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Assessment of combined tools and strategies for *Aedes aegypti* control with low environmental impact

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Abstract

The control of the mosquito vector *Aedes aegypti* L. (Diptera: Culicidae) is the main action against dengue, chikungunya, and Zika. The excessive use of conventional insecticides has promoted the development of other control methods and strategies with lower environmental impact. We evaluated the effectiveness of applying triflumuron 1 ppm and emptying water-filled containers in a field trial in temperate Argentina. Both control methods were implemented either individually or combined and regularly from the beginning of the mosquito reproductive season or once it reached peak abundance. The impact on a non-target midge of the genus *Chironomus* was also tested. The highest reductions of *Ae. aegypti* were achieved in treatments which included triflumuron. This effect was stronger when applied from the beginning of the reproductive season, with < 1.3% of positive containers throughout the entire season. No enhancing effects were obtained when combining both control methods. Treatments with triflumuron were not completely innocuous for the non-target species, with *Chironomus* sp. more susceptible to treatments including triflumuron applied from the beginning of the reproductive season than all others. Sharp reductions of mosquito populations in urban environments with high density of water-filled containers are possible with minimum container management efforts, by applying triflumuron 1 ppm every 6 weeks. In temperate urban settings, better results can be obtained when applications begin early in the reproductive season of the mosquito vector *Aedes aegypti*.

Keywords Dengue · Mosquito control · Triflumuron · Container management · Non-target species · *Chironomus*

Introduction

The increasing incidence of dengue, the geographic spread of chikungunya and Zika, and the threat of yellow fever are being reported in urbanized settings worldwide (Wilder-Smith et al. 2017). These diseases are all transmitted through the bite of the mosquito *Aedes aegypti* L. (Diptera: Culicidae), which

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has adapted to domestic environments, developing its preimaginal stages in artificial containers filled with water that people accumulate for many purposes (Powell and Tabachnick 2013). These mosquito borne illnessess present similar clinical manifestations hindering a clear diagnosis. Furthermore, the slow development of vaccines and antiviral treatments (Kantor 2018) draws the efforts to the control of the mosquito to hamper viral transmission via humans and vectors contact (World Health Organization 2009).

The Integrated Vector Management framework highlights the need to implement a more efficient and rational use of resources for vector control. The improvement of effectiveness and efficiency of available control tools with sustainable and ecologically sound long-term approaches are required, emphasizing the judicious management of pesticides used in combination and synergistically, and integrating non-chemical and chemical methods (World Health Organization 2012). However, the mainstay of *Ae. aegypti* control during outbreaks is still the use of conventional chemicals as organophosphates and pyrethroids for adult mosquitoes, which are poorly complemented with other environmentally safe

methods (Chang et al. 2011). It is desirable, at least during non-epidemic periods, to focus on preventive actions against the immature stages of the vector and their aquatic habitats through environmental management like the cleaning and manipulation of containers. Furthermore, chemical or microbiological treatment with ovicides, larvicides, or pupicides could enhance the effect of container management (World Health Organization 2009). Using multiple vector control methos simultaneously can help to reduce the vector population and disease transmission in a more efficient way than using a single intervention (Rafikov et al. 2015; Oki et al. 2011). The selection of the optimal control method combination and the application strategies both require comprehensive knowledge of the target vector ecology. For instance, seasonal variation of *Aedes* population should be considered when planning control actions, to maximize cost-effectiveness and minimize human, environmental, and financial impacts.

The application of larvicides is unavoidable in some situations, like in the use of water storage containers in some communities. In those cases, it is desirable the use of long-lasting effect, high selectivity, and low toxicity both for humans and other non-target organisms chemicals (Achee et al. 2015). Insect growth regulators (IGRs) are developmental inhibitors that have emerged as larvicidal agents with environmentally safer attributes than traditional neurotoxic insecticides. They are highly efficient against mosquitoes with low impacts on birds and mammals (Belinato et al. 2009), although it is important to consider possible damaging effects on aquatic fauna (Soltani et al. 1999). Triflumuron is an IGR that interferes with insect molting, reducing larval survival, adult emergence, physical and reproductive fitness of the adults, and egg viability (Belinato et al. 2009). Furthermore, under laboratory conditions, triflumuron is effective against *Ae. aegypti* (e.g., Batra et al. 2005; Belinato et al. 2013), but there are hardly any studies regarding its effects in natural conditions (Sulaiman et al. 2004; Giraldo-Calderón et al. 2008; Jacups et al. 2014; Rubio et al. 2018). Triflumuron was only tested against *Ae. aegypti* in field conditions in South America, specifically on streetside storm drains in Colombia (Giraldo-Calderón et al. 2008) and in cemetery flower vases in Argentina (Rubio et al. 2018). Field integrated approaches including triflumuron and its potential synergy with other control tools have not yet been explored.

