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Evaluation of the manhole physical pest management system UNFO-PLS for mosquito control in southern Switzerland

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RESEARCH ARTICLE

Abstract

The expanding distribution of the *Aedes albopictus* mosquito (also known as the Asian tiger mosquito) throughout Europe is a health and safety threat due to its potential to spread tropical and sub-tropical arboviruses to temperate climate regions. Therefore, it is becoming increasingly important to broaden the use of all tools within integrated pest management systems to control this threat. Since this mosquito species in Europe breeds mainly in manhole drains, managing them is therefore essential. The UNFO-Pest Lock System (UNFO-PLS) mechanical control device fits in siphoned manholes and prevents mosquitoes from entering the manhole and breeding in the water below. We tested and quantified the efficacy of these UNFO-PLS devices against standard control manholes (no mechanical devices attached) in Balerna, Switzerland (Canton Ticino). Weekly counts of five developmental stages (larval stages L1-L4 and the pupal stage) were conducted at 12 experimental and 12 standard control manholes during the mosquito breeding and activity periods of 2020 and 2021. For each developmental stage, we compared the counts of mosquitoes in manholes fitted with and without the UNFO-PLS devices using generalised mixed-effect models. Results show that these devices reduced the presence of mosquitoes at all developmental stages between 92.6-97.2%. The use of the UNFO-PLS mechanical control device, or similar devices, should be considered for use in integrated mosquito pest management plans, especially in places that might present challenges for maintaining adequate larvicide applications, such as around schools and secondary homes.

Keywords: mechanical mosquito control, mosquito management, Aedes albopictus, Culex pipiens, urban mosquitoes

1. Introduction

The container-breeding invasive exotic mosquito *Aedes(Stegomyia) albopictus* (Skuse, 1894), also known as the Asian tiger mosquito, has spread and established itself rapidly over the last few decades across Europe (Knudsen, 1995; Mosquito Maps, http://ecdc.europa.eu/; Müller *et al.*, 2020). This species is a nuisance because of its repeated bites, mostly during the day (ECDC, 2016), but it is also a major health concern due to its ability to transmit at least 26 arboviruses such as dengue, chikungunya, and Zika (Bonizzoni *et al.*, 2013; Medlock *et al.*, 2012). In fact, many cases of local transmission of dengue and chikungunya in Europe are linked to the tiger mosquito (ECDC, 2022).

Several control systems have been in place in Europe to reduce or limit the presence of tiger mosquitoes for many years. Although recent efforts have been made to standardise surveillance and control activities (Bellini *et al.*, 2020), each regional surveillance system adopts the strategies that best suit its particular situation: political support for the project, climatic, territorial and cultural characteristics, availability of biocides, laws, etc. In general, there are several control measures that can be applied to reduce the presence of the tiger mosquito. Targeted actions are those that affect the aquatic phase of the mosquito, such as removal of larval breeding sites (various small water containers, such as saucers, drums, etc.) and/or larvicidal treatments, while adult traps and/

or adulticidal treatments can affect the adult phase (Bellini et al., 2020; Flacio et al., 2015). There is no single solution, but the combination of different strategies can lead to its containment (Ravasi et al., 2021). One difficulty with any control system is that the mosquitoes are present on both private and public lands, and therefore it is necessary to protect both areas. Manhole drains are present on private and public land and are considered among the main breeding sites for tiger mosquitoes (Bellini et al., 2020). Several mechanical systems are commercially available that aim to prevent mosquitoes from gaining access to the water in manholes, thus preventing reproduction in these locations. The University of Applied Sciences and Arts of Southern Switzerland (SUPSI) collaborated in the scientific development and subsequently tested one of these physical pest management systems proposed by the Italian company UNFO PLS s.r.l. (https://www.unfo-pls.com) and distributed in Switzerland by the company ACO Passavant AG (http://www.aco.ch/).

