An Operational Evaluation of 3 Methoprene Larvicide Formulations for Use Against Mosquitoes in Catch Basins

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ABSTRACT: Effectiveness in controlling mosquitoes in storm water catch basins in the North Shore Mosquito Abatement District (northeastern Cook County, Illinois) was determined for 3 formulations of methoprene-based larvicides (Altosid XR 150-day Briquets, Altosid 30-day Pellets, Altosid 30-day Granules) in 2017 using a pass/fail evaluation criterion, in which emergence of a single adult from pupae collected from the basin constituted a control failure. Over the course of the 16-week study, basins receiving the 150-day briquets were treated once and basins receiving the pellet and granular formulations were treated every 4 weeks, with the first treatment occurring during the last week of May. Untreated basins were also observed for comparison with the treated basins. Over the course of the study, adult mosquitoes emerged from pupae collected in 94.2% of the untreated basins that contained pupae. All of the formulations evaluated in the study demonstrated some degree of control compared with the untreated basins, with pupae successfully emerging as adults in 64.6%, 55.5%, and 21.8% of samples from 150-day briquet, 30-day tablet, and 30-day pellet–treated basins that contained pupae, respectively. Pellets reapplied every 28 days provided significantly more effective control than the other formulations. The simple pass/fail criterion for evaluating control effectiveness proved to be a useful procedure for comparing effectiveness to untreated basins and among treatments.

KEYWORDS: Methoprene, mosquito, catch basin, larvicide

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Introduction

Mosquito control programs in urban settings have routinely applied larvicides to storm water catch basins for decades.[1,2] These efforts are made to reduce *Culex* spp. mosquitoes, particularly *Culex pipiens* (Lin.) and *Culex quinquefasicatus* (Say), that may contribute to West Nile virus infections.[3] The North Shore Mosquito Abatement District (NSMAD), located in northeastern Cook County, Illinois, applies larvicides to approximately 40 000 catch basins each year from June through September. As suggested by the US Centers for Disease Control and Prevention (CDC),[4] the NSMAD has performed routine quality control evaluations of these larvicides.[5–9]

To better standardize these quality control evaluations across larvicide formulations and to simplify procedures for field technicians, the NSMAD has adopted a "pass/fail" criterion for evaluating bacterial-based catch basin larvicides such as those containing spinosad or *Bacillus sphaericus* that kill larvae shortly after contact with or ingesting the active ingredient.[9] In the NSMAD quality control procedure, if no larvae or only early-stage larvae are present in 2 dip samples of a basin, this is considered evidence of effective control or "pass" in that basin. If late-stage larvae (third or fourth instar) or pupae are present, this is considered evidence of a control failure or "fail," indicating that retreatment of that basin would be necessary. If >25% of catch basins fail in a treatment area, NSMAD procedure is to retreat all catch basins in the area. This "pass/fail" criterion has been found to be operationally useful and is based on procedures used in previous work.[9–11] It is also consistent with guidance from the World Health Organization (WHO)[12] that states, "The frequency of larvicidal treatment is determined based on the reappearance of fourth instar larvae or pupae, in the case of common larvicides and bacterial larvicide products." This implies that retreatment is recommended when any adult emergence occurs. The WHO guidance goes on to state that retreatment should be based on the day "inhibition of emergence falls below 90% for IGRs [insect growth regulators]." Therefore, evaluations of IGR larvicides, such as methoprene, commonly involve collecting samples of pupae from IGR-treated habitats and holding them to determine what proportion successfully undergo pupal-to-adult eclosion.[10,11,13–20] This also implies that retreatment is recommended when >10% of the pupae in an IGR-treated basin emerge. This is a less stringent threshold for retreatment compared with that recommended for the direct-kill larvicides, where no adult emergence would be acceptable. For example, if there were 1000 mosquito pupae in an IGR-treated basin, 100 mosquitoes could emerge and the basin would not meet the threshold for retreatment using the WHO criteria. Considering the thousands of basins a mosquito control program may routinely treat and the large numbers of mosquitoes found in many of the basins, survival of 10% of pupae to the adult stage may not achieve desired control outcomes.