Weekly emptying and cleaning of water storage containers such as flower vases, water drums, and pet water bowls are recommended by WHO (2009) to reduce *Ae. aegypti* populations. Although this measure rapidly reduces the number of mosquitoes breeding sites, as proven in tropical regions from Asia and America (Fernández et al. 1998; Phuanukoonnon et al. 2005), its effect is short-lived. When an IGR is used in combination, previous emptying of containers can enhance the larvicide expected effectiveness and extend residual effects. The active compound of some IGRs can be adsorbed

onto container surfaces contributing to a longer residual activity as it is released gradually to the water (Batra et al. 2005). It was demonstrated that the IGR triflumuron has an improved performance against *Ae. aegypti* when applied as a pretreatment on dried containers, arguing that adherence or binding to the surface of buckets strengthens control effects (Jacups et al. 2014). These auspicious results suggest that the simple emptying of containers coupled with the use of triflumuron could have a greater effect than the cumulative effect of each method, optimizing cost-effectiveness of interventions by both reducing container management frequency and improving larvicide results.

The potential impacts on vector coexisting non-target species in aquatic habitats should be considered in the searching for better tools to control *Ae. aegypti*. The bulk of literature about dipteran communities in artificial containers, such as tires and flower vases, points at Chironomidae as the most abundant family in mosquito habitats (Yee 2008; Rubio et al. 2012, 2013). Although these non-biting midges are non-disease vectors, several IGRs have been evaluated as control tools when their populations reach nuisance proportions (Ali 1991). Notwithstanding this, field studies assessing the effect of triflumuron against Chironomidae in natural water bodies are outdated (Ali and Lord 1980; Johnson and Mulla 1982).

In order to improve the control of *Ae. aegypti*, we combined the application of the IGR triflumuron with environmental management during a field trial in temperate Argentina. The aim of the study was to evaluate the effectiveness of two control methods, applying triflumuron and emptying water containers. Both methods were implemented either individually or combined, and regularly from the beginning of the mosquito reproductive season or once it reached peak abundance. The impact of these interventions was also tested on a non-biting non-target midge of the genus *Chironomus*, which is one of the most abundant insects in water-holding containers in the region.

Materials and methods

Study area

The study was conducted in Greater Buenos Aires, Buenos Aires Province, Argentina, where the climate is temperate with annual mean temperatures ranging 11–25 °C and annual precipitation averaging 1200 mm (Servicio Meteorológico Nacional https://www.smn.gob.ar/caracterización-estadísticas-de-largo-plazo). The trial was conducted in three public cemeteries located in the districts of San Isidro (34°29′34.96″ S; 58°34′46.92″ W), Morón (34°39′44.80″ S; 58°37′38.00″ W), and San Martín (34°35′3.82″ S; 58°33′1.50″ W). All cemeteries have similar size (between 11.9 and 14 ha) and

population density in their surroundings (between 5734 and 7396 inh/km^2; Instituto Nacional de Estadística y Censos de la República Argentina www.indec.gov.ar/nivel4_default.asp?id_tema_1=2&id_tema_2=41&id_tema_3=135). These cementeries have similar graves arrangement with water-filled containers in which visitors offer fresh-cut flowers. *Ae. aegypti* is one of the most abundant mosquito species, as has been reported for other cemeteries of the study area (Vezzani and Albicócco 2009; Rubio et al. 2012) and most urban cemeteries worldwide (revised by Vezzani 2007). Likewise, non-biting midges of the genus *Chironomus* are among the most abundant dipterans in flower vases from cemeteries and discarded tires in the study area (Rubio et al. 2012, 2013).

