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OPERATIONAL NOTE

STANDARDIZED BACK CHECKS OF CATCH BASIN LARVICIDES ACROSS THREE MODES OF ACTION IN THE NORTH SHORE SUBURBS OF CHICAGO, USA

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ABSTRACT. During June through September 2018, the North Shore Mosquito Abatement District evaluated a quality control protocol for larvicide-treated catch basins that allowed for comparisons of larvicides with different modes of action. Results suggest that when applied at a rate of 2 tablespoons (approximately 20 g of product) per basin, these larvicides (VectoLex® FG *Lysinibacillus sphaericus*, Natular™ G30 Spinosad, and Altosid® Pellets Methoprene) could reduce the need for retreatment for up to 4 wk or longer. Using this same protocol, Spheratax® SPH (50G) *L. sphaericus* was applied to a subset of approximately 900 basins and met the threshold for retreatment 1.1 wk postapplication. Despite these larvicide applications, pupae continued to be observed in non-Altosid® treated basins, suggesting 100% mortality may not be attainable.

KEY WORDS Back check, catch basin, effectiveness, larvicide, mosquito

Among mosquito control programs, there is currently no widely accepted standardized quality control protocol for evaluating the effectiveness of larvicides, nor is there agreement on what constitutes an acceptable level of control. During the summer of 2018, the North Shore Mosquito Abatement District (NSMAD) utilized a standardized "pass/fail" quality control protocol to evaluate the effectiveness of larvicides applied to approximately 40,000 catch basins as part of routine operations. This protocol is based on that described by Nasci et al. (2017) and functions by scoring basins as "pass" or "fail" based on the presence of late-stage larvae and pupae or, in the case of larvicides utilizing methoprene, the successful emergence of an adult from treated larvae. These quality control evaluations or "back checks" are used to help guide decisions of timing and locations of larvicide retreatments and to compare effectiveness between larvicides and treatment methodologies.

For the 2018 season, 3 larvicides (VectoLex® FG [Vecto; Valent BioSciences, Libertyville, IL], AI: *Lysinibacillus sphaericus* Meyer and Neide, Natular G30 [NG30; Clarke Mosquito Control Products, Inc., Roselle, IL] Spinosad, and Altosid® Pellets [Altosid; Wellmark International, Schaumburg, IL], AI: methoprene) were applied to approximately 40,000 catch basins distributed across 58 operational treatment maps that comprise the ~70 square miles of the NSMAD. Vecto was applied to 19,543 basins in maps in the southwest and upper portion of the NSMAD, Altosid was applied to 6,010 basins in maps in the central portion, and NG30 was applied to 16,293 basins in maps located in southeast portion. Maps were retreated with larvicides every 3 to 6 wk. In mid-July, approximately 900 basins within a single map received an application of Spheratax® SPH (50G) *L. sphaericus* to evaluate this product in comparison to the other 3 larvicides. All larvicides were applied at a dose of 2 tablespoons (approximately 20 g) per basin.

The active ingredient of both Vecto and Spheratax® SPH (50G; Advanced Microbiologics, LLC., Mitchell, SD) is *Bacillus sphaericus* strain 2362, serotype H5a5b, which has been reclassified as *L. sphaericus* (Ahmed et al. 2007). *Lysinibacillus sphaericus* kills larvae by destroying their midgut when they ingest toxins in parasporal bodies attached to the bacterial spores (Su et al. 2018). Spinosad is the active ingredient (AI) in Natular™ G30 and kills larvae by overexcitation of the nervous system when ingested or upon contact (Hertlein et al. 2010, Raghavendra and Velamuri 2018). Methoprene, the AI in Altosid pellets, is a juvenile hormone analogue that hinders maturation. Thus, most mortality becomes evident when mosquitoes fail to emerge successfully as adults from pupae, days after the larvicide was applied (Mulla 1995). For the 2018 NSMAD quality control back checks of basin treatments, up to 20 basins in 4 to 9 operational treatment maps per treatment material were sampled each week with 2 dips of a standard 350-ml dipper. For basins treated with any of the larvicides, if no larvae or only 1st or 2nd instars were observed, the basin was scored as a "pass."

