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OPERATIONAL NOTE

EFFICACY OF AQUATAIN® AGAINST *CULEX PIPIENS* COMPLEX AND *AEDES ALBOPICTUS* IN CATCH BASINS IN ITALY

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ABSTRACT. Aquatain® is an alternative larvicide formulation to the currently used larvicides. Its efficacy can be assessed monitoring emerging adults with a floating device that was recently developed for use in catch basins. In this study, the efficacy of Aquatain in controlling *Aedes albopictus* and *Culex pipiens* complex was investigated by comparing the adults emerging from 25 treated catch basins with that of 25 control basins in northeastern Italy. Basins were monitored weekly for 9 times and the efficacy was evaluated using the Mann–Whitney *U*-test and calculating the inhibition of emergence at each sampling. Aquatain was effective in reducing the number of emerging mosquitoes for both species, but its duration was affected by rainfall. Intensive showers (>10 mm daily) seem to reduce the efficacy of the product, allowing an increase in emerging adults after about 2 wk. This finding suggests that climatic factors should be taken into account to decide the right time for reapplication of Aquatain during routine larval treatments.

KEY WORDS Aquatain, catch basins, efficacy, floating system, mosquitoes

Mosquito control in Italy was mainly aimed at reducing the nuisance of these insects until some years ago (Romi et al. 2008). However, recently the introduction and persistent circulation of West Nile virus in some areas of the country (Engler et al. 2013), coupled with outbreaks of chikungunya virus in 2007 and 2017 (Rezza 2018), drew the attention of public health authorities on the importance of controlling the mosquito vectors of these diseases. Among different potential vector species, *Aedes albopictus* (Skuse) and *Culex pipiens* L. play a primary role because of their widespread diffusion (Busani et al. 2012) and their proven vectorial capacity. These 2 species commonly breed in catch basins, where conditions are suitable for their development. In Italy typical catch basins are small structures to collect rainwater and could contain as much up to 40 liters of water (Bellini et al. 2009). The periodic treatment of nonremovable larval sites, such as catch basins, during the period at risk (April–September) is part of the national strategy to reduce the mosquito population and decrease the risk for pathogen transmission. Implementation of the Biocide legislation (EU 1998) caused the reduction in availability of many insecticides (e.g., fenitrothion, malathion, temephos, tralomethrin, trichlorfon, triflumuron), due to more strict rules on toxicological risk assessment and authorization of products for commercial use. Besides, some authorized molecules are at risk of developing resistance, as recently proved for diflubenzuron (Grigoraki et al. 2017). In this scenario, innovative mosquito control agents, such as Aquatain®, are increasingly used for mosquito control due to their demonstrated larvicidal and pupicidal activity against different genera of mosquitoes (Webb and Russell 2009, 2012; Bukhari et al. 2011). A new system for monitoring the emergence of adult mosquitoes from catch basins has been recently developed and tested, demonstrating to be an effective approach for this purpose. The tool consists of a floating system (FS) designed to collect the emerging mosquitoes (Drago et al. 2017). The aim of this study was to assess the duration of efficacy of Aquatain against the 2 most important mosquito species (*Ae. albopictus* and *Cx. pipiens* complex) detected in urban catch basins in Italy, using the newly proposed system.

The study was carried out in catch basins located in the town of Legnaro, Padova District (45°21′18″N, 11°57′07″E), northeastern Italy. The summer climate is hot and humid with high risk of intensive showers. All catch basins present in the study site were not treated since the previous year. Climatic parameters (average temperature at 2 m aboveground, and rainfall in millimeters) were recorded daily in the nearby weather station of Legnaro. Water temperature was measured with an immersion thermometer during each sampling in about 20% of monitored catch basins and the average temperature calculated. Seventy catch basins present in the study site were initially monitored using dip sampling as previously described (Drago et al. 2017), and the ones with the highest infestation were included in the trial. The pretreatment sampling was performed on July 13, 2017, and the 1st application on July 17, 2017, for

