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UPDATES IN PARASITOLOGY FROM ARGENTINA (A SANCHEZ AND A TOLOZA, SECTION EDITORS)

A Review of the Use of Pyriproxyfen for Controlling *Aedes aegypti* in Argentina

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Abstract

Purpose of Review *Aedes aegypti* (L.) is the main vector of dengue, yellow fever, Zika, and chikungunya viruses in many parts of the world affecting millions of people worldwide each year. Its control is mostly aimed at the larval stages (removal of breeding sites, larviciding, and community education) to reduce the population of new adults. This review is a compilation of recent studies carried out in Argentina with the insect growth regulator (IGR) pyriproxyfen applied for controlling different life stages of the mosquito *Aedes aegypti*.

Recent Findings Pyriproxyfen (PPF) is an increasingly used hormone analog that prevents juvenile *Aedes* mosquitoes from becoming adults and being incapable of transmitting dengue. It exhibits a high level of activity against mosquito larvae inhibiting adult emergence at very low doses. Additionally, effects on adult mosquito fecundity and fertility after IGR treatments were identified.

Summary IGRs have been reported to be selective against target species, with considerable safety to the environment and nontarget organisms, including mammals. Its high efficacy as a larvicide at very low doses, together with its proven effects on adult reproduction, makes it an excellent candidate for use within an integrated vector management program.

Keywords *Aedes aegypti* · IGR · Pyriproxyfen · Argentina

Introduction

Severe human arboviral diseases can be transmitted by the mosquito *Aedes aegypti* (L.), including dengue, chikungunya, Zika, and yellow fever; hence, it is considered a species of international concern. *Ae. aegypti* is widely distributed in Argentina, living in close association with people, mainly breeding in artificial containers in domestic habitat [1]. The most commonly utilized strategy to reduce *Ae. aegypti* densities is aimed at the larval stages (removal of breeding sites, larviciding, and community education) to reduce the population of new adults. Adult control using spatial sprays with adulticides is recommended when dengue outbreaks occur [2]. Vector control therefore remains a key part of any dengue control program, and the integration of locally accepted and effective methods is needed [3].

Treatment of larval production sites has led to the emergence of insecticide-resistant populations, particularly against the main larvicide used in recent years, the organophosphorus temephos, highlighting the requirement for new larvicides for mosquito control [4–8].

Particularly in Argentina, differences have been registered in the susceptibility levels to temephos in the cities of Clorinda, Puerto Iguazú, and Ledesma [6, 9, 10]. Besides temephos, the WHO recommends the biolarvicide *Bacillus thuringiensis var. israelensis* (*Bti*), spinosad, and insect growth regulators (IGRs) diflubenzuron, novaluron, methoprene, and pyriproxyfen to treat drinking water [11]. IGRs differ widely from the commonly used insecticides, in that they exert their insecticidal effects through their influence on development, metamorphosis, and reproduction of the target insects by disrupting the normal activity of the endocrine system [12]. There are three major groups of IGRs:

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the juvenile hormone analogs, the ecdysone agonists, and the chitin synthesis inhibitors [13, 14].

The direct effects of IGRs on immature stages, such as mortality or adult emergence inhibition (EI), are the subject of several reports [15–17, 18•, 19]. Additionally, after receiving IGR treatments, adult mosquitoes showed changes in adult fecundity (the amount of eggs laid) and fertility (the hatchability or viability of their eggs) [20–23]. However, notably, in *Ae. aegypti*, the effects of their direct usage on adult females and their implications for vector fitness are less taken into account [24, 25].

Use of Pyriproxyfen as Larvicide in Argentina

A particularly effective insecticide against a variety of insects of public health importance is pyriproxyfen, a juvenile hormone (JH) mimic [26–28]. Pyriproxyfen interferes with mosquitoes' hormonal balance and, in some circumstances, strongly inhibits embryogenesis, metamorphosis, and adult growth [29–31]. When natural juvenile hormones or juvenile hormone mimics are applied at the right periods, adult emergence is either reduced or its normal development disrupted [25].