The main goal of this study was to test and quantify the efficacy of this newly developed mechanical system under realistic conditions. Specifically, counts of larval- and pupal-stage mosquitoes in manholes with siphons fitted with UNFO-PLS devices were compared to counts from standard control manholes with siphons in southern Switzerland.

2. Materials and methods

Study site

The experiment was done in the municipality of Balerna, Canton Ticino, Switzerland. Balerna was chosen due to it being representative of urban and industrial areas in Ticino, and its size which allowed for distinct experimental and control areas. The region around Balerna is characterised by sunny, dry winters, spring and autumn with periods of northern foehn wind with occasional heavy snow- and rainfall, and sunny summers with violent downpours. The landscape features foothills and typical components of Lombard agriculture (Ravasi *et al.*, 2021). Balerna and the surrounding areas are within the normal breeding range of the tiger mosquito in southern Switzerland, as confirmed by long-term studies (Flacio *et al.*, 2015).

Three study zones (urban and industrial zones with old style and new style manholes) were selected (Figure 1, Supplementary Figure 1, 2 and 3) where the manholes were not connected to each other through the sewer system. Each zone contained four experimental and four control manholes with siphons, for a total of 12 experimental and 12 control manholes. An UNFO Pest Lock System (UNFO-PLS) was placed in the experimental manholes, while control manholes had no device.



Figure 1. Map of study area in Balerna, Switzerland. The three study zones are marked with red points (map source: Swiss Federal Office of Topography).

UNFO-PLS mechanical pest control system and installation

UNFO PLS s.r.l. and SUPSI collaborated to develop the UNFO-PLS mechanical, chemical-free pest control system (Agropoli, Italy; Patent Number: 102015000066852 Nr. PCT / IT2016 / 000258) to physically prevent pests from entering municipal storm drains. The device fits over the drain hole inside of a manhole and consists of a floating ball that rises to allow rainwater to flow into the drain but sits securely over the drain hole in dry periods to prevent the passage of pests into the drainage system (Figure 2). Siphoned manholes were used for both experimental and control manholes to prevent mosquitoes from entering the manholes from other places within the public sewer system. Without siphons, mosquitoes could fly through connective pipes from one manhole to another despite the UNFO-PLS device and colonise the water.

All experimental and control manholes were cleaned (walls and siphons) on the same day the devices were installed (18 May 2020; 27 May 2021) to prevent existing eggs from hatching or mosquitoes accumulating from other parts of the public drainage system. Each manhole was cleaned using an iron brush to remove any remaining diapause eggs from the previous season (eggs that survive in winter that can hatch in spring), as well as to remove dirt and



Figure 2. UNFO-PLS manhole device. Diagram of a UNFO-PLS device installed in a manhole (A), manhole grate shown in red and the metal side edges of the frame shown in light blue) and a 3-dimensional image of the UNFO-PLS device (B).

grease. After cleaning, all manholes were treated with a biological larvicide without persistence (VectoBac[®] G, Valent Biosciences, 30 grains/manhole). This was done to kill any larvae present in the manholes. The larvicide treatment was repeated on the first control lap, one week after the cleaning and the first treatment and before the experiment began, in order to kill any larvae that could have hatched as a result of the first cleaning, i.e. diapause eggs scratched with the iron brush and falling into the water. Between the two years of testing, for the winter period, the device was removed and only the support frame was left in the manhole.

Manhole sampling

Manholes were sampled for mosquitoes and five developmental stages (the 4 instar larvae and pupae) were assessed without differentiating the species. In Canton Ticino, Ae. albopictus, Aedes (Hulecoeteomyia) japonicus (Theobald, 1901), Aedes (Hulecoeteomyia) koreicus (Edwards, 1917) and Culex (Culex) pipiens Linnaeus, 1758 are commonly found in manholes. Sampling (experiment and control) was carried out between June and September on a weekly basis starting 2 June 2020 and 9 June 2021 until the beginning of winter diapause or until no mosquitoes were observed in any manhole within the study area (28 September 2020; 13 October 2021). This methodology resulted in a total of 19 sample surveys in 2020 and 20 sample surveys in 2021. General sampling procedures followed the standard and proven effective procedures described by the European Centre for Disease and Prevention Control (ECDC, 2018). In order to maximise the capture of juvenile stages, sampling was done both using a standard dipper with three catches (model 1132, BioQuip Products, Rancho Dominguez, CA, USA) and using a fine mesh aquarium net (2 mm) making a figure 8 motion in the water column of the manhole. Once the juvenile stages were counted, they were returned to the manhole. During the samplings, the manholes and UNFO devices were removed for a short amount of time (ca. 5 min.) in order to conduct the sampling. No further manipulations (including cleaning) were conducted.

