Soil presence reduces the control effectiveness of a slow release formulation of pyriproxyfen on *Ae. aegypti* (Diptera: Culicidae) larvae

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Abstract

Objective: To assess the influence of soil on the effectiveness of two new slow-release formulations (floating and nonfloating) of pyriproxyfen coextruded with low-density polyethylene.

Methods: Two slow-release devices were developed using low-density polyethylene, pyriproxyfen as larvicide, and calcium carbonate as filler. A factorial design was used to evaluate the effect of soil presence on the performance of each device. Weekly bioassays were performed.

Results: Soil presence affected treatment effectiveness, but this effect was associated with device type. The tablets were effective for nearly 3 months.

Conclusion: Treatment effectiveness could be reduced because of the loss of pyriproxyfen by several physico-chemical processes such as adsorption into the soil.

Keywords: insect growth regulator, larvicide, vector control.

Introduction

The insect growth regulator (IGR) pyriproxyfen is a very effective tool for the control of several vectors affecting human health, such as *Aedes (Stegomyia) aegypti* (Linnaeus; Mulla 1995). Treatment with pyriproxyfen against *Ae. aegypti* has proved to be more effective than other larvicides under laboratory and semi-field conditions (e.g. Seccacini *et al*. 2008, Lau *et al*. 2015), and pyriproxyfen has become more

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important for mosquito control in the last decades in part because the insects' susceptibility to several larvicides such as temephos (PAHO 1997) is waning.

Pyriproxyfen effectiveness is due to its high specificity and low impact on non target organisms (Mulla 1995) and depends on formulation type. Slow-release formulations, such as granules or sand, are more effective than emulsifiable concentrate or wettable powder (Kawada *et al*. 1988, Seccacini *et al.* 2008). The material used for the development of the formulation is also an important factor. Plastics, particularly polyolefins, are based on organic chemicals, which offer a huge range of physical properties to manufacturers (Tolinski 2015). The optimal material for each application is identified by developing a formulation according to the desired performance. Polymers are easily modified with the help of different fillers and additives depending on what is required in the process and for the final product application.

Pyriproxyfen effectiveness is also due to its persistence. Among seven methods for the control of *Ae. aegypti*, including four IGRs (methoprene, diflubenzuron, cyromzine, novaluron) and temephos (Lau *et al*. 2015), pyriproxyfen had the highest persistence, lasting even after water replacement with untreated water (Yapabandara & Curtis 2002, Sihuincha *et al*. 2005, Vythilingam *et al*. 2005, Seng *et al*. 2006). Pupal mortality was registered even when pyriproxyfen was no longer detected dissolved in the water (Schaefer *et al*. 1988). Pyriproxyfen has low water solubility (Sullivan & Goh 2008) and thus tends to be adsorbed onto soil particles, suspended solids and sediment (Sullivan & Goh 2008). Dissolved pyriproxyfen concentration in water is lower at high levels of organic pollutants (Schaefer *et al*. 1988).

In this work, we evaluated the effect of soil on the control of *Ae. aegypti* larvae under semi-field conditions with two new slow-release devices formulated with pyriproxyfen in a thermoplastic polymeric matrix.

Materials and Methods

Design and development of slow release active plastic devices

Two formulations were designed: a floating slow-release device (FSRD) and a non-floating slow-release device (NFSRD). For the formulation of both low density polyethylene (LDPE; Braskem PB 208) was used. The larvicide used was pyriproxyfen (2- (1-methyl-2- (4-phenoxyphenoxy) epoxy) pyridine; 0.5% w/w). Pyriproxyfen technical grade 97% was kindly supplied by Chemotecnica S.A, Argentina.

To adjust the density of the NFSRD, calcium carbonate ($CaCO_3$; Arcolor 4453 concentrate (LDPE $CaCO_3$ 60%)) was used. Calcium Carbonate ($CaCO_3$) is one of the most widely used mineral fillers in the plastics industry. The addition of calcium carbonate to plastics increases performance, improves processing and improves sustainability of the finished part. Carbon black LDPE concentrate was used to differentiate the NFSRD from the FSRD during the trial.

