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EXPERIMENTAL USE OF NATULAR™ XRT TABLETS IN A NORTH SHORE SUBURB OF CHICAGO, IL

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ABSTRACT. In the northern suburbs of Chicago, stormwater catch basins are the primary source of vector mosquitoes targeted by the local mosquito abatement district, and therefore effective catch basin larvicides are needed. From June to September 2011, Natular™ XRT was applied at label rate to a cluster of 20 catch basins and compared to a cluster of 20 untreated basins all within a small 0.21 km^2 area of catch basins known historically to have high levels of mosquitoes. All monitored basins held immature stages of mosquitoes at least once; however, significantly fewer immatures overall were observed from Natular-treated basins than in untreated basins during 14 wk of monitoring. During the study a portion of Natular-treated catch basins was cleaned as part of scheduled maintenance, and this appeared to remove both the larvicide and any control effects. Better timing of catch basin maintenance events is suggested, particularly if, as the results of this study suggest, a single larvicide treatment may be effective for an entire season.

KEY WORDS Spinosad, larvicide, catch basin, mosquitoes, maintenance

INTRODUCTION

There is growing evidence that in many areas of the USA stormwater structures are the predominant sources of vector mosquitoes (Stockwell et al. 2006; Allen and Shellito 2008; Metzger et al. 2011, 2012). In the Chicago, IL, metropolitan region, an area that encompasses more than 2,500 km^2 (1,000 mi^2), storm drains and catch basins are the primary sources of potentially disease-carrying *Culex* mosquitoes (Tedesco et al. 2010). As such, mosquito control efforts in the city of Chicago focus almost exclusively on larviciding upwards of 200,000 stormwater catch basins annually to reduce the incidence of West Nile virus (WNV) (CDPH 2007). For the North Shore Mosquito Abatement District (NSMAD), the local mosquito abatement agency serving the predominately suburban area (205 sq. km [79 sq. mi]) just north of Chicago, catch basins are also the primary target of control operations. The bulk (approximately 75%) of NSMAD control work consists of applying larvicides to up to 60,000 catch basins once or twice a season. These applications generally begin in late May and June and, if needed, in late July and August. Because a significant portion of the NSMAD annual budget can be spent on the purchase and application of catch basin larvicides, it is preferable to find a treatment that remains effective throughout an entire season without the need for a 2nd application.

A recent study in Connecticut demonstrated that catch basins treated with Natular™ XRT (Clarke Mosquito Control Products, Roselle, IL), a tablet formulation of 6.25% spinosad, held generally lower numbers of immature mosquitoes for 8 wk (Anderson et al. 2011). Spinosad, a mixture of 2 neurotoxins (Spinosyn A and D from *Saccharopolyspora spinosa* Mertz and Yao), has been reviewed elsewhere for use as a larvicide (Bond et al. 2004, Hertlein et al. 2010, Anderson et al. 2011). In light of these results there was a desire to evaluate the potential of Natular to reduce numbers of immature mosquitoes in NSMAD catch basins using a small experimental study. The objective of this study was to compare a cluster of 20 catch basins treated with Natular at label rate to a cluster of 20 catch basins with no treatment by monitoring both numbers of immatures in catch basins and numbers of adults captured in a gravid trap placed among each cluster of catch basins. Additionally, maintenance of some basins during the study period allowed for a simple assessment of catch basin cleaning on the larvicide's effectiveness.

MATERIALS AND METHODS

All trials were conducted between June and September 2011 in a residential area located near the center of the NSMAD service area. The section was divided into 2 similarly sized zones (approximately 0.21 km^2 [0.08 mi^2] each). The study area was chosen because historical data and prestudy monitoring suggested that catch basins installed in this section generally held higher numbers of mosquitoes during summer months than other parts of the district and thus could allow for better detection of any variance in mosquito numbers. The area also had an added benefit of being located away from high-traffic roads, allowing for safer access of basins. On June 13, each catch basin and belowground vaults (a total of 119 structures) in the southern half received a single Natular tablet (as per label;

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Fig. 1. Application of a Natular™ XRT tablet to a study catch basin.