Statistical analysis

In order to evaluate the pest control efficacy of UNFO-PLS with regard to standard manholes, we compared the abundance of mosquitoes counted in the surveys for the experimental treatment versus controls (i.e. manholes fitted with and without UNFO-PLS devices, respectively) while accounting for the main features of the sampling design and seasonal effects.

From the total sample size generated by sampling 39 times 12 experimental and 12 control manholes, survey data that consisted only of mosquito number estimates instead of

specific counts were not included in the statistical analysis (i.e. converted to NA; n=46). Furthermore, survey data from manholes that were accidentally treated with pesticide (i.e. two manholes that were accidentally treated once by a municipal worker in 2021), and the corresponding weeks related to pesticide efficacy, were also removed from the statistical analysis (n=39).

For all validated data, the five developmental stages (i.e. L1-L4 and pupal stage) were analysed separately with a generalised mixed-effect model with a negative binomial distribution to account for the skewed distribution of the survey counts. Each model included the number of larvae or pupae counted during each sampling event as the response variable and manhole type (two level factor: control manholes or experimental treatment manholes fitted with UNFO-PLS devices) as the fixed effect of primary interest. Each model also included the study zone (three level factor: zones 1-3), year (two level factor: 2020 and 2021), and day of year of each sample as fixed effects to account for sampling design and seasonal effects. Day of year was included as an orthogonal polynomial of second degree to allow for a possible quadratic relationship between survey counts and time, which is consistent with the reproductive seasonality of mosquitoes. Additionally, each model included an interaction of year and day of year to allow for possible differences in seasonality between the two sampling years. Finally, manhole identity (factor with 24 levels) was included as a random effect due to the repeated measures at each location over time. Except manhole type, all other explanatory variables were kept into the final model irrespectively of their statistical significance because induced by the sampling design.

For all models, two alternative parametrisations (linear and quadratic) of the negative binomial distribution were compared using Akaike information criteria (AIC) and Bayesian information criterion (BIC) to identify the preferable model (Hardin and Hilbe, 2007). For each developmental stage, the regression model estimated the treatment effect of the UNFO-PLS manhole as compared to the control manholes while taking all other predictors into account. Due to the log link function, the estimated treatment effect can be naturally interpreted as the percent change in mosquito counts due to the use of UNFO-PLS as compared to standard manholes. The 95% confidence intervals for these estimates were calculated using profiling likelihood methods. For all regression models, model fit was assessed visually by plotting and comparing observed and predicted counts over time.

Furthermore, we assessed the statistical evidence in favour of more complex models in two additional ways. Firstly, given that a large number of survey counts were zero, we assessed whether the primary models suffered from zero-inflation by comparing them to hurdle models. These extend the negative binomial models from the main analysis by modelling the survey counts using two parts: one focused on whether the count was zero or not, and another part of the model focused on modelling counts that were not zero. Hurdle models are therefore much more suited to deal with excessive zeros and should be favoured by AIC and BIC in the presence of zero-inflation. Both parts of the hurdle model were fit with the same predictor combinations used in the main analysis. Secondly, we evaluated whether additional predictor interactions were needed by comparing AIC and BIC from the model of the main analysis and the more complex model including all possible interactions between manhole type, year, and day of year.