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each of the 25 treated catch basins. Further treatments were performed when necessary. The same operator performed all applications and conducted all assessments for the entire study. Aquatain AMF™ (Blueline, Italy; http://www.bleuline.it/) was applied at the rate of 2 ml per catch basin to 25 catch basins, following the manufacture's instructions. Twenty-five additional catch basins located nearby to the treated ones were selected to act as control basins and never treated during the study period. After the 1st application, all 50 catch basins were assessed at weekly intervals, using the FS, as previously described (Drago et al. 2017). It was decided that the product had to be reapplied when at least 25% of the treated basins were found with 2 or more emerging adult mosquitoes of both species.

The difference in numbers of 1st–2nd and 3rd–4th instars (*Ae. albopictus* and *Cx. pipiens*) at pretreatment sampling between treatment and control catch basins was evaluated by the Mann–Whitney *U*-test. The mean number of adults for treatment and control catch basins was calculated for each sampling. Descriptive statistics showing the trends of the different mosquito stages were assessed using Excel® 14.7.7 (Microsoft Corporation, Redmond, WA). The differences in numbers of adults of the 2 test species emerging in treatment and control catch basins were evaluated by the Mann–Whitney *U*-test, using the IBM SPSS Statistics 24 (IBM Corporation, Armonk, NY). The overall level of statistical significance was set to $P < 0.05$ (significant) and $P < 0.01$ (highly significant). Product efficacy was also estimated using the inhibition of emergence formula (%IE), adapted from the World Health Organization guidelines (WHO 2005): $\%IE = [(C - T)/C] \times 100$, where C = mean number of emerging adults from untreated catch basins, and T = mean number of emerging adults from treated catch basins.

As per the study protocol, reapplication of the product was performed after the 3rd wk, on August 7, 2017, since emergence was recorded in a number of basins with 2 or more adults, equal or higher than 25% for both species. No significant differences in 1st–2nd and 3rd–4th larval instars were found between treatment and control catch basins, according to the Mann–Whitney *U*-test ($P > 0.05$) for both species investigated. The mean numbers of emerging adults of *Ae. albopictus* and *Cx. pipiens* for each sampling, including the level of significance of the differences between treatment and control basins, are shown in Fig. 1, and reported in Table 1, together with the %IE and the percentage of basins with 2 or more emerging adults. During the trial, an average of <5% of the basins were not assessed in the analysis because of a car parked on top of it, or FS was broken or overturned. Figure 1 also shows the rainfall pattern, which was characterized by showers at the beginning of wk 2 after the 1st application and in wk 4, 5, and 6 after the 2nd application. Surface temperature did not show sharp changes during the study period, but only limited reduction after the showers, and ranged between 15.3 and 30.0°C. Average water temperature in the basins was constantly high (29.2–30.4°C) during the whole 1st treatment period, whereas it decreased progressively at the end of the 2nd treatment (from 29.1°C at 3rd wk after 2nd application to 21.4°C at the last sampling). The monitoring of adult *Ae. albopictus* showed high level of emergence inhibition (95–100%) for 2 wk after the 1st application and for 3 wk after the 2nd application (Table 1). In only 1 case (5th wk during the 2nd treatment) it decreased abundantly under 90%. In accordance with this finding, highly significant differences ($P < 0.01$) between treated and control basins were recorded in all samplings, except for the last two (Fig. 1). As far as *Cx. pipiens*, the percentage of inhibition was high (95–100%) for 2 and 3 wk after the 1st and the 2nd applications, respectively (Table 1). Highly significant differences ($P < 0.01$) in the mean numbers of adults emerging between control and treated basins were found for only 1 wk after the 1st application and 3 wk after the 2nd (Fig. 1). The number of emerging *Ae. albopictus* adults in control basins was significantly higher than that of *Cx. pipiens*, considering the whole period ($P < 0.001$) and specifically at wk 3 ($P = 0.01$) after the 1st application, and at wk 1–2 and wk 4 after the 2nd application ($P < 0.01$).