Fig. 1), and all catch basins and belowground vaults (a total of 92 structures) in the northern portion received no larvicide treatment (Fig. 2). Because maintenance (e.g., vacuum pumping) of catch basins is not uniformly performed throughout the district and many parts of the district are prone to flooding, each half was chosen so catch basins within each section could only backflow or overflow into catch basins of the same experimental treatment, reducing the risk of cross-contamination. Twenty catch basins from each half that permanently held water were monitored weekly by taking 2 dip samples from each basin. All catch basin sumps were approximately 1 m diameter with water surface generally within 0.6 m of ground level. The dip samples consisted of using a standard 350 ml dipper and recording the number of larvae and pupae collected by each dip. As per common sampling protocol, time was given after removing the catch basin grate and between dips to allow for resettling of immatures. During each weekly monitoring, 1 to 24 4th instars from 1 to 3 basins in each zone were collected and identified to species using Darsie and Ward (2005). Immature monitoring continued for 14 wk after treatment, with the last monitoring event occurring on September 19.

Because the primary adult mosquito surveillance data for the NSMAD are aboveground gravid trap catches, in the week following Natular application, a Reiter/Cummings gravid trap (Reiter 1987) was placed within a right-of-way along the western border in approximately the middle of each experimental zone to observe if trends in catch basin numbers would be similar to any trends observed in gravid trap catches. If trends in aboveground and belowground populations were indeed comparable, this might suggest that aboveground gravid trap catches may adequately predict mosquito populations in nearby catch basins and preclude the need for more labor-intensive surveillance such as dip samples. Each catch basin was located within 380 m (1,240 ft) of its respective trap, and each gravid trap was run for 24 h twice a week. All captured adult mosquitoes were removed, counted, and identified using Darsie and Ward (2005) after each 24 h sampling period.

Gravid trap monitoring continued for 7 wk after treatment until most catch basins and belowground vaults were cleaned during a maintenance event initiated by the local municipality from August 3 to 5. Basins and vaults were cleaned by vacuum pumping most water and debris out of sumps using a Vactor truck (Federal Signal Corporation, Streator, IL). This cleaning occurred in all basins and vaults in the untreated area and all but 18 basins and 6 vaults in the Natular-treated area (6 of these "uncleaned" basins received weekly monitoring). Cleaning was performed as part of routine maintenance and in response to relatively high amounts of recent precipitation and flooding. When possible, efforts were made to visually verify that the vacuum pumping did remove Natular tablets. Because no larvicide was placed in untreated basins, these structures were still considered to be untreated after cleaning. On June 13, using truck-mounted equipment, the NSMAD performed ultra-low-volume spraying of the area using Anvil® 2+2 (Clarke Mosquito Control Products). The study area was included in the adult mosquito control operation map. Numbers of mosquitoes and WNV-positive pools of mosquitoes did not warrant further adult mosquito control in the study area during the rest of the 2011 season. Precipitation data were collected from a weather station of the NOAA National Weather Service Forecast Office located at Chicago's O'Hare Airport (http://www.nws.noaa.gov/climate/index.php?wfo=lot).

Statistical analyses were conducted using Stata 9.2 (StataCorp, College Station, TX). Results of a Shapiro-Francia test for non-normality suggested observations of immatures per dip were not normally distributed; therefore nonparametric tests were appropriate. Kruskal-Wallis rank tests were used to compare differences in the pooled mean ranks of immatures collected per dip between untreated catch basins and Natular-treated basins that were not cleaned out over the entire study period. These tests were also used to compare pooled mean ranks of immatures collected per dip during the last 7 wk of the study among untreated basins and cleaned and not cleaned Natular-treated basins. Associated values of $P < 0.05$ were considered statistically significant.

RESULTS

During the study period all monitored basins were observed at least once to hold immature

