria for favoring the presence of *Lu. longipalpis*. Henhouses and their surrounding area were treated, with the formulation (100 mg of permethrin and 2 mg/m$^2$ of pyriproxyfen) applied using a hand pump sprayer. Untreated henhouses were used as controls. Phlebotomine abundance was monitored before treatment and then weekly, using Centers for Disease Control and Prevention light traps. *Lutzomyia longipalpis* was the only phlebotomine species captured. A male/female ratio of 2.5 was observed. The more chickens there were in the henhouses, the greater the number of phlebotomines captured. The treatment resulted in a significant decrease in the number of individuals, which persisted for at least 2 wk. This encouraging result provides a baseline for further studies evaluating the possibility of using Dragon Max as a tool for *Lu. longipalpis* control.

**KEY WORDS** *Lutzomyia longipalpis*, permethrin, pyriproxyfen, visceral leishmaniasis

## INTRODUCTION

Leishmaniasis is a disease that affects 12 million people in 90 countries around the world (WHO 2012). There are more than 1 million new cases and 25,000 deaths reported annually. This neglected disease is caused by the protozoan *Leishmania* parasites, which are transmitted to humans by female phlebotomine sandflies. In the cycle of disease transmission some 70 animal species, including humans, act as reservoirs of the parasite (WHO, 2015). Among *Leishmania* species, phenotypic diversity within and among species leads to the existence of 3 forms of the disease: cutaneous, mucocutaneous, and visceral (WHO 2015). Cutaneous leishmaniasis is the most common form of this disease, while visceral leishmaniasis is the most serious (it affects internal organs and is fatal if left untreated).

The first indigenous case of visceral leishmaniasis in Argentina was detected in 2006 (Salomón et al. 2008). Most cases reported since then have occurred in the province of Misiones, with a few being reported in the provinces of Corrientes, Santiago del Estero, and Salta. In these cases, the vector was *Lutzomyia longipalpis* Lutz & Neiva (Salomón et al. 2012).

Hens and chickens are refractory to *Leishmania infantum* Nicolle infection, so they do not act as reservoirs; however, they are a food source that attracts adult phlebotomines (Alexander et al. 2002, Fernández et al. 2010). The presence of henhouses can amplify phlebotomine populations and provide an ideal environment for sandfly breeding (Salomón et al. 2015), threatening human health when henhouses are close to human peridomiciles.

Since there are neither vaccines nor chemoprophylaxis for leishmaniasis, the main strategy available for reducing its incidence is vector control. Different methods have been tested: residual sprays, spatial sprays, use of insecticide-impregnated netting or clothing, application of topical repellents, sticky traps, treatment of dogs with ectoparasiticides, and environmental management (Alexander and Maroli 2003, Claborn 2010). Sugar baits containing the insecticide dinotefuran were recently evaluated in Morocco: either applied to vegetation or in bait stations, they significantly reduced densities of *Phlebotomus papatasi* Scopoli and *P. sergenti* Parrot over 5 wk (Qualls et al. 2015).

In urban areas, the most common and most effective technique of vector control is residual treatment of the walls of human dwellings and/or animal shelters. Formulations of residual dichlorodiphenyltrichloroethane (DDT) and malathion are effective in controlling adult phlebotomines (Kishore

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et al. 2006); DDT is banned in many countries. Positive results have also been obtained with pyrethroids (Kelly et al. 1997, Feliciangeli et al. 2003). In general, no treatments specifically target phlebotomine larvae, because their breeding sites are difficult to locate (Casanova et al. 2013).

A formulation containing permethrin and pyriproxyfen had previously been evaluated by our group for controlling *Aedes aegypti* (L.). Ultra-low volume application of this formulation in the town of Wanda (province of Misiones, Argentina) was successful and showed good adulticidal and larvicidal effects on adult mosquitoes and aquatic larvae (Lucia et al. 2009). We considered that previous evidence could support the idea of testing this formulation with adulticidal and larvicidal effect in the control of *Lu longipalpis*.

Pyriproxyfen is a mimic of insect juvenile hormone (Pener and Dhadialla 2012). Its efficacy against the phlebotomine *Phlebotomus perniciosus* Newstead has been investigated in dogs, using an ectoparasiticide composed of pyriproxyfen, dinotefuran, and permethrin (Liénard et al. 2013). Recent results from our laboratory showed a high larvicidal effect of pyriproxyfen in 2nd-stage larvae of *Lu. longipalpis* exposed to filter papers impregnated with 5 or 50 mg/m$^2$ (Pettersen and Juan, unpublished data). Permethrin is a pyrethroid whose effectiveness against adult *Lu. longipalpis* has previously been evaluated in laboratory bioassays (Juan et al. 2014).