Treatments

Six treatments, consisting of a combination of two mosquito control methods and two application strategies, along with a control treatment without any intervention were designed (Table 1).

The first control method against *Ae. aegypti* was the application of IGR triflumuron (Starycide® SC, 48%, Bayer CropScience Germany) in a dose of 1 ppm (1 mg a.i./L) (Rubio et al. 2018). All containers with or without water were treated following the methods of Jacups et al. (2014), by spraying the product evenly on the water surface (if any) and the walls of the container with a hand sprayer. The second mosquito control method was the container management, consisting on emptying all containers except those with fresh-cut flowers (i.e., placed up to 3 days before the treatment).

The two application strategies assessed considered the type (individual or combined use of both mosquito control methods) and the time of the intervention (since the beginning of the *Ae. aegypti* reproductive season or from its peak abundance). For the combined mosquito control method, all

containers were emptied and then sprayed with triflumuron except containers with fresh flowers in which only triflumuron was applied.

Field and laboratory work

The entire experiment required a continuous sampling effort of 39 weeks, from mid September 2015 to mid June 2016, covering the reproductive season of *Ae. aegypti* in the region (Vezzani and Albicócco 2009). A total of 13 entomological surveys (every 3 weeks) and 6 mosquito control interventions (every 6 weeks) were performed (Fig. 1). The frequency of the control interventions followed the previous field study recommendations (Sulaiman et al. 2004; Batra et al. 2005; Rubio et al. 2018). As expected for temperate regions in the Southern Hemisphere, seasonality determines the presence of *Ae. aegypti* larvae from October to June and peaks abundance in February–March (Vezzani and Albicócco 2009). Therefore, applications begun the last week of September, and trends of the entomological survey in the control treatment (C) were monitored to establish the peak of abundance.

Within each cemetery, 35 consecutive rows of graves were selected. The mean number of containers per row was 127 ± 4 (min 52, max 217), and mean percentage of containers with water were $55 \pm 8\%$ (min 40%, max 76%). The six treatments and the control were randomly assigned among the first seven rows of graves, and this arrangement was repeated in the remaining area giving a total of five rows per treatment in each cemetery. This design minimized the effects of environmental heterogeneity in the working area.

Each entomological survey involved the inspection of 50 water-holding containers per treatment in each cemetery. These were selected in groups of 10 consecutive water-holding containers in each row of graves, starting at randomly selected points. Containers with fresh-cut flowers were excluded from the survey to prevent visitors and cemetery

Table 1 Treatment codes resulting from the combination of *Ae. aegypti* control methods and application strategies

	Mosquito control method		Application strategy			
			Form		Time	
Treatment	Triflumuron (T)	Emptying (E)	Individual (I)	Combined (C)	Beginning (B)	Peak (P)
TIB	●		●		●	
TIP	●		●			●
EIB		●	●		●	
EIP		●	●			●
TECB	●	●		●	●	
TECP	●	●		●		●
C (control)						

Month	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN
Week from Sep 15th, 2015	1 2	3 4 5 6 7	8 9 10 11	12 13 14 15 16	17 18 19 20	21 22 23 24	25 26 27 28	29 30 31 32	33 34 35 36 37	38 39
Entomological survey	● ●	●	●	●	●	●	●	●	●	●
Treatments										
From the beginning	↑	↑	↑	↑	↑	↑				
From the peak						↑	↑			

Fig. 1 Field work timeline

authority annoyance. To estimate the availability of water-holding containers, the number of containers without water observed during the search of the 10 containers with water was recorded. Containers were emptied in a white plastic tray (31 × 26 × 5 cm), and the water was examined for third/fourth instar larvae and/or pupae of *Ae. aegypti* and *Chironomus* sp. Field identification was made by a single person. For laboratory confirmation, the water of one in ten randomly selected positive containers was filtered with a fine mesh strainer and fixed in 70% ethanol for further identification. *Ae. aegypti* was identified to species according to Rossi et al. (2002) and *Chironomus* following Epler (2001) and Wiederholm (1983). Coincidence between field and laboratory identifications was 100%. All specimens of *Chironomus* belonged to the same unidentified species.