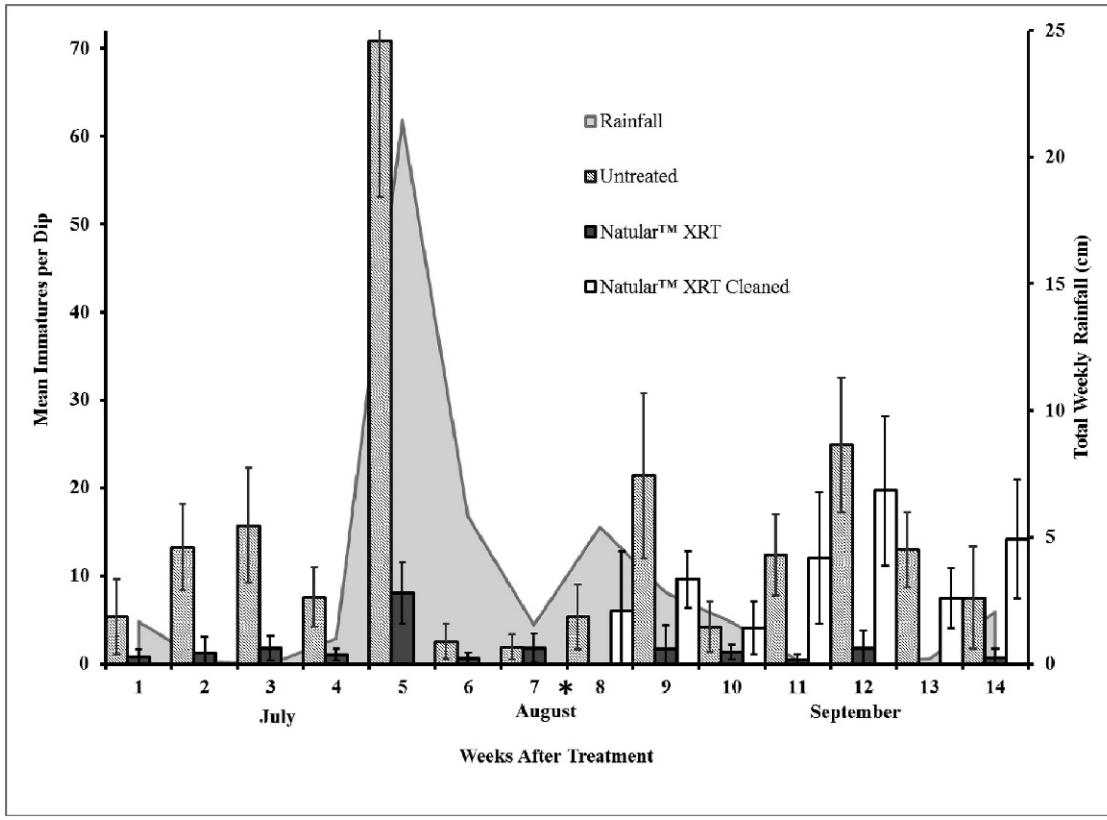

Fig. 2. Comparison of average immatures per dip (with 95% CI) from weekly monitoring of 20 catch basins treated with Natular XRT tablets on June 13, 2011, to 20 untreated catch basins in the test area with weekly rainfall from June 21 to September 19, 2011. * indicates between weeks 7 and 8, 14 Natular-treated basins and all untreated basins were cleaned by vacuum pumping sumps.

mosquitoes; however, Natular-treated basins consistently held fewer immatures per dip than basins in the untreated area (Fig. 2). A total of 8,251 immatures (2,165 4th instars [26.2%] and 775 pupae [9.4%]) were found in dip samples from the 20 untreated catch basins and 2,736 (772 4th instars [28.2%] and 120 pupae [4.4%]) collected from the 20 Natular-treated basins. The pooled average immatures per dip for untreated basins was 14.7 ± 2.3 (95% CI, n = 560) and 1.9 ± 0.6 (95% CI, n = 364) for basins treated with Natular that were not cleaned (H = 157.39, 1 df, $P < 0.001$). A total of 526 4th instars were identified from 79 samples from 32 basins. From these samples 453 (86%) were *Culex pipiens* L. and 73 (14%) were *Culex restuans* Theobald. *Culex restuans* dominated samples for the first 2 wk (100% in week 1 and 79.2% in week 2 of total larvae identified) and then disappeared by the seventh wk (the 1st week in August).