All analyses were performed with the R statistical package, version 3.6.3 (R Core Team, 2021). All regression analyses were performed with the glmmTMB function from the glmmTMB package (Brooks *et al.*, 2017). Zero-inflation was assessed using the bbmle package (Bolker and R Development Core Team, 2022). Inference was performed with likelihood ratio tests (for p-values) and profiling likelihood methods (to estimate confidence intervals). The level of significance was set at α =0.05. We used the ggplot2 package (Wickham, 2016) for plotting model output and visualising model fit. All R code and output is provided in Supplementary Materials and Methods and data are available upon request.

3. Results

For all generalised mixed-effect models fit separately for developmental stages, the linear parameterisation of the negative binomial distribution provided a better model fit than the quadratic parameterisation in terms of AIC/ BIC and was subsequently used for all models in the main analysis. Model fit visualisations at the population level revealed that fitted values captured the general pattern of the observed data, but were not able to fully reproduce the observed spikes in survey counts (see Figure 3 and Figure 4 for larval L1 and pupal stage as examples, see R code in Supplementary Materials and Methods for model fit visualisations of larval stages L2-L4). Furthermore, neither the hurdle model analysis to address possible zero-inflation nor the models including all possible statistical interactions provided evidence in favour of more complex models (see R code in Supplementary Materials and Methods).

Based on the final models derived through the model validation process, mosquito counts at all developmental stages were significantly lower at manholes fitted with UNFO-PLS devices compared to control manholes (Table 1, Figure 5). The percent reduction in mosquito counts at the L1 stage was 95.2% (95% confidence interval (CI)=90.3-97.9%, P<0.001), at the L2 stages was 96.3% (CI=91.8-98.6%, P<0.001), at the L3 stages was 97.2% (CI=93.0-99.2%, P<0.001), at the L4 stages was 95.0% (CI=88.8-98.2%,



Figure 3. Population level predicted and observed count values for larval L1 stage mosquitoes at control and UNFO-PLS manholes by day of year within study years. Gray lines represent observed values and the bold black line depicts the fitted value at the population level for standard control manholes (A) and manholes fitted with UNFO-PLS devices (B).



Figure 4. Population level predicted and observed count values for pupal stage mosquitoes at control and UNFO-PLS manholes by day of year within study years. Gray lines represent observed values and the bold black line depicts the fitted value at the population level for standard control manholes (A) and manholes fitted with UNFO-PLS devices (B).

Table 1. Results summary from main models for each developmental stage.

Predictor variable	Larval stage 1	Larval stage 2	Larval stage 3	Larval stage 4	Pupal stage
Manhole type	***	***	***	***	***
Zone (2)	NS	NS	NS	NS	NS
Zone (3)	NS	NS	NS	NS	NS
Year (2021)	NS	NS	NS	*	NS
Poly(day of year)1	***	*	NS	NS	NS
Poly(day of year)2	***	***	***	***	***
Year(2021): poly(day of year)1	***	***	***	**	**
Year(2021): poly(day of year)2	NS	**	***	NS	NS
Number of observations	872	886	895	896	897

¹ Significance levels of estimated parameters were determined using generalised mixed-effect models with negative binomial distributions. NS = not significant, * = *P*<0.05, ** = *P*<0.01, *** = *P*<0.001.



Figure 5. Treatment effect estimates of the UNFO-PLS manhole type across mosquito developmental stages. Values represent the percent reduction of mosquito counts at UNFO-PLS manholes compared to control manholes. Point estimates and 95%-confidence intervals are represented.

 $P{<}0.001),$ and at the pupal stage was 92.6% (CI=83.1-97.6%, $P{<}0.001).$ See R code in Supplementary Materials and Methods for model output details.

For all developmental stages, there was a significant association between mosquito counts and the day of year that samples were taken as well as between counts and the interaction of day of year and year (Table 1, Supplementary Materials and Methods). Study year was only a significant predictive factor in larval L4 stage mosquito counts while study zone location did not have a significant association with mosquito counts at any developmental stage (Table 1, Supplementary Materials and Methods).