Vecto-, NG30-, or Spheratax-treated basins were scored as "fail" if late-stage (3^{rd}- and 4^{th}-stage) larvae or pupae were observed in the 2 dips taken during sampling. For basins treated with Altosid

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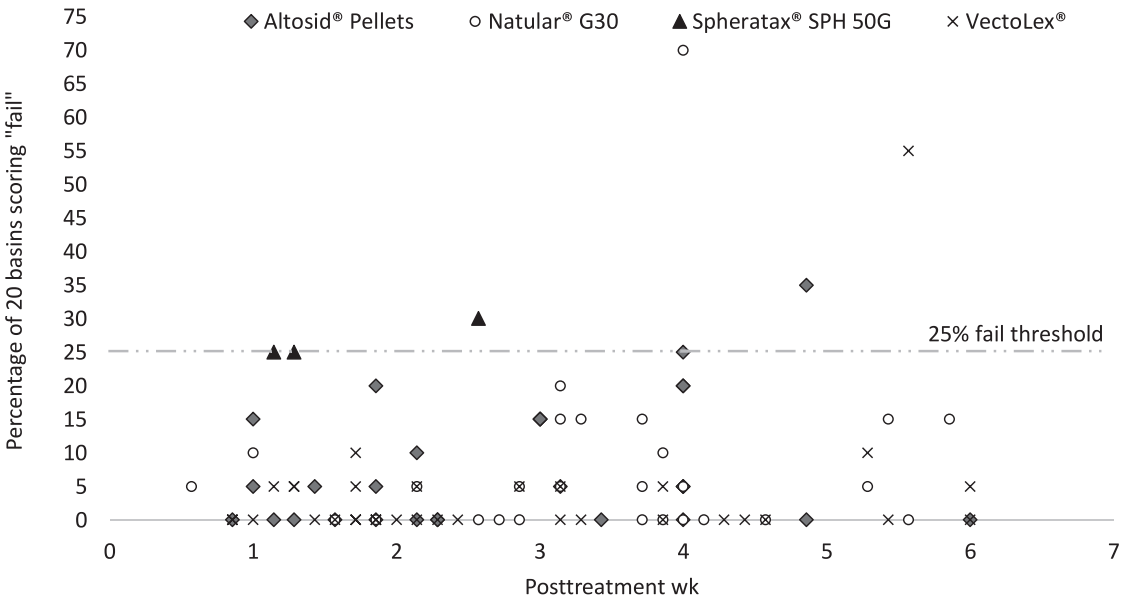

Fig. 1. Percentage of basins scoring "fail" during back checks of 20 catch basins across the North Shore Mosquito Abatement District (NSMAD) during June to September 2018. The 25% fail threshold used by the NSMAD indicates the need for retreatment.

pellets, if late instars and/or pupae were present in these dip samples, they were placed in a Dart Solo UltraClear 16-oz. clear polyethylene terephthalate Plastic Squat Cold Cup along with water from the catch basin, and covered with a Dart Solo Clear Flat Lid with Straw Slot Cup. In these cases, 2 to 3 extra dip samples were taken in an attempt to get at least 10 late instars or pupae. Cup samples were brought back to the laboratory and held at room temperature (approximately 22°C) for 4 days. If any adult successfully emerged within a cup during the 4-day holding period, the basin was scored as a "fail." If no adults emerged from a cup sample, the basin was scored as "pass."

This "pass/fail" scoring is somewhat different than past effectiveness studies of insect growth regulators (IGRs) such as methoprene. These often focus on comparisons of emergence inhibition (Mulla et al. 1971, WHO 2005). However, we've found that catch basin water can be extremely murky and laden with debris, hindering accurate counting of pupae and/or 4th instars found in dip samples for emergence inhibition trials. Aside from being a bit more simple and less time-intensive alternative, the pass/fail scoring also allows us to evaluate both direct-kill and IGR larvicides similarly, requiring what amounts to 100% emergence inhibition from basin samples for both groups of pesticides.

Currently, the NSMAD considers 25% or more of the 20 sampled basins scoring "fail" to be an indication of widespread control failure and the need for retreatment. By utilizing a standardized threshold for determining pass or fail, new control products or methodologies can be quickly and reliably compared. Additionally, for all basins treated by Vecto, NG30, or Spheratax, the number of pupae found in the 2 monitoring dips per sampled basin was recorded to help infer the amount of mosquitoes generally produced in larvicide-treated basins.

Aside from Spheratax-treated basins, all 3 main larvicide formulations (Vecto, Altosid, and NG30) appeared to keep the number of basins scoring fail below the 25% threshold for up to 4 wk or longer (Fig. 1). During all 3 monitoring events of Spheratax-treated basins (1.1, 1.3, and 2.6 wk posttreatment), the proportion of basins scoring "fail" met or exceeded the 25% retreatment threshold indicating widespread control failure. These results were somewhat surprising given those same basins had been treated with Vecto 4.6 wk previously. As seen in these 2018 results and in 2017 (Harbison et al. 2018), Vecto can remain effective (i.e., <25% of sampled basins containing late-stage larvae or pupae) in some areas for 5 wk or longer. Thus even with the potential for residual and additive control from the prior Vecto treatment, Spheratax still appeared to have a shorter duration of control relative to the other 3 larvicides.