This study demonstrated the efficacy of Aquatain in significantly reducing the number of emerging adults of both *Ae. albopictus* and *Cx. pipiens* immediately after treatment. This reduction is more evident and significant (Fig. 1) for *Ae. albopictus*, but this may probably be due to the different densities of the 2 species in untreated catch basins. In fact, the number of emerging *Ae. albopictus* adults was significantly higher in control basins as compared with *Cx. pipiens* adults. This is in agreement with a previous study (Marini et al. 2017) that demonstrated a different temporal dynamic of the 2 species in temperate climate regions, with *Cx. pipiens* declining earlier during the warm season. Notwithstanding an initial efficacy of both applications, a clear difference in its duration between the 1st and 2nd treatments has been recorded. The efficacy of the 2nd application was high (>95%) for 3 wk, instead of 2 wk during the 1st treatment. More importantly, the mean number of emerging adults was kept at very low level for 4 wk after the 2nd application, and for 2 wk after the 1st application. Most probably, external factors, especially the rainfall pattern, may influence the duration of treatment efficacy. It is clear that showers (in particular >10 mm daily precipitation) had a strong influence on control basins, which recorded a decrease in the number of emerging adults immediately. This is particularly evident for *Cx. pipiens* in our study. The reason for this finding can be attributed to the removal of larvae and pupae, which are washed away by the rainwater. The different diving ability of the immature stages of the 2 species (Koenraadt and Harrington 2008), which