The active formulations (LDPE and 0.5% pyriproxyfen (FSRD); LDPE, filler masterbatch and 0.5% pyriproxyfen (NFSRD)) were processed in a single screw extruder (Killion KL-100 L / D 25; optimized hopper-head temperature profile was 145-165-165-160 °C). The material was pelletized and active slow-release devices were obtained by injection molding (ENGEL ES 75 ST; optimized hopper-head temperature profile was 160-165-170-180-195 °C).

Experimental design and bioassay

The experiments were carried out in cylindrical glass vases (11.5 cm diameter x 22 cm high; capacity: 2.3 l). A factorial design was used: four treatments were defined from the combination of soil (presence or absence) and type of slow release plastic device (FSRD or NFSRD). Soil presence consisted of the addition of 200 cm^3 of commercial soil (Biofertyl SRL, fertilized land: 70% compost-30% humus). Three replicates of each treatment and two controls (with and without soil) were considered, in a total of 14 vases. Two liters of dechlorinated water and 60 mg of the device were added to each vase. The final concentration was expected to be lower than 0.01 ppm of active ingredient. The vases were left outside, under a roof so rain did not affect them, and were covered by a voile top to avoid the influence of external organic matter and intervention of birds and insects.

Once a week 250 ml of water were extracted from each container and placed in individual plastic cups. Water was extracted close to the middle of the vase and soil was not removed during water extraction. The vases were then refilled to 2 l with dechlorinated water. Ten to fifteen late third or early fourth instar *Ae. aegypti* larvae were added to each plastic cup.

Biological material

Immatures belonging to a susceptible CIPEIN strain of *Ae. aegypti* (L.), originating from the Rockefeller strain from Venezuela and kept in the laboratory since 1996, were used for all bioassays. The laboratory colony has been maintained at 25–30 ºC and 80–90% relative humidity under a photoperiod of 12:12 h. This colony is maintained free of exposure to pathogens, insecticides, or repellents (Lucia *et al*. 2007).

The number of emerged adults was registered once a week until the emergence/death of all individuals. Weekly bioassay continued until two consecutive weeks without emergence inhibition in all treatments had been reached.

Statistical analysis

We used generalized linear mixed models (GLMM) for data analysis. GLMM allow for non-normal errors and account for correlation among experimental units through the inclusion of a random term (Crawley

2007). The response variable was defined as adult emergency success per vase, and was estimated as the proportion *a/b*, with *a* being the number of emerged adults in treated vases and *b* the number of emerged adults in the control. When there were fewer emerged adults in the control (with control mortality less than 20%) than in the treatment, the proportion *a/b* was considered as one. As the response variable is a proportion, binomial error and the logit link function were used for the models (Crawley 2007). A maximal model was constructed with soil, formulation and their two-way interaction as fixed factors, and time and vase as random factors. Random effects allow considering for the correlation induced by each vase and each week. Different random effects structures were tested: time and vase, vase alone, time alone, time given vase. Colinearity among explanatory variables was tested with the variance inflation factors (VIF, Car package). A VIF value below five was considered to indicate absence of multicolinearity (Zuur *et al*. 2010). A backwards stepwise procedure was performed in which fixed and random terms were removed one by one from the maximal model to identify and keep those that explained more variance and gave the better goodness of fit. The goodness of fit was evaluated in terms of the Akaike's information criterion (AIC: Akaike, 1974); the model that yielded the lowest AIC was selected from all possible models (Zuur *et al*. 2009). Models with $\Delta AIC \leq 2$ were considered equivalent. Graphical verification of the selected model was performed. The final model parameters were bootstrapped (1000 replications) to sort out very influential observations and outlayers by verifying that their 95 % confidence interval did not include the zero value. The explanatory power of the model was estimated by the proportion of the total variance explained by the fixed and random terms. Analyses were performed using the open-source R 3.1.2 software with MuMIn, car, boot and lme4 packages (R Core Team 2015).