The objectives of this study were to evaluate 3 methoprene-based products (one 150-day duration and two 30-day duration formulations) to determine how control effectiveness in catch basins compared with the maximum control durations stated on the product labels, to determine whether retreatment at label-specified intervals provided effective control, and to develop and use a "pass/fail" criterion for evaluating IGR catch basin larvicides similar to that used with bacterial larvicides elsewhere.[9–11]

Methods

In total, 40 catch basins where chosen from each of 2 villages (42°03′04.2″N 87°46′16.2″W and 42°04′39.0″N 87°43′42.6″W) within the NSMAD operational area for weekly monitoring. These basins were specifically chosen because they were among the most productive basins monitored the prior year in an evaluation of 5 non-IGR larvicides.[9] The hope was that these basins would be highly productive for consistent collection of sufficient numbers of pupae needed for the methoprene evaluation. During the last week in May 2017, 10 basins were treated with Altosid XR Briquets (up to 150-day maximum label duration, 2.1% methoprene), 10 basins treated with Altosid Pellets (up to 30 days, 4.25% methoprene), 10 treated with Altosid XR-G Granules (up to 30 days, 1.5% methoprene), and 10 left untreated for each of the 2 village locations (Altosid formulations produced by Central Life Sciences, Schaumburg, IL, USA). Basins were treated on May 30, 2017, with a single briquet (1.05 g methoprene/application), 1 tablespoon of pellets (10 g of pellets or 0.425 g of methoprene per application), or 1.5 teaspoons of granules (10 g of granules or 0.3 g of methoprene per application). Pellet and granular-treated basins were retreated every 4 weeks. After the initial granular application, the amount was increased to 1 tablespoon (20 g of granules or 0.6 g of methoprene per application) to increase the amount of active ingredient applied with the granules to a similar amount provided by the pellet formulation.

All 80 catch basins were sampled weekly for 16 weeks from the second week of June through the last week of September. Sampling was accomplished by removing the lid grate and taking 2 dips with a standard 350-mL dipper. The dip samples were observed to determine whether pupae were present in the basin. In a similar protocol to Phillips et al[15] if one or more pupae were present in a basin's dip samples, these pupae were placed with sump water in a Dart Solo UltraClear 16 oz (473 mL) Clear PET (polyethylene terephthalate) Plastic Squat Cold Cup and covered with a Dart Solo Clear Flat Lid with Straw Slot. All basins received 2 dip samples during weekly monitoring. When pupae were observed in at least one of the 2 dip samples, subsequent dips were taken in an attempt to collect at least 10 pupae.

The sample date and unique catch basin identifier number were recorded on the collection cup with a permanent marker. The number of pupae collected in each cup was also recorded. Cup samples were brought back to the NSMAD laboratory and held at approximately 22°C for 48 hours. During that time, cups containing the pupae were monitored daily for the presence of adults. If no adults successfully emerged within 2 days (100% emergence inhibition), this was considered evidence of effective control and the associated basin was scored as a "pass." If at least one adult was observed to have successfully emerged within a cup (eg, resting on the cup wall, flying) within 2 days, the associated basin was scored as a "fail." The number of cup samples failing for each of the 4 basin treatments (untreated, briquets, pellets, and granules) was recorded and compared across treatments. During 4 of the monitoring weeks, samples of mosquito larvae and pupae were collected from 1 to 2 of the untreated basins and placed into 21 cm × 12 cm rearing containers ("Mosquito Breeders": BioQuip Products) and allowed to emerge. The number, sex, and species of emerged adults were recorded. The total proportion of weekly samples scoring "fail" was compared among treatments using a χ^2 test for comparison of proportions.[21]

Results

Over the 16 weeks of monitoring, pupae were found in 421 of the 1249 (33.7%) basin visits to the 80 study basins. More basins containing pupae were found during the second half of the study when the rainfall decreased and *C pipiens* abundance typically increases (data not shown). On average, 5.8 pupae ± 0.2 SE were collected in each cup with a range of 1 to 53; the mode was 5 pupae (N = 65). Most cup samples contained 9 or less pupae (N = 442, 91.7%). All mosquitoes obtained from the 4 collections that were reared to adults were identified as *C pipiens* or *Culex restuans* based on morphological characteristics. Thus, it is likely that all of the pupae that were collected in the catch basins were one of these species.

Overall, pupae were found and collected for cup samples in 81 of 309 (26.2%) untreated basin visits performed during the 16 weeks of the study. This percentage was lower than expected, as study basins were specifically chosen because they were among the most productive in the previous year. In the samples from untreated basins, adult mosquitoes emerged from pupae in more than 90% of these samples within 2 days, meeting the criteria for a "fail" score and suggesting that most pupae from untreated basins successfully emerge as adults (Table 1). Among the insecticide-treated basins, samples from briquet basins had the highest percentage of samples scoring "fail" (64.6%) followed by granules (55.5%) and then pellets (21.8%). All of "fail" percentages from the 3 treatments were

Table 1. Results of catch basin inspections showing the number of basin samples containing pupae and the number of samples from which adults successfully emerged.