A study carried out in Argentina observed that the mortality of individuals occurs mainly in the pupal phase or during the moult to adult in *Ae. aegypti* [32]. On the other hand, Harburguer [33] showed that the L4 stage is the most susceptible to pyriproxyfen, reaching an EI% value greater than 90% after only 15 min of exposure to the fumes of a fumigant tablet containing 0.2 g/kg of this IGR (Fig. 1). These results are in accordance with the work of Lan and Grier [34] who found that fourth instar larvae (L4) are more sensitive to methoprene (an analog of the juvenile hormone) than L3.

The study by Seccacini et al. [10] undertaken in Argentina developed experimental emulsifiable concentrate formulations of pyriproxyfen and diflubenzuron in the laboratory and obtained EI_{50} values of 0.01 and 0.02 ppb, respectively. They also obtained 100% adult emergence inhibition with 0.1% slow-release experimental sand formulations of pyriproxyfen and diflubenzuron. Moreover, in a simulated field study, the granular sand formulations of pyriproxyfen, diflubenzuron, and temephos remained active for over 4 months. The use of 0.1% or 0.2% of diflubenzuron or pyriproxyfen in sand granules provided highly effective control of the immature stages of *Ae. aegypti* in oviposition sites, giving a good alternative to temephos sand granules in places where mosquito resistance to the latter has been reported [35]. A large-scale field trial would be necessary to establish cost-effectiveness of these products for use in *Ae. aegypti* control programs.

Use of Pyriproxyfen on Adults in Argentina

Because *Ae. aegypti* adults rest inside the dwellings, indoor treatments using portable machines are particularly effective and are the only option when vehicle access is not possible.

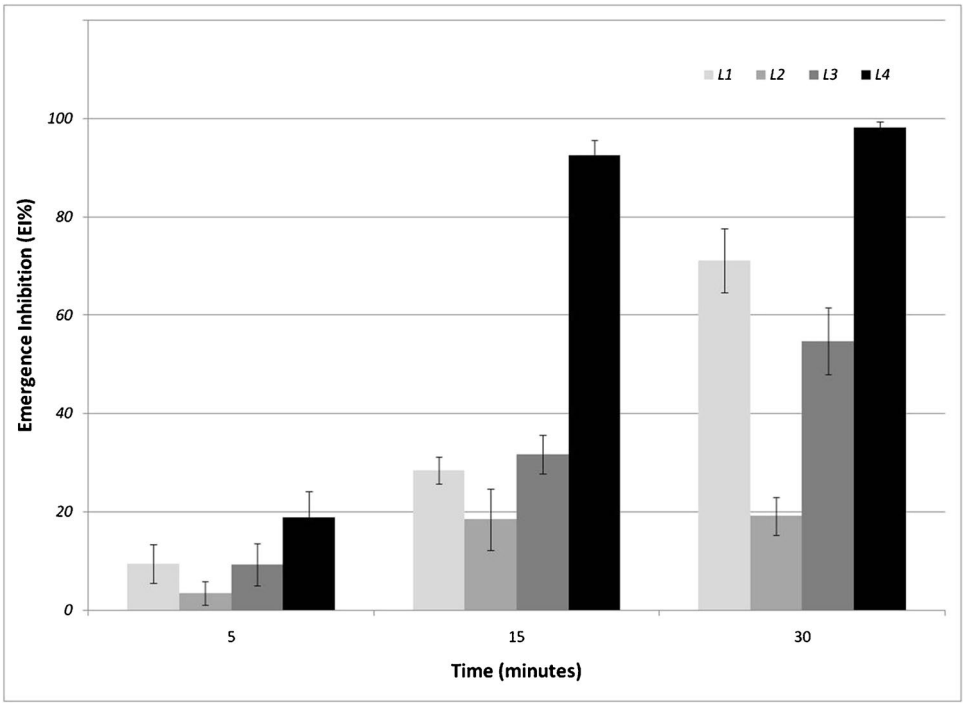

Fig. 1 Emergence inhibition (% ± SE) of *Ae. aegypti* larvae (L1, L2, L3, or L4) of exposed to the fumes of a 300-mg fumigant tablet containing 0.2 g/kg of pyriproxyfen during different exposure times