The aim of this study was to evaluate the effectiveness of a new field control strategy against phlebotomines, which involves the application to the peridomicile of an insecticide formulation containing pyriproxyfen and permethrin. The work was conducted in the city of Posadas (Misiones, Argentina), where domestic populations of *Lu. longipalpis* and human cases of visceral leishmaniasis have been reported (Fernández et al. 2010).

## MATERIALS AND METHODS

### Study site

Field evaluations were conducted in the city of Posadas, located in northeastern Argentina, near the border with Paraguay (27°22′00″S, 55°53′49″W). Posadas is the capital of Misiones province and has just over 277,500 inhabitants.

### Insecticide formulation

The effectiveness of the commercial insecticide Dragon Max® (Chemotecnica, Spegazzini, Argentina) was evaluated. This product contains 2 active ingredients, the pyrethroid permethrin (10%) and the insect growth regulator pyriproxyfen (2%).

### Trapping and identification of phlebotomines

Trapping was performed using small, battery-powered Centers for Disease Control and Prevention (CDC) light traps (BioQuip, Rancho Dominguez, CA), whose effectiveness in catching phlebotomines has already been demonstrated (Garlapati et al. 2012). Traps were placed in roofed henhouses (1 per henhouse), 1.5 m above the ground, between 6:00 p.m. and 8:00 a.m. for 2 consecutive nights. This monitoring method was previously used in epidemiological studies performed in the city of Posadas, where the study area is located (Fernández et al. 2010, Santini et al. 2010).

The captured phlebotomines were preserved dry. To facilitate taxonomic identification and sexing, phlebotomines body's hairs were removed with 1% lactophenol. Taxonomic identification was according to the key by Galati (1995).

### Choice of homes

To establish appropriate application sites for Dragon Max, "worst scenarios" were identified, being places that have a high probability of harboring phlebotomines. These areas of Posadas City were previously described with high trees, organic debris, bush abundance around the houses, and a clear association with the presence of chickens (Fernández et al. 2010, Santini et al. 2010). Using this criterion, 20 households with henhouses were identified and monitored for the presence of phlebotomines on October 28 and 29, 2014.

Based on the trapping results, 7 households positive for phlebotomines were chosen for further testing. These households were separated from each other by at least 500 m. Before starting the tests, the householders were informed of the objective of the work and what treatments would be applied in each case. Informed consent was obtained from all the householders.

### Study design and timing

From the 7 households selected after trapping, 4 henhouses and surrounded areas were chosen at random to be treated with Dragon Max, while the remaining 3 received no treatment (negative controls). Treatments were performed on November 5, 2014 in the morning. Temperature and humidity during the experimental period were recorded using a thermohygrometer (model 30.5005; TFA, Wertheim, Germany).

Dragon Max was applied using a 10 liters hand pump sprayer (model 1165-4; Guarany, Sao Paulo, Brazil). Dragon Max 1.2% in water was applied with a mean flow of 1,135 ml/min to obtain a dose of 100 mg of permethrin and 20 mg/m$^2$ of pyriproxyfen. Spraying covered the floor and walls of henhouses, and the ground, plants, and trunks of trees outside, covering a height of 2 m and a radius of 3 m outside each henhouse. These distances were chosen because phlebotomines tend to stay near their food sources and rarely fly at a height of over 1.5 m (Santini et al. 2010).

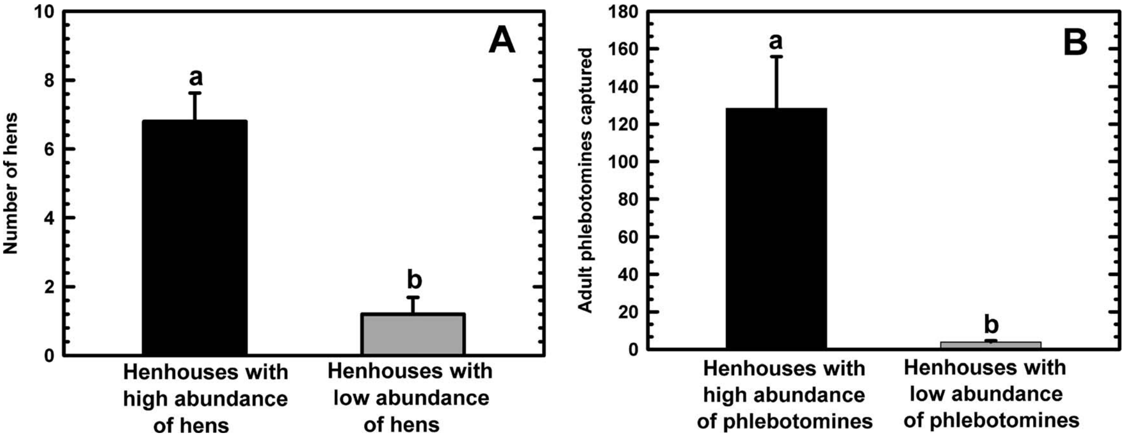


Fig. 1. (A) Abundance of hens in henhouses, and (B) mean number of adult *Lutzomyia longipalpis* trapped. Vertical lines are SE. Bars marked with different letters are significantly different ($P < 0.05$).