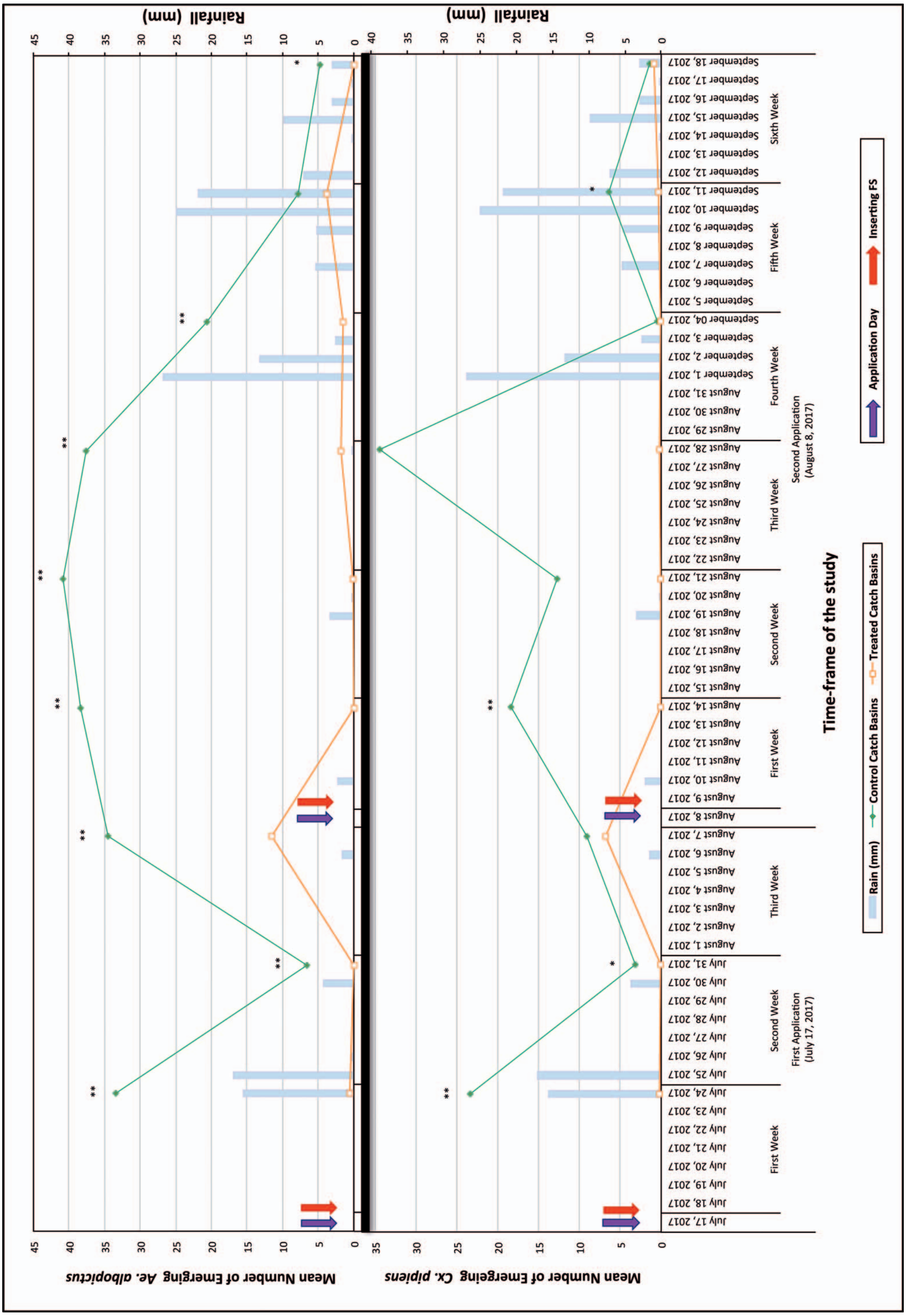

Fig. 1. Mean number of emerging adults of *Aedes albopictus* (upper) and *Culex pipiens* (lower) in treated and control basins at each sampling, and rainfall pattern. The level of significance of the difference within each sampling (control versus treated) is indicated by asterisks: * $P < 0.05$; ** $P < 0.01$ (Mann–Whitney U-test).

Table 1. Mean number of emerging adult mosquitoes in control (C) and treated (T) catch basins, percentage of basins with 2 or more emerging adults (b%) among basins of the treated group, and inhibition of emergence (IE%) at each sampling. Pretreatment sampling was performed on July 13, 2017.

Species and parameter	1st application: Jul. 17, 2017			2nd application: Aug. 8, 2017					
	S[1] 1: 1st wk, Jul. 24	S 2: 2nd wk, Jul. 31	S 3: 3rd wk, Aug. 7	S 4: 1st wk, Aug. 14	S 5: 2nd wk, Aug. 21	S 6: 3rd wk, Aug. 28	S 7: 4th wk, Sep. 4	S 8: 5th wk, Sep. 11	S 9: 6th wk, Sep. 18
Aedes albopictus									
Mean no. C	33.5	6.6	34.5	38.4	40.8	37.5	20.6	7.8	4.8
Mean no. T	0.6	0.0	11.5	0.0	0.2	1.7	1.5	3.8	0.0
IE%	98.3	100.0	66.8	100.0	99.6	95.4	92.9	50.9	100.0
b%	4.2	0.0	54.2	0.0	4.0	24.0	20.8	28.0	0.0
Culex pipiens									
Mean no. C	23.4	3.2	9.0	18.5	12.8	34.5	0.4	6.3	1.4
Mean no. T	0.3	0.0	6.8	0.0	0.0	0.2	0.1	0.4	0.9
IE%	98.9	100.0	24.5	100.0	100.0	99.6	71.3	93.9	38.2
b%	4.2	0.0	25.0	0.0	0.0	4.0	4.2	8.0	8.3

[1] S, sampling.

is less developed in *Cx. pipiens*, can justify the difference in magnitude of the reduction between the 2 species. This aspect does not represent a problem for treatment efficacy, but may complicate the data interpretation of the %IE parameter. Besides, it can be argued that these showers have an effect also on treated catch basins, since there is an evident increase in emerging adults 2 wk after the rain, at the end of wk 3 after the 1st application for both species and at the end of wk 5 after the 2nd application for *Ae. albopictus* (Fig. 1). This is probably due to the effect of rain on the silicon-based film, which could be disaggregated and partly washed away, thereby losing its full efficacy in the medium term.

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