Data analysis

Temporal variations in *Ae. aegypti* and *Chironomus* sp. infestation levels under each treatment were characterized by the Container Index, defined as CI = 100 × positive containers / water-holding containers inspected. The CIs of the three cemeteries were averaged for each survey and treatment.

Effects of treatments on *Ae. aegypti* and *Chironomus* sp. were tested with generalized linear models (GLM) and their extension including random effects (GLMM). These models allow the use of error distributions other than Normal, and the inclusion of random terms to control for correlations that arise from grouped observations. The response variable was the Difference in Positive Containers of each treatment relative to the control (DPC = no. of positive containers in control − no. of positive containers in treatment). Higher DPC values correlate with favorable outcomes of the treatment on mosquito population control. As the number of water-holding containers inspected was lower than 50 in some treatment/cemetery/survey (minimum 38) due to water scarcity, the number of positive containers was relativized to 50 per treatment/cemetery/survey.

The following environmental variables known to affect mosquitoes life cycle were included in the modeling: proportion of containers with water, mean daily temperature, accumulated precipitation, and days since the first intervention (Table 2). Temperature and precipitation were added up from 1 to 4 weeks prior to each entomological survey to determine the lag with highest correlation with the response variable. Daily temperature and precipitation from the Buenos Aires meteorological station (34°35′0″ S, 58°30′0″ W) were provided by the National Oceanic and Atmospheric Administration (https://www7.ncdc.noaa.gov/CDO/dataproduct).

All continuous variables were standardized and squared. Prior to multivariate modeling, preliminary univariate analysis without random terms was run for each explanatory variable, assuming a quasi-Poisson error distribution to account for potential overdispersion and log function connecting the response variable with the linear predictor. All non-correlated variables (variance inflation factors ≤ 4; Zuur et al. 2009) were included in the multivariate full model. Explanatory variables retained in the final multivariate models were selected by a stepwise backward procedure, in which all two-way interactions and quadratic relations were evaluated. To simplify the models, the levels for the treatment variable that were not significantly different were merged together; decision rules for dropping variables and joining season levels were based on a chi-square test comparing the deviance ratio of the nested models at each step. Multivariate models assumed a Poisson distribution of errors and log function interconnecting the response variable and the linear predictor; over-dispersion was discarded on both models.

To account for correlations from grouped observations, once the best GLM for each species group was obtained, the cemetery (cem; as spatial grouping variable) and the number of entomologic survey (sur; as temporal grouping variable) were tested as a random intercept (1|cem) and as a random intercept plus varying slopes (sur|cem). The selected model for species group was the one that yielded the lowest Akaike information criterion (AIC) value (Zuur et al. 2009). Data bootstrapping and parameter re-fitting (1000 replications) were performed to discard the effect of influential observations. To assess the accuracy of the selected models, marginal and conditional R^2 (variance explained by fixed factors only and by fixed plus random factors, respectively) were calculated (Bartón 2013).

All analyses were performed using the open-source software R 3.2.3 (R Development Core Team, www.r-project.org) with lme4, MuMIn, and Car packages.

Results

A total of 13,580 water-holding containers were inspected, and the overall CI was 5.0% for *Ae. aegypti* and 3.2% for *Chironomus* sp.

Table 2 Variables tested for modeling the reductions in the infestation levels of target and non-target dipterans (*Aedes aegypti* and *Chironomus* sp., respectively). Descriptions, range values, and the random (R) or fixed (F) factors which were used are detailed. Deviance of the null model for each taxon is reported between brackets