Results

The whole experiment took 18 weeks, and 204 observations were used in the model. The mean proportions of emergence for the controls were 0.77 with soil and 0.80 without soil (standard deviation of 0.25 in both cases).Week 5 was excluded from the analysis because of a problem with mosquito breeding in that week. The fixed effects retained in the final GLMM model are shown in Table 1. Of the four random effects structures tested, vase (12 levels, 0.298 SD) and time (17 levels, 1.277 SD) were kept in the best model. This final model explained 59.6% of the total variance, with the fixed effects accounting for 38.2%. The presence of an interaction shows that the effects of both fixed factors are not additive, so each combination should be separately analyzed. In the absence of soil, NFSRD were the effective in inhibiting adult emergence. In contrast, in the presence of soil, FSRD were more effective. Without soil, estimated emergence probability was 6% higher with the FSRD (Table 1). With soil, estimated emergence probability was 20% lower with the FSRD. With FSRD, estimated emergence probability increased 45% because of soil presence. With NFSRD, estimated emergence probability increased 71% because of soil presence.

The general pattern in adult emergence decayed towards week 4 and peaked in week 12 (Figure 1). The increase was sudden in the absence of soil and oscillatory with soil. After week 12 a plateau was seen. When soil was present, more adult emergence was registered overall. When soil was absent, less than 20% of larvae reached adult stage throughout almost the entire experiment.

Discussion

Two slow-release formulations were developed in the present study. There was a clear detrimental effect of soil on the efficiency of both devices. In previous studies, dissolved pyriproxyfen remained below 2 ppb after 7 days using an ovitrap also made of low-density polyethylene (Harburguer *et al*. 2016). This small amount of pyriproxyfen could be rapidly reduced due to pyriproxyfen adsorption by soil particles. Pyriproxyfen has low water solubility (0.367 mg/l) and high organic carbon partition (log KOC=5.6; Sullivan & Goh 2008, Fenoll *et al*. 2011). A high KOC indicates a tendency to be adsorbed and, once in the soil, to have low mobility. Adsorbed pyriproxyfen persistence in soil is reduced by rapid degradation via biological catalysis under static conditions (Schaefer *et al*. 1988, Sullivan & Goh 2008, Fenoll *et al*. 2011, Liu *et al*. 2017). With the NFSRD, the highest concentration was closer to the soil surface. Thus, the adsorption of pyriproxyfen would have more influence over the NFSRD effectiveness. To test this hypothesis, it would be necessary to measure pyriproxyfen concentration in the soil every week.

Without soil, pyriproxyfen effect on adult emergency was lower with the NFSRD. Pyriproxyfen presence in the water could be affected by several processes such as volatilization and photolysis (Sullivan & Goh 2008). For example, pyriproxyfen has moderate vapor pressure ($1x10^{-7}$ mm/Hg), and tends to slightly volatilize to the atmosphere from water surface. The loss of pyriproxyfen due to these processes could be determined by the physical properties of the devices. However, the loss of pyriproxyfen due to processes such as volatilization is very small, so it can be considered insignificant for mosquito control. Further studies are necessary to evaluate whether these processes can affect the performance of the two devices. Nevertheless, the effect of soil was greater than the effect of the difference in device formulation. Thus it follows that the loss of pyriproxyfen due to adsorption is stronger than the loss due to processes such as volatilization.

There was a change in adult emergence behavior at week 12, which may indicate that the slow-release devices are effective for close to 3 months. This persistence is higher than other formulations, including granular ones (Kawada *et al*. 1988, Schaefer *et al*. 1988, Yapabandara & Curtis2002, Ritchie *et al*.

2013, Mbare *et al*. 2013). An initial delay in inhibition was observed in the three first weeks of our experiments in vases with soil. Kamimura & Arakawa (1991) registered an increase in the adult emergence inhibition at the beginning of the experiment in containers with mud. The slow release behavior of the devices used in our study may need this time to saturate the soil and build up a significant concentration in the water to affect the emergence of adults.

Adsorption of pyriproxyfen was described before as beneficial for treatment effectiveness due to an increase in pyriproxyfen persistence. Pyriproxyfen affected adult emergence even with the active ingredient only present in the organic matter and not dissolved in the water (Schaefer *et al*. 1991). Notwithstanding the high effectiveness of the devices used in the present study for the control of *Ae. aegypti*, soil presence significantly reduced the effect of pyriproxyfen over adult emergence. The slow-release effect could be the reason of the opposite response of our system to the soil presence. In the vases with soil, most of the pyriproxyfen in the water could have been rapidly adsorbed and, once in the soil, been exposed to biodegradation . However, pyriproxyfen remained inside the device and was released at a low rate to the water, in contrast to other studies. So, even though at the beginning the loss of pyriproxyfen due to biodegradation could be sufficient to reduce treatment effectiveness, higher long-term effects could be achieved since pyriproxyfen remained "protected" inside the device. In the field, treatment effectiveness could be higher. Pyriproxyfen effectiveness was associated with container materials (Suman *et al*. 2013). In this study, the effectiveness of pyriproxyfen was analyzed in glass, which does not adsorb or retain pyriproxyfen. In the field plastic containers would be common, and release of pyriproxyfen from the device itself and from the container would be expected.