WEEKS AFTER TREATMENT	UNTREATED[a], FAIL/TOTAL	150-DAY BRIQUETS[a], FAIL/TOTAL	TREATMENT ROUND[a]	UNTREATED[b], FAIL/TOTAL	30-DAY[b] PELLETS, FAIL/TOTAL	30-DAY[b] GRANULES, FAIL/TOTAL
4	12/13	8/18	1	12/13	4/17	13/21
8	21/22	17/31	2	9/9	5/12	13/16
12	39/40	37/54	3	18/18	9/23	23/37
16	81/86	64/99	4	42/46	4/49	26/61
			Total	81/86	22/101	75/135
Percent of total samples scoring “fail”[c]	94.2[d]	64.6[e]		94.2[d]	21.8[f]	55.5[e]

Results from basins treated with 150-day briquets are shown as cumulative numbers of the course of the 16-week study. Results from basins treated with the 30-day formulations are shown as the totals for each of the four 4-week treatment rounds. Results from the untreated basins are shown for both groups but are temporally separated to allow comparison with each treatment schedule.
[a]Cumulative number of basins failing/sampled at the end of each 4-week period through the 16-week study.
[b]Number of basins failing/sampled for each 4-week treatment round.
[c]Percentages with different letters are significantly different ($P<0.05$) within the row.

significantly less than untreated basin. Compared with samples from untreated basins, there was evidence of some degree of control with all of Altosid formulations.

Discussion

Using this study’s simplified “pass/fail” evaluation protocol, all 3 Altosid formulations were observed to have significantly lower “fail” scores than untreated basins, suggesting that some degree of control was achieved for all the larvicides during the 16 weeks of the study. Pellet formulations had the lowest total percentage of fail scores and therefore appeared to provide the best control of the 3 formulations. The potential reasons for these differences in control effectiveness were not investigated as part of this study. Briquet formulations may be more prone to becoming completely flushed out of basin sumps or completely buried in sump debris to a greater degree than pellet or granule formulations that spread the active ingredients over many pellets or granules instead of a single briquet. During 2 weeks in August, it was possible to see the entire bottom of 5 shallow Altosid Briquet–treated basins and a complete search for the applied briquets was performed. Four of the 5 appeared to be missing briquets, highlighting a potential issue for this type of formulation. This contradicts suggestions from laboratory simulations that pellets are more likely to be flushed from catch basins than briquets[22] but is consistent with field observations from others indicating that briquets and tablets are frequently lost from catch basins, presumably from flushing or other mechanisms.[7,8]

As methoprene has a delayed control effect on mosquitoes, evaluating the effectiveness of Altosid formulations cannot be done through more simple and direct on-site observations of catch basin dip samples, as performed in studies of bacterial-based formulations.[9–11] Instead, evaluations of Altosid and other IGR-based larvicides require the collection and rearing of samples of larvae and pupae from basins. This study’s “pass/fail” protocol was specifically designed to be a simpler and more stringent alternative to trials used to determine what specific proportion of a sample successfully undergoes pupal-to-adult eclosion. As is, this study’s IGR protocol requires 100% emergence inhibition in a sample to achieve a “pass” score and thus is more in line with the implied 100% emergence inhibition expected for bacterial larvicides suggested by the WHO[12] and used in other studies.[9–11] Operationally, another benefit of this study’s simplified IGR evaluation protocol is that it is not necessary to leave basins untreated to identify if an area’s catch basins need to be retreated. As noted previously, if >25% of larvicide-treated catch basins fail in a treatment area, NSMAD procedure is to retreat all catch basins in the area. In addition, the study’s protocol using 16-oz plastic cups to transport and rear pupae did not appear to cause undue mortality of pupae, with more than 90% of untreated samples scored as “fail” (at least 1 adult successfully emerged) within 2 days.

A major challenge with this study was that during at least the first half of the study, it was difficult to find pupae in the study basins. Increased rain events may have temporarily reduced the presence of mosquitoes in many of the study catch basins[17,20,23–27] and thus decreased the number of weekly samples collected during that time period. When considering the mosquito production in this study’s untreated basins, only a little more than one-fourth of the basin visits yielded pupae over the entire study period. This seemingly low degree of mosquito production in basins is further accentuated by the fact that these basins were specifically chosen for this study because they appeared to be highly productive the previous year. For comparison, during the previous year and in the same study area, 1 or more pupae were found in 43.8% (113/258) of the basin visits to untreated basins. This variability in mosquito production highlights the changes in relative importance catch basins may have in producing local populations of *Culex* mosquitoes.

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Author Contributions

JEH, MH, ABR, and RSN conceived and designed the experiments. JH and RSN analyzed the data. JH wrote the first draft of the manuscript. ABR, MH, BH, AM, HJ, and RSN contributed to the writing of the manuscript and agree with manuscript results and conclusions. All authors reviewed and approved the final manuscript.

Disclosures and Ethics

As a requirement of publication, authors have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality, and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. The external blind peer reviewers report no conflicts of interest.

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