Name	Description	Range values and units	Factor	Residual deviance	
				Ae. aegypti (1854.3)	*Chironomus* sp. (812.9)
cem	Cemetery	3 levels: 1 for each cemetery	R		
sur	Number of entomologic survey	1–13	R		
trt	Treatment	6 levels: TIB, TIP, EIB, EIP, TECB, TECP	F	1754.6*	780.7 ns
pww	Proportion of containers with water	0.8–1	F	1785.3*,a	812.1 ns
days	Days accumulated from the first trial day	0–264 days	F	957.4***,a	738.4***
t1w	Average of the mean daily temperature of the last week	9.58–26.94 °C	F	1761.7**,a	744.7***,a
t2w	Average of the mean daily temperature of the two last weeks	11.13–25.51 °C	F	1798.5*,a	783.2**
t3w	Average of the mean daily temperature of the three last weeks	11.26–25.79 °C	F	1853.8 ns	773.7***
t4w	Average of the mean daily temperature of the four last weeks	11.20–25.95 °C	F	1789.1*,a	788.1**
pp1w	Accumulated precipitation in the last week	0–4.61 mm	F	1814.6 ns	788.2*,a
pp2w	Accumulated precipitation in the two last weeks	0.2–5.16 mm	F	1838.1 ns	772.9**,a
pp3w	Accumulated precipitation in the three last weeks	0.87–8.54 mm	F	1839.6 ns	796.3*
pp4w	Accumulated precipitation in the four last weeks	0.87–9.61 mm	F	1796.7*,a	721.3***,a

F test comparing the deviance ratio of the univariate (linear relation: x) or bivariate (quadratic relation: $x + x^2$) model with the null model

TIB triflumuron individual beginning, *TIP* triflumuron individual peak, *EIB* emptying individual beginning, *EIP* emptying individual peak, *TECB* triflumuron emptying combined beginning, *TECP* triflumuron emptying combined peak, *ns* not significant

Significant at ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

[a] Best fit is for quadratic ($x + x^2$) relations

Seasonality

Ae. aegypti showed a typical unimodal trend, with a maximum infestation level (CI = 36.7 ± 5.9%) in April around the 30th study week (Fig. 2). Among the treatments applied since the beginning of the reproductive season, those including triflumuron (TIB, TECB) maintained CI values below 1.3% throughout the study, whereas emptying containers (EIB) had a slight effect on the infestation levels, noticeable since the fourth application of the treatment. Until the first intervention, infestation levels in all treatments applied (TIP, EIP, TECP) were similar to the control, once peak abundance was attained. Thereafter, the CI of the treatments with triflumuron (TIP, TECP) reached values below 2.7% until the end of the study, whereas emptying alone (EIP) barely kept CIs around 20% and reached minimum values only after the second intervention but prevented a further rise in infestation levels.

Chironomus sp. presented maximum CI values at weeks 13 (CI = 11.3 ± 3.7%) and 35 (CI = 15.3 ± 7.7%) (Fig. 2). In response to the application of mosquito control treatments, reductions in infestation levels of this non-target insect were observed with a similar pattern to those of *Ae. aegypti*. In treatments with triflumuron, the CI was drastically reduced both when applied from the start (CI < 1.3%) or since the peak (CI < 2.7%), whereas emptying alone (EIB, EIP) was associated with a moderate reduction of the CI.

Univariate models

Univariate relationships between the "Difference in Positive Containers" (DPC) for *Ae. aegypti* and *Chironomus* sp. and the explanatory variables are presented in Table 2. The treatments (trt) and the proportion of containers with water (pww) were good predictors of the variability in DPCs values of *Ae. aegypti*. For both taxa, time since the first intervention (days), temperature during the last week (t1w), and precipitation during the last month (pp4w) were among the best predictors.

Multivariate models

The best model for *Ae. aegypti* explained 76% of the total variance, mostly attributable to variables included as fixed factors (Table 3). The reduction of *Ae. aegypti* infestation levels was greater with triflumuron treatments applied from the beginning of the season (TIB, TECB), followed by those including triflumuron since the abundance peak (TIP, TECP),