Three to 5 days after 34 monitored basins were cleaned; all held water, and 23 (70.6%) of these held immatures. No tablets were observed during visual inspections of the 14 cleaned Natular-treated basins, and weekly averages of immatures from these basins were similar to untreated basins, suggesting tablets were removed during cleaning, and no residual treatment remained (Fig. 2). For the final 7 wk of monitoring, the average of immature mosquitoes collected per dip for untreated basins, cleaned Natular basins, and basins where Natular tablets remained was 12.7 ± 2.4 (95% CI, n = 280), 10.5 ± 2.3 (95% CI, n =196), and 0.85 ± 0.5 (95% CI, n = 84), respectively. A significant difference in mean ranks was found between pooled numbers of immatures per dip in Natular basins and cleaned Natular basins (H = 64.15, 1 df, $P < 0.001$) and between Natular basins and untreated basins (H = 74.39, 1 df, $P < 0.001$). No significant difference in mean ranks was found between cleaned Natular basins and untreated basins (H = 1.04, 1 df, P = 0.30)

A total of 315 adults were collected from both traps during the first 7 wk of the study; 57 females (18.1%) were identified as *Cx. restuans,* 239 females (75.1%) were *Cx pipiens*, and 1 female (0.3%) as *Aedes triseriatus* Say. Eighteen males (5.7%) were also collected in traps but not identified to species. *Culex pipiens* was found

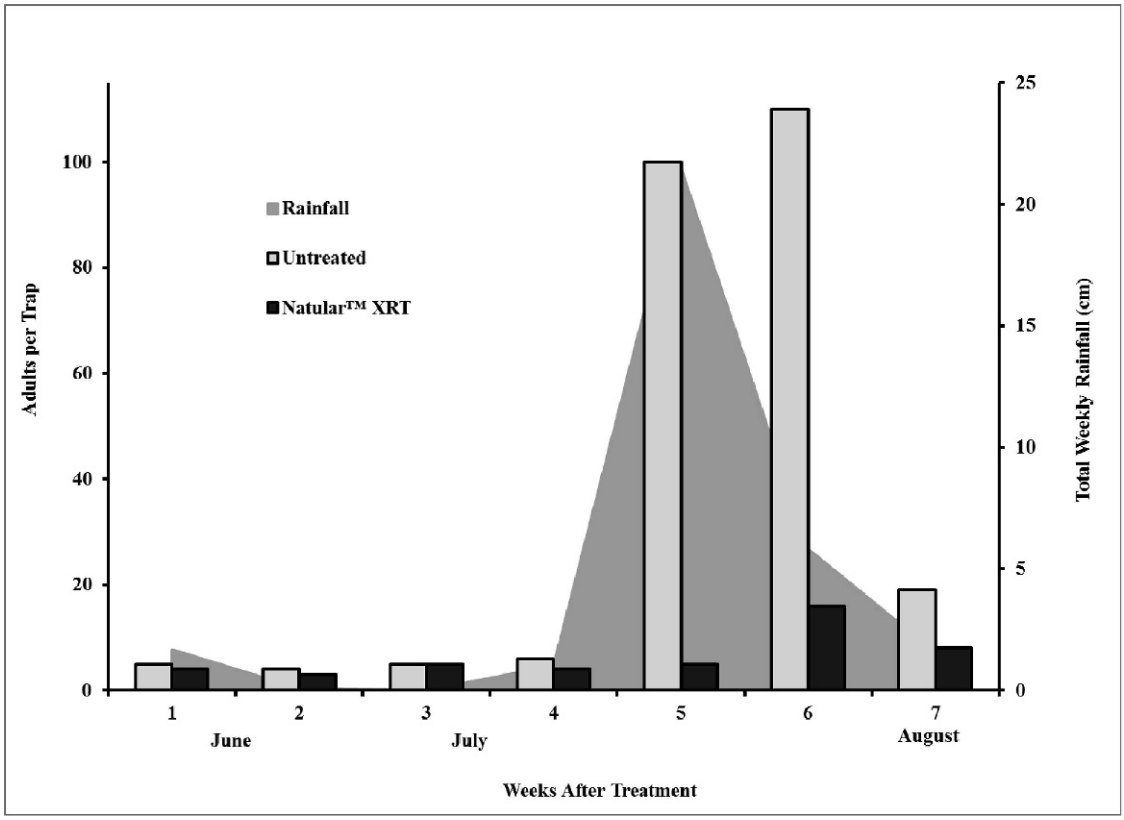

Fig. 3. Comparison of weekly trap catches from a gravid trap within an area treated with Natular XRT tablets to one located in an adjacent untreated area (approximately 0.21 km^2 each) and weekly rainfall from June 21 to August 3, 2011.

predominately in the 7 wk of trap catches except for the 1st wk, in which 55.5% of adults were identified as *Cx. restuans*. Little difference was observed in adult catches between the Natular-treated area (mean 2.9 ± 2.7, 95% CI, $n = 14$) and the untreated area (mean 18.3 ± 16.7, 95% CI, $n = 14$) until the last 3 wk of gravid trap monitoring (Fig. 3).