4. Discussion

This study found that UNFO-PLS devices are highly effective at reducing the presence of L1-L4 larval stage and pupal stage mosquitoes in sewer system drainage holes in Canton Ticino (southern Switzerland) under real world conditions. The UNFO-PLS devices reduced mosquito counts for these developmental stages between 92.6 to 97.2% compared to control manholes. Furthermore, counts for all developmental stages were also significantly associated with day of year and the interaction of day of year and year. This outcome is most likely related to the seasonal reproductive cycle of mosquito breeding and local environmental conditions (e.g. rainfall, temperature, etc.; Alto and Juliano, 2001a,b; Fischer et al., 2014; Medlock et al., 2006; Neteler et al., 2013; Roiz et al., 2011). Counts of the larval L4 developmental stage were also significantly reduced in 2021 compared to 2020 (Chapter 8 in Supplementary Materials and Methods 'R code'), although other stages were not affected.

Sometimes debris (e.g. sticks, leaves, etc.) carried by the rain inside the manhole did not flow completely through the device, resulting in these materials becoming trapped between the sphere and the outflow cone and preventing the hole from closing completely. These occasional openings were millimetric, but in some cases may have allowed mosquitoes to enter. This may explain the occasional findings of larvae in manholes with the device.

However, the strong reduction in mosquito counts at UNFO-PLS manholes indicates that such devices should be further considered for an integrated pest control system for *Ae. albopictus* and other mosquito species throughout Ticino, and the use of this or similar devices should be investigated throughout other areas of Switzerland, Europe, and the world. In the municipality of Balerna alone, there are many manholes where this device could be installed

in publicly accessed areas such as schools and homes for the elderly, as well as privately around second homes and industrial areas. While not all potential breeding sites would be addressed, manholes represent a major source of mosquito reproduction (Bellini *et al.*, 2020) and thus targeting these locations could strongly impact mosquito populations.

Current management plans in Canton Ticino and much of Europe focus on removal of breeding sites, larvicide applications, and occasionally adulticide applications (Bellini *et al.*, 2020; Flacio *et al.*, 2015). While UNFO-PLS devices cannot eliminate the use of larvicides in mosquito pest management strategies, it could be a very useful complementary tool to be used in public and private spaces to help reduce the population of container-breeding mosquitoes (Ravasi *et al.*, 2021). For instance, it could be highly beneficial in areas where it is difficult and timeconsuming to apply biocides, which can lead to inconsistent application. Further studies should consider the cost-benefit ratio for the introduction of such a tool in an integrated pest management strategy.

5. Conclusions

The UNFO-PLS devices have shown promising results in containing all stages of mosquitoes in siphoned manholes in Canton Ticino (southern Switzerland). These devices can provide another useful tool to combat *Ae. albopictus* in an integrated pest control system. In the case of manholes, which represent main breeding sites that cannot be removed from the environment, it can complement the application of larvicides or even replace it in some situations. The use of such a mechanical tool could be especially useful in situations where the application of biocides is difficult due to the absence of those who can regularly perform the application during mosquito breeding and activity periods (e.g. secondary homes, schools, etc.). However, the cost-benefit ratio against the use of the larvicides must be evaluated.

Supplementary material

Supplementary material can be found online at https://doi. org/10.52004/JEMCA2022.0007.

Figure S1. Map of study zone 1 containing experimental manholes T1-T4 and control manholes C1-C4.

Figure S2. Map of study zone 2 containing experimental manholes T5-T8 and control manholes C5-C8.

Figure S3. Map of study zone 3 containing experimental manholes T9-T12 and control manholes C9-C12.

Supplementary Materials and Methods. Evaluation of UNFO-PLS devices: R code for statistical analyses.

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Conflict of interest

SUPSI has collaborated over the years with the inventor of the product and later with the producing company by suggesting improvements to the instrument without receiving any profit. SUPSI has only requested compensation from the productor company for the year 2021 for field analysis. SUPSI does not receive any profit from the sale or non-sale of the product.

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