Fig. 2 Mean Container Index (± SE) per survey for *Aedes aegypti* and *Chironomus* sp. Arrows indicate each treatment application. Dots are slightly out of phase of the corresponding entomological survey for a better understanding of the figure
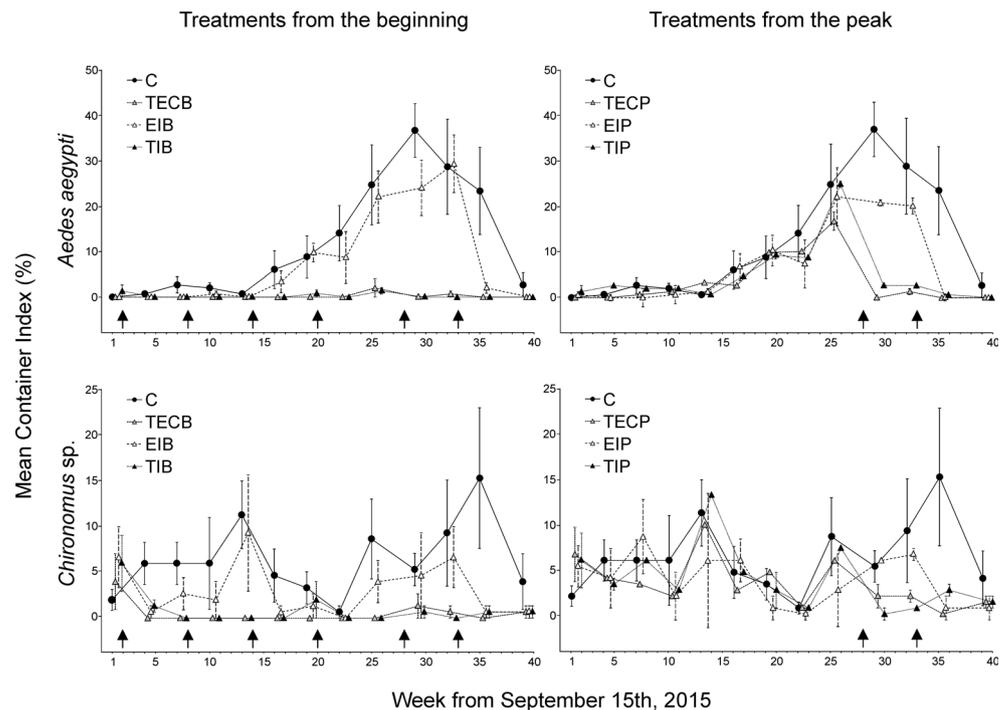
and smaller in emptying treatments (EIB, EIP). In addition, mosquito control effects increased as the season progressed. Generally, intermediate mean daily temperatures of the last week (18–24 °C) were associated with higher effects. Nevertheless, interaction terms indicate enhanced control effects when lower temperatures were combined with lower proportions of water-filled containers, and higher temperatures with lower precipitations within 4 weeks. The cemetery and the number of entomologic survey were both included in the random factor, indicating that absolute DPC values differ per cemetery in a random way and that the relationship between DPC and the explanatory variables exhibits a different shape for each cemetery.

Regarding *Chironomus* sp., the overall model accounted for 62% of the total variance. Most of it was explained by both grouping factors, spatial (cemetery) and temporal (survey), and to a lesser extent by the treatments and environmental variables (Table 3). A greater reduction in infestation levels was obtained with the triflumuron application once the season begins (TIB, TECB). Higher effects were also associated with a lower proportion of water-filled containers, higher values of accumulated precipitation in the last 4 weeks, and intermediate values of mean daily temperatures of the last week.

Discussion

The search for new application strategies of known control methods with low environmental impact was the main goal of the current research. Here, we presented the results of a

field study that combined the application of the IGR triflumuron with emptying of containers to control *Ae. aegypti*, the main dengue vector. The highest reductions in the infestation levels of *Ae. aegypti* were achieved in treatments including triflumuron. In previous field studies, triflumuron has been proven to be more effective than other IGRs such as pyriproxyfen and methoprene (Sulaiman et al. 2004) or bioinsecticides based on the mixture of *Bacillus thuringiensis* and *Bacillus sphaericus* (Giraldo-Calderón et al. 2008). Likewise, triflumuron performed better against *Culex quinquefasciatus*, another mosquito vector, when compared to diflubenzuron, lufenuron, and azadirachtin (Suman et al. 2010). There is no previous antecedent on the comparative analysis of the effectiveness of triflumuron in combination with any container management tool. Our results indicate that relying solely on the periodic emptying of water-holding containers every 6 weeks did not reduce considerably the *Ae. aegypti* infestation levels. This periodicity may not compensate the filling of containers by rainfall and visitors, reinforcing the fact that the frequency of application of any control method is an essential strategy component. We have previously demonstrated that a single intervention with triflumuron 1 ppm near the mosquito peak abundance reduces the Container Index (CI) to 10% (Rubio et al. 2018). In the present study, interventions every 6 weeks since the onset of peak in mosquito abundance maintained CI below 2.7% until the end of the mosquito reproductive season, suggesting that triflumuron at this dose and frequency could be an optimal *Ae. aegypti* controlling method in small water-filled containers.