In Argentina, the organophosphate fenitrothion, the pyrethroids permethrin and deltamethrin, and more recently the *cis* isomer of permethrin have been used for ultra-low-volume (ULV) spraying [36, 37]. The Center for Research on Pests and Insecticides (CIPEIN) has carried out studies with a new formulation for space spraying containing an adulticide such as permethrin and a larvicide from the IGR group, pyriproxyfen. This trial, performed in the city of Puerto Libertad, Misiones (Argentina), has shown excellent results for controlling the adults and larvae of this vector [38]. For example, with the combined adulticide-larvicide (AL) formulation, the EI% had an initial mean value of 96% and maintained a high level up to 35 days after the ULV spraying. At this point, EI% reached a value of 71%, but a week later, the efficacy of the product was quickly reduced to a value of 2%. On the other hand, adult mortality was greater than 80% and no differences were found between the use of the adulticide-larvicide (AL) formulation and the formulation with the pyrethroid (permethrin) alone. Similar results were obtained using this formulation by Dantur Juri et al. in Tucumán, Argentina [39].

As an additional control tool, different from liquid formulations, our laboratory has also carried out studies in Argentina with a smoke-generating tablet containing pyriproxyfen and permethrin as discussed by Harburguer et al. [40]. This evaluated in field conditions the efficacy against *Ae. aegypti* of an experimental nonprofessional fumigant formulation containing permethrin + pyriproxyfen; they also investigated the residents' acceptance of this tool together with its role in community participation. In this study, adult insect mortality 24 h after treatment was more than 95% while EI% was 90%. Also, when the fumigant tablet was used, the adult index fell from three/four *Ae. aegypti* adults per house (in 10 min of search) to almost zero immediately after application and remained lower than the control values for 8 weeks. Regarding the acceptance of the community of this new tool for mosquito control, the results showed that 88% of residents of Puerto Libertad (Misiones, Argentina) were very concerned about dengue disease; however, it is apparent they did not consider a training workshop as part of a community participation program because only 16% of the residents attended. However, the fumigant tablet was widely accepted: 81% of the people applied it, and entomological surveillance showed that it had the same effect when applied by the community as when applied by professionals.

In *Ae. aegypti* adult females, the increase in the juvenile hormone (JH) indicates that the ecdysis process has ended and processes related to reproduction should begin. JH levels increase during the first day after adult emergence. This initial increase is essential for reproductive maturation to occur and JH acts on various tissues, including the ovaries, the fat body, and the midgut, rendering them competent to perform their specific functions in adults [41]. After the initial increase, the JH titer decreases slowly and, after blood ingestion, the JH level falls rapidly, reaching its lowest point after 24 h [42].

Exposure to IGRs causes a reduction in adult emergence but they also appear to have secondary effects, particularly on female reproduction, as indicated by studies using chitin synthesis inhibitors and JH analogs [21, 43]. Since the strict regulation of the JH titer is critical in the processes of oogenesis and vitellogenesis, it is of interest to know the effect of the application of a JHA in sublethal doses. On the other hand, *Ae. aegypti* is a domestic mosquito whose breeding sites (mostly drinking water storage containers) experience frequent variations in water volume, resulting in a decreased insecticidal effect. In this regard, detailed studies of the effects of sublethal doses of IGRs on viability, fecundity, and adult fertility should be considered.

The effect of pyriproxyfen on fertility and fecundity of *Ae. aegypti* females who survived treatment with a sublethal dose during the larval stage was examined in the study by Harburguer et al. [23] conducted in Argentina. The number of eggs laid per female was substantially lower in the treated group (33.7 ± 5.5) than in the control group (77.4 ± 1.4) when a dose corresponding to EI_{40} was employed. Additionally, there was a 20% decrease in egg hatch. However, neither before nor after the females were blood feed, there were changes in the amount of ovarioles per female between the control and treatment groups.

Other studies have demonstrated the impacts of applying sublethal doses of various IGRs to larvae. As indicated in "Introduction," numerous studies have demonstrated that sublethal doses of several IGRs have an impact on the fecundity and fertility of *Ae. aegypti* and other Culicidae larvae. Additionally, Itoh et al. [44] demonstrated that this IRG was still detectable in adult females 5–6 days after being exposed for 30 min to a surface containing 1 g/m^2 of pyriproxyfen. When the larvae treated with a sublethal dose of pyriproxyfen become adults, it is likely that they still contain this substance in their bodies as discussed by Harburguer et al. [23].