Most commonly (32 out of 64 back checks), no pupae were found in non–Altosid-treated basins during monitoring (Fig. 2). When considering basins not treated with Altosid, the number of pupae increased as the proportion of basins scoring "fail" in a map increased as expected. Only on 2 separate back checks did the total number of pupae observed in a 20-basin back check exceeded 6, once when the percentage of basins "failing" was 55% (9 total pupae, 5.6 wk posttreatment, Vecto) and once when

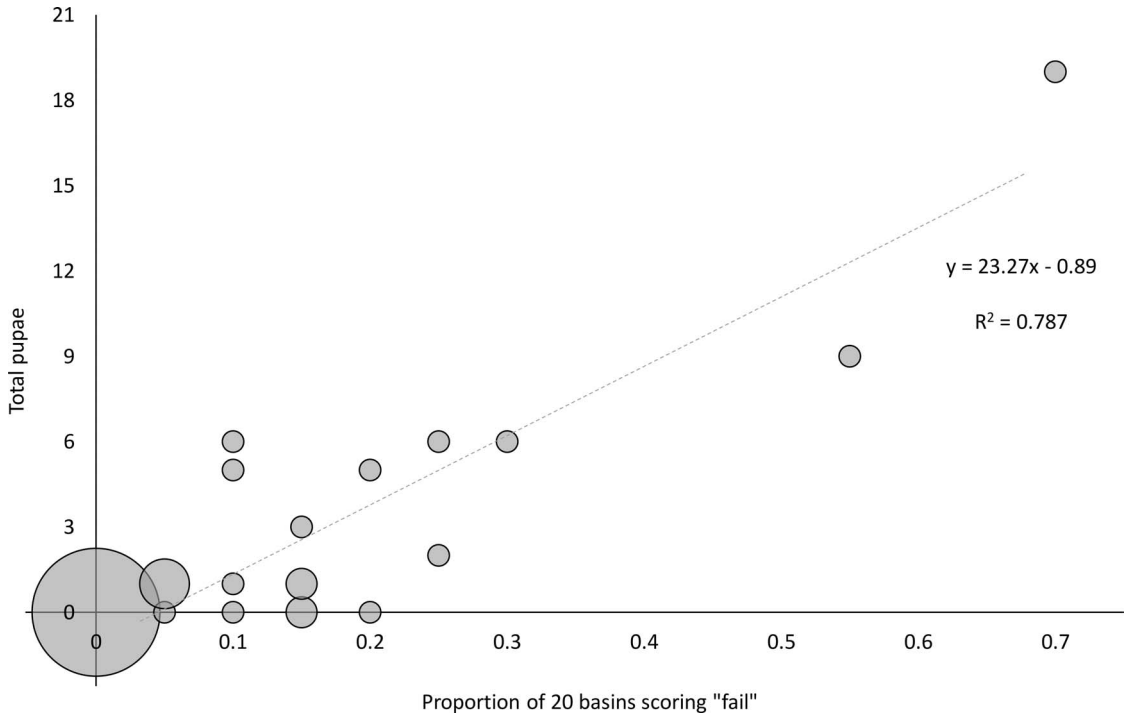

Fig. 2. Total pupae observed during back checks (inspections of 20 basins per map) of 31 operational maps treated with VectoLex® Natular G30®, or Spheratax® SPH (50G) by the proportion of basins scoring "fail" during June to September, 2018. Size of bubbles corresponds with the frequency of observations. Most (32 of 64) observations were of 0 pupae found in 0 of 20 basins scoring "fail" or 100% scoring "pass."

the percentage "failing" reached 70% (19 total pupae, 4 wk posttreatment, NG30). On average, 1.1 total pupae (± 0.37, $n = 64$) per 20 basin-visits was observed in the back checks of Vecto-, NG30-, and Spheratax-treated maps. This suggests that it may be possible to raise the retreatment threshold from 25% without significantly increasing the amount of pupae produced in basins. However, more observations are needed to confirm this. These results also highlight the idea suggested by Nasci et al. (2017) that 100% control (no pupae) in treated catch basins is an unrealistic expectation. The use of the "pass/fail" protocol and the 25% failure threshold allowed for a straightforward comparison of Spheratax, a larvicide new to NSMAD, to the other 3 larvicides. Standardized evaluation protocols, such as the one utilized by Nasci et al. (2017) can be useful tools to inform operational decisions and to generate comparable data across mosquito control programs.

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