To evaluate the results of treatments, a CDC light trap was placed in each henhouse as described above (considering that 7 henhouses were included in this experiment, a total of 7 traps per night were used).

During the treatment period, the minimum and maximum average temperatures were 19.1°C and 27.8°C (first week), and 23.9°C and 29.8°C (second week). The minimum and maximum average relative humidity levels were 39.6% and 69.5% (first week), and 57.7% and 63.4% (second week).

### Statistical analysis

The sex ratio value was calculated using the quotient M/F, where M is the number of males and F the number of females. The results failed to meet the assumption of normality required for analysis of variance (ANOVA); therefore, they were analyzed using the Mann–Whitney rank sum test. The numbers of phlebotomines trapped before and after the treatment were analyzed using repeated-measures ANOVA. In all cases, differences were considered significant when $P < 0.05$.

## RESULTS

### Phlebotomine identification, abundance, and sex ratio

In the monitoring carried out prior to insecticide treatment, 2,646 phlebotomines were captured. *Lutzomyia longipalpis* was the only species of phlebotomine captured. The sample consisted of 1,899 males and 747 females, giving a sex ratio (M/F) of 2.5. The number of phlebotomines captured was directly proportional to the number of hens present in the henhouses (Fig. 1). In henhouses with 3 or fewer hens, an average of 7.8 ± 1.8 sand flies were captured, while in henhouses with 4–12 hens, an average of 128.4 ± 27.5 individuals were captured. The difference between the 2 averages was highly significant ($P < 0.001$).

### Efficacy of treatment with Dragon Max

Seven henhouses with high levels of sandfly infestation (henhouses containing 4 or more hens) were chosen for the insecticide test (i.e., 4 treated and 3 controls). Dragon Max treatment resulted in a drastic decrease in the number of phlebotomines captured (Fig. 2). Before treatment there were no significant differences in the mean number of phlebotomines captured between treated or control henhouses ($P = 0.248$). After treatment, the mean number of phlebotomines captured in the treated henhouses was significantly lower compared with controls ($P = 0.001$).

Results from control and treated henhouses shown in Fig. 2 were also analyzed separately. In the henhouses belonging to the control group, the number of phlebotomines increased slightly during the 2 wk following treatment, but compared with the pretreatment trapping, differences in the number of phlebotomines captured were not significant ($P = 0.579$). In peridomiciles treated with Dragon Max, the number of phlebotomines decreased significantly from 344 ± 135 (before treatment) to 1.4 ± 0.68 (1 week after treatment) ($P = 0.017$). Two weeks after treatment, the number of phlebotomines remained very low (25 ± 17.9) and was not significantly different from the value of the first week ($P = 0.05$).

The original design of this study was to monitor phlebotomine abundances until those in the treated henhouses began to increase, to determine the length of time over which Dragon Max treatment worked. However, because of heavy rain in the weeks subsequent to the final observations, it was not possible to continue monitoring.

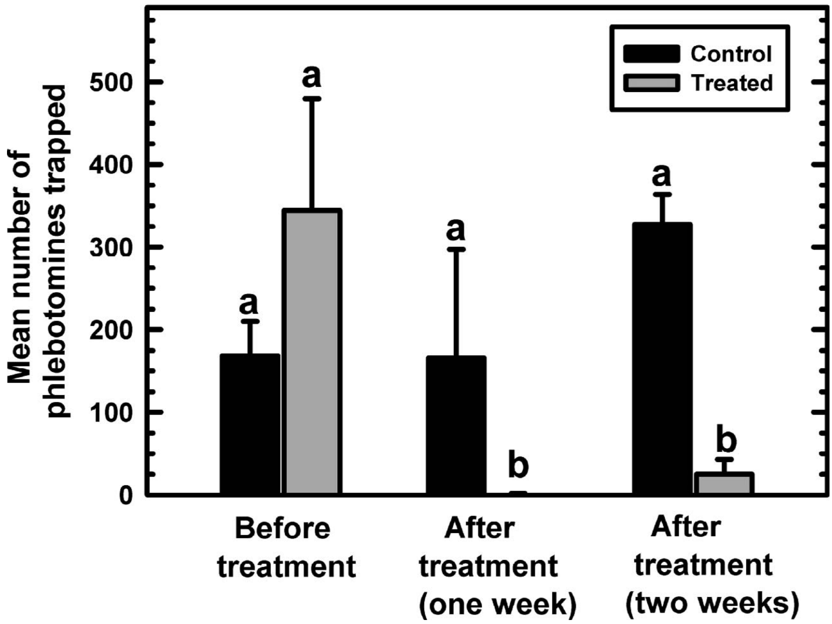


Fig. 2. Effect of Dragon Max® application for controlling *Lutzomyia longipalpis* in henhouses. Each bar is the mean of the number of insects trapped in 3 (control) or 4 (treated) henhouses. Vertical lines are SE. In each group, bars marked with different letters are significantly different ($P < 0.05$).