The preference of *Ae. aegypti* for container-like breeding sites provides an opportunity to control this species using ovitraps. Ovitraps are containers constructed to imitate natural breeding sites and to attract egg-laying females [45]. Ovitraps have been have been used for many decades as a sensitive, inexpensive, passive surveillance tool for detecting container-inhabiting mosquitoes as well as for providing a relative measure of temporal changes in adult abundance. In addition to their value for vector surveillance, various ovitrap devices can be used to suppress *Ae. aegypti* populations [46–49].

Currently, there are a number of promising alternative mosquito control agents that can be used in ovitraps such as *Bacillus thuringiensis var israelensis* [50] and insect growth regulators [51]. Pyriproxyfen can be used as an

autodissemination agent and dispersed into breeding sites by contaminated adult females [52]. Female mosquitoes can acquire pyriproxyfen crystals when landing on a treated surface and can then transfer them to breeding sites [44, 53], thereby killing larvae in these sites.

Because *Ae. aegypti* is a skip ovipositor (i.e., lays small numbers of eggs in multiple sites), pyriproxyfen can be used as an autodissemination agent for "mosquito-driven larval control" using the gravid female to disperse the larvicide and to contaminate multiple breeding sites [54••, 55, 56].

In Argentina, Harburguer et al. [57] examined the biological and chemical characteristics of a new larvicide ovitrap prototype manufactured by injection molding of low-density polyethylene (LDPE) and incorporated pyriproxyfen. The aim of the study was to develop a new generation of ovitraps for effective *Ae. aegypti* control. These ovitraps should take advantage of the reproductive ecology of *Ae. aegypti* to reduce mosquito vector abundance by preventing breeding in them. This study demonstrated that pyriproxyfen was immediately released from the LDPE into the water. Even water taken from the ovitrap after 1 min had an amount of pyriproxyfen sufficient to cause an EI of 100%. In fact, the chemical analysis demonstrates that this larvicide concentration in water after 1 min was 0.74 ppb, > 10 times the dose required to achieve an EI_{95} [58]. Residual activity assays show that the ovitraps remain active for the 30 weeks evaluated with 100% of EI even with weekly water replacement.

Conclusions

The development of targeted, ecologically safe insecticides as well as innovative application techniques is necessary to meet future difficulties in integrated pest management. These pesticides are more effective at controlling arthropod pests; the more specifically they work, the less harm they do to beneficial insects and the environment.

Pyriproxyfen is a juvenile hormone (JH) mimic that is highly active against a wide variety of insects of public health importance [59, 60]. It affects their hormonal balance and in some cases generates a strong suppression of embryogenesis, metamorphosis, and development to adults [61, 62].

The "autodissemination" approach is one of the new tactics being tried out to control vector mosquitoes. It is based on the idea that wild adult mosquitoes exposed to artificial resting sites contaminated with IGRs (so far pyriproxyfen, a juvenile hormone analog) may spread insecticide to larval breeding sites, preventing adult emergence [52, 63–65]. The "autodissemination" approach has the potential to target the numerous cryptic larval breeding sites that are inaccessible by conventional larvicidal applications by acting as both a "push" (i.e., dispersal of contaminated mosquitoes and dissemination of IGR to larval habitats) and "pull" (i.e., attraction of wild mosquitoes to contaminated sites) control strategy.

For mosquito vector control strategies, the Ministry of Health of Argentina currently uses temephos and *Bti* as larvicides. As mentioned above, an incipient resistance to temephos has been found in Argentina, and on the other hand, it is known that formulations based on *Bti* have low residuality [66]. Consequently, new formulations based on pyriproxyfen and other IGRs are needed.

To the best of our knowledge, Argentina is the only country in Latin America where some of the formulations used for controlling *Ae. aegypti* have been developed locally. The Center for Research on Pests and Insecticides (CIPEIN), to which the authors of this review belong, has been a pioneer in the region in the development of new formulations based on the use of IGRs, which are currently used by other Latin American countries, such as Bolivia and Paraguay [67].

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Declarations

Conflict of Interest The authors declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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