DISCUSSION

Since 1928 the NSMAD staff has treated catch basins with a variety of products to prevent the spread of mosquitoes and the diseases they transmit. Today the NSMAD catch basins generally receive larvicide treatments once or twice a season, depending on the estimated size of local mosquito populations and incidence of WNV. Finding a larvicide that can be effective for the entire season could reduce the need for a 2nd treatment and its associated costs. Throughout the 14 wk of this study, monitored catch basins holding Natular tablets consistently were observed with fewer immature per dips than untreated basins, suggesting a single treatment of this larvicide may indeed be sufficient over 3 months and even an entire season (usually May to October). Additionally, lower percentages of late immature stages (4th instars and pupae) were observed in treated than untreated basins, suggesting a lower eclosion rate in treated basins. What still remains to be better clarified is the nontarget effects of the larvicide. Although Natular meets the US Department of Agriculture's National Organic Standard and in 2010 Clarke Mosquito Control Products received a Presidential Green Chemistry Challenge Award from the US Environmental Protection Agency for the product (EPA 2010), more studies are needed to identify any potential effects this larvicide formulation of spinosad may have on nontargeted aquatic species. Although the polluted nature of stormwater collected in basins reduces species diversity found in these structures, such studies would be of particular interest in areas like the Chicago region that can be prone to flooding and catch basin overflow.

There was less of an apparent difference noted when comparing adult numbers captured in the gravid traps placed within each experimental zone. The gravid trap within the Natular-treated area had lower numbers only during the last 3 wk

of the 7 wk adult monitoring period. While useful for estimating adult mosquito numbers and testing pooled mosquitoes for WNV, the aboveground Reiter gravid traps may not be as sensitive to changes in belowground immature numbers as dip samples. This idea is supported by the observation of an *Ae. triseriatus* female in one gravid trap catch, a species that was not found in dip samples and generally not associated with catch basins. Our results suggest aboveground gravid traps may not accurately predict *Culex* populations in nearby catch basins despite being a commonly utilized mosquito and WNV surveillance tool. If this occurs on a larger scale, such a finding could be important for mosquito control programs like the NSMAD that focus much of their control operations on catch basins yet rely heavily on aboveground adult *Culex* trap catches for mosquito surveillance. Not surprisingly, *Cx. pipiens* dominated both larval samples and adult catches. This species is the most common mosquito found in the NSMAD catch basins during warmer months and is considered to be the primary vector of WNV for the region (Fonseca et al. 2008, Ruiz et al. 2010, Messina et al. 2011). *Culex restuans* can be found sporadically throughout July, August, and September but is generally associated with cooler spring months.

Although catch basin cleaning was initiated in response to a relatively high amount of rainfall and as part of routine maintenance, it did provide the opportunity to directly evaluate the effect of this common practice on the existing Natular treatment. Because a small portion of water can remain in basins after vacuuming, it is unknown if catch basins treated with a larvicide prior to cleaning can retain enough active ingredient in this remnant water to sustain any control effects. In the present study, all monitored basins that were cleaned (both untreated and Natular treated) appeared to have significantly more larvae and pupae than those basins where Natular treatment remained. This suggests cleaning removes most, if not all, larvicide treatment and can have a noticeably negative effect on control even 8 to 14 wk after an application.

Because abatement districts such as the NSMAD can spend a large portion of their resources on the purchase and application of catch basin larvicides, it would be beneficial to coordinate catch basin cleaning events in a manner that minimizes the need for retreatment. This could be, for example, cleaning basins either early in the season prior to larvicide applications or late in the season when mosquito numbers have decreased. Because a single treatment of Natular to catch basins may be sufficient for an entire season, appropriately timed cleaning events will be of great value to the NSMAD and other abatement districts that focus control efforts on these structures. Other benefits of coordination between mosquito abatement agencies and agencies charged with maintenance of stormwater catch basins, including safer access and easier identification of structure locations, have been discussed at length elsewhere (Harbison and Metzger 