## DISCUSSION

This paper presents the first evaluation of an insecticide containing both a larvicide and an adulticide compound, against *Lu. longipalpis* in the peridomicile.

In this work, *Lu. longipalpis* was the only species of phlebotomine captured in henhouses, with numbers being proportional to the number of hens present (Fig. 1). In several previous studies on phlebotomines in and around homes, most individuals were captured in the peridomicile (Michalsky et al. 2009, Costa et al. 2013). Males and females of *Lu. longipalpis* are strongly attracted to hens (Alexander et al. 2002), meaning large numbers of individuals are captured in henhouses.

Hens are refractory to *Leishmania* infection. The exact reason is unknown, but it has been speculated that the relatively high body temperature of birds (41°C) may be incompatible with the establishment of a parasite population in their blood (Alexander et al. 2002). Thus, chickens do not act as reservoirs, but as a food source that draws phlebotomines near to people, threatening the human health (Alexander et al. 2002).

The spraying of henhouses and surrounding areas with Dragon Max was effective at reducing the abundance of *Lu. longipalpis* adults. The application of pyrethroids has previously been reported as an effective method of residual control for phlebotomines. For example, applying lambda-cyhalothrin emulsifiable concentrate (EC) indoors in houses in the Island of Margarita, Venezuela, reduced *Lu. longipalpis* abundance to very low levels (Feliciangeli et al. 2003). In a Brazilian village in Pará State, peridomiciliated *Lu. longipalpis* were well controlled by spraying lambda-cyhalothrin (microencapsulated formulation, 20 mg/m$^2$) on animal pens (Kelly et al. 1997). In a similar study, applying deltamethrin EC (25 mg/m$^2$) to the external walls of houses, peridomestic dwellings, and tree trunks reduced the abundance of *Lu. longipalpis* 7 days after spraying, but the abundance had increased 14 days later (Santini et al. 2010).

Our study provides the first evaluation of simultaneous application of a larvicide and adulticide against *Lu. longipalpis*. A similar formulation containing permethrin and pyriproxyfen had previously been evaluated by our group for controlling *Aedes aegypti* (L.). Ultra-low volume application of this formulation in the town of Wanda (province of Misiones, Argentina) was successful and showed good adulticidal and larvicidal effects on adult mosquitoes and aquatic larvae (Lucia et al. 2009).

The use of pyriproxyfen aimed to control *Lu. longipalpis* larvae, complementing the effect of permethrin on adults. The only way to identify the presence and abundance of larvae in breeding sites is by using soil emergence traps, which detect adults. One of the best known emergence traps was designed by Casanova. Larval presence is inferred from the presence of adult phlebotomines on an adhesive paper (Casanova 2001, Casanova et al. 2013). The Casanova trap needs a trapping period varying between 14 and 29 days to collect adult sand flies from larvae present in the soil (Casanova 2001). Considering a mean time of 40 days for development of immature stages of *Lu. longipalpis* under laboratory conditions (Casanova et al. 2013), the emergence traps needs a long trapping period. This requirement makes the use of emergence traps to monitor larval populations in studies of insecticide efficacy impossible.

Given the known larvicidal effect of pyriproxyfen, it is hypothesized that its application on the ground surrounding the henhouses could have a lethal effect on *Lu. longipalpis* larvae, decreasing the adult population. Our group demonstrated in laboratory bioassays that development of *Lu. longipalpis* is interrupted in the pupal stage after exposure to 5 or 50 mg/m$^2$ of pyriproxyfen (Pettersen and Juan, unpublished data).

Results of this field study suggest that spraying Dragon Max is an effective way of controlling phlebotomines in peridomestic areas, where henhouses can support populations of adult *Lu. longipalpis*, and the soil surrounding henhouses may be a breeding site for phlebotomines.

This work is the first study examining the possibilities of residual treatment with a formulation containing a larvicide and an adulticide for controlling *Lu. longipalpis* in henhouses and surrounding areas. Because of the close relationship between hens and phlebotomines, the efficacy of treatments focused on these areas of housing is promising. Further studies are needed to evaluate the persistence of the residual effect of Dragon Max and to establish the real role of pyriproxyfen in controlling the larvae of *Lu. longipalpis*.

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