Table 3 Best generalized linear model for the reductions in the infestation levels of target and non-target dipterans (*Aedes aegypti* and *Chironomus* sp., respectively). Akaike information criterion (AIC) values for the null and final models and the marginal and conditional R^2 are informed in each case. Explanatory variables included in the random and fixed part of the models are reported, for the latter parameters along with their corresponding standard error (SE) and p value are given

Species	AIC		Variance explained		Explanatory variables				
	Null	Best model	Fixed (marginal R^2)	Fixed+random (conditional R^2)	Random	Fixed	Parameter	SE	p value
Aedes aegypti	2310.9	910.3	0.69	0.76	sur|cem	Intercept (TIB,TECB)	1.531	0.219	< 0.001
						TIP,TECP	−0.358	0.074	< 0.001
						EIB,EIP	−0.878	0.086	< 0.001
						days	1.443	0.193	< 0.001
						t1w	0.365	0.066	< 0.001
						t1w^2	−0.560	0.044	< 0.001
						pp4w	−0.213	0.045	< 0.001
						pww	−0.053	0.039	0.184
						pww x t1w	0.226	0.035	< 0.001
						pp4w x t1w	−0.321	0.066	< 0.001
Chironomus sp.	1229.7	756.9	0.19	0.62	sur|cem	Intercept (TIB,TECB)	0.273	0.304	0.369
						TIP,TECP, EIB,EIP	−0.488	0.089	< 0.001
						pp4w	0.464	0.062	< 0.001
						pww	−0.455	0.048	< 0.001
						t1w	−0.495	0.077	< 0.001
						t1w2	−0.238	0.062	< 0.01

Variables: *cem* cemetery, *sur* number of entomologic survey, *pww* proportion of containers with water, *days* days accumulated from the first trial day, *t1w* average of the mean daily temperature of the last week, *pp4w* accumulated precipitation in the four last weeks

Treatments: *TIB* triflumuron individual beginning, *TIP* triflumuron individual peak, *EIB* emptying individual beginning, *EIP* emptying individual peak, *TECB* triflumuron emptying combined beginning, *TECB* triflumuron emptying combined peak

No enhancing effects were obtained when combining triflumuron and emptying of containers. It has been suggested that integrating several control methods can result in synergistic effects, achieving maximum cost-effectiveness and environmental benefits (Luz et al. 2009). To our knowledge, there are no previous studies focused on evaluating the summative effect of triflumuron with another *Ae. aegypti* control action. However, Jacups et al. (2014) concluded that triflumuron sprayed on dried containers with post-flooding delay induced lower levels of pupae emergence and higher residuality than those without delay. The apparent linked reason is the binding of triflumuron to the containers substrate. Although our results did not support the previously described effects, there are some issues to highlight. First, we found that the action of emptying alone the water-filled contaiers had a barely perceptible impact on the infestation levels of *Ae. aegypti*, so additive effects are not expected. The quick re-filling of treated containers is an uncontrollable fact in field conditions. Second, we only measured the effects of treatments on the percentage of positive containers (CI) but not on the percentage of pupae emergence or larval density, as was performed in previous controlled conditions (Braga et al. 2005; Jacups et al. 2014). The CI is an operationally plausible variable in a field design with a large number of containers, repeated control interventions and systematic entomological monitoring. However, it is less sensitive to the effects of the treatments since the presence of a single individual makes a container positive. Nevertheless, the CI is still a useful entomological index to predict the occurrence of outbreaks and the implementation of vector control actions (Chang et al. 2015).