Pyriproxyfen is an efficient tool for *Ae. aegypti* control (Mulla 1995). Both devices tested in the present study showed advantages for *Ae. aegypti* control. Most important is its longer persistence compared to other devices, which would increase the time between applications and thus reduce treatment costs. The devices also allow the treatment of containers of different sizes. Small pieces of standardized size may be applied proportional to container capacity to achieve desired concentrations. Finally the devices can be floating or submersible according to specific needs.

FSRD floats because it has a lower density than water. FSRD can be used in water reservoirs with a water outlet at the bottom without the risk of obstruction. In contrast, NFSRD has a higher density than water and remains at the bottom of the container, where it will not interfere with accessible containers such as water reservoirs placed on the surface. Both slow-release devices were designed to be used in drinking water tanks: all materials from which they were manufactured complied with the requirements of the legislation for materials in contact with foodstuffs.

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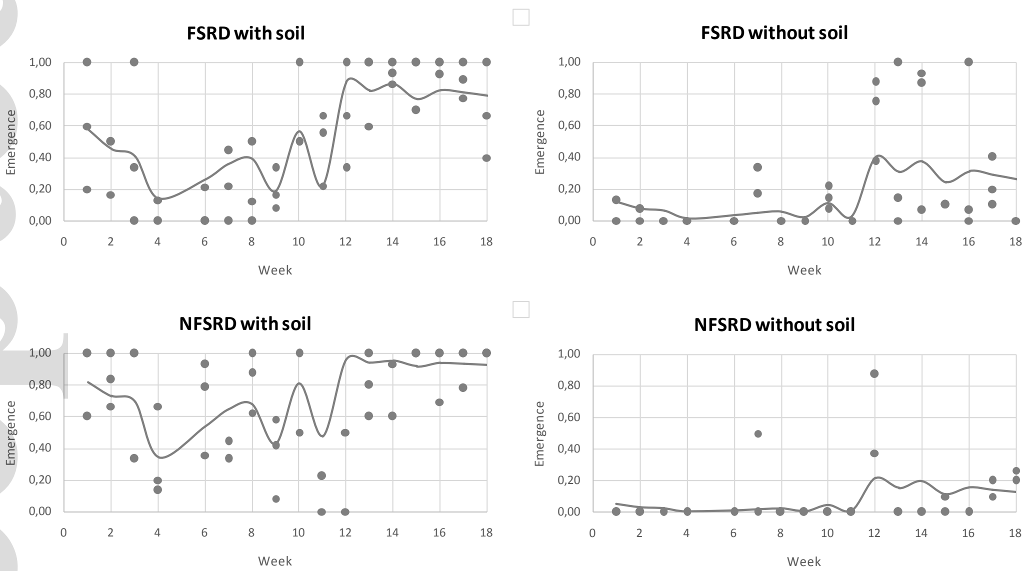
Table 1. Generalized linear model parameters for the proportion of emerged adults per treatment (n=204). Non floating slow release device, NFSRD; floating slow release device, FSRD.

Treatment	Model Parameter (Std. error)	Mean proportion of emergence in respect to control
NFSRD without soil	-3.171 (0.404)	0.04
FSRD without soil	1.017 (0.341)	0.10
NFSRD with soil	4.338 (0.350)	0.75
FSRD and with soil	-1.919 (0.463)	0.55

Figure 1. (A) Proportionof adult emergence (number of emerged adults in treated vases/ number of emerged adults in the control) by week for each treatment. Observed values, symbols; proportions estimated by the model, lines. (B) Proportion of adult emergence (number of emerged adults / number of larvae) by week for each control.

(A)



(B)