The effect of interventions involving triflumuron was stronger when applied from the beginning of the reproductive season of *Ae. aegypti*. Nevertheless, the two selected mosquito timings are very effective and maintain CI values as low as 2.7% when applied since its peak abundance. The optimal timing to carry out strategies against dengue vectors has been reported within their reproductive season elsewhere, at the onset of the period of maximum growth of the mosquito population (Guzzetta et al. 2017), and when the dengue transmission season begins (Luz et al. 2009) or reaches its peak (Bang and Pant 1972). However, the period of the intervention is the most variable issue because it is closely related to unpredictable factors, such as climatic conditions, vector infestation levels, and the occurrence of cases or active transmission of pathogens. Other factors such as financial and human resources can also condition the beginning of interventions. Regardless of the exact moment, all studies agree that few applications at optimal times have high effectiveness, prevent insecticide resistance, and avoid continuous larval control policies which are expensive, laborious, and harmful to the environment.

Field studies allow the assessing of control actions effectiveness in a real context and the setting of quantitative relationships among infestation levels and environmental factors. Temperature was the strongest meteorological variable affecting magnitude of effects for all control treatments. Intermediate temperatures were associated with high vector control effects, while at extreme values, the effects diminished. The efficacy of IGR compounds varies with temperature (Amarasekare and Edelson 2004), and their active ingredient is strongly affected by photolysis due to solar radiation (Hu et al. 2009). Furthermore, at low temperatures (which occur at both peaks of the reproductive season of *Ae. aegypti* in temperate Argentina), the control effects increased when the proportion of water-filled containers was low, indicating a low availability of potential aquatic habitats for immatures. In this context, the probability of gravid females using pretreated containers to lay their eggs could increase due to the scarcity of water-filled containers. These effects could be accentuated by the small size of the vector population at the beginning and at the end of the reproductive season. At higher temperatures, vector control effects also increased when rainfall was scarce, highlighting the influence of evaporation processes due to the combination of high temperatures and the absence of refilling by rainfall. Both named conditions promote the preservation of triflumuron concentration and the maintenance of empty containers, whether applied in the individual or combined form. Finally, we verified a strong spatio-temporal variability in the response of *Ae. aegypti* to all treatments. The magnitude of the control effect increased as the season progressed, suggesting a cumulative effect of the successive interventions along the study period. An absolute infestation levels reached for each control treatment varied among cemeteries and during the course of the intervention period. Therefore, the maintenance of a systematic entomological survey is fundamental when control interventions are performed under natural conditions.

Previous studies have revealed the effectiveness of triflumuron (Ali and Lord 1980; Johnson and Mulla 1982) and other IGRs (e.g., Tassou and Schulz 2011) against nuisance Chironomidae species from large water bodies, but this is the first study dealing with triflumuron or environmental management effects on Chironomidae species in artificial containers. Our results revealed that variations in the infestation levels of *Chironomus* sp. were strongly explained mainly by trial conditions, related to site and temporality, while treatments and environmental factors played a secondary role. Although the treatment was not significant in the univariate model, differential effects of the control measures appeared when environmental factors were included in the multivariate modeling. This reveals a high susceptibility of the non-target organism to treatments including triflumuron applied from the beginning of the reproductive season. These results, coupled with those presented for *Ae. aegypti*, highlight the complexity of vector control that pretends to be environmentally safe and points out that the choice of foresight control measures and

strategies to be implemented is essential, as the most efficient intervention against *Ae. aegyti* involving the application of triflumuron 1 ppm will not be completely innocuous for a non-target species of *Chironomus* coexisting with the vector in aquatic habitats. Future studies should establish susceptibility levels of other non-target species of typical insect communities in artificial containers.

Applying any control method that implies a container-by-container effort is certainly expensive, not only in human and time but also in financial resources. Though, our results strongly suggest that combined control methods are not always better and that testing the possible strategies to be implemented on a small scale can reduce the costs of vector control programs at greater scales and longer periods. In temperate Argentina, a significant reduction of *Ae. aegypti* larval population in cemeteries is achievable with minimum container management effort, without emptying pre-treatment, by applying triflumuron 1 ppm every 6 weeks during the reproductive season. With this control method, infestation levels below 1.3–2.7% (depending on application timing) can be achieved. In the future, greater efforts should be focused to elucidate if such reductions are sufficient to avoid or disrupt diseases transmission. Finally, there is a need to develop other application strategies to reduce the dose of larvicide and the frequency of applications, aimed to ensure the safety of interventions in non-target insects, minimize the accumulation of triflumuron in the environment, and prevent the development of resistance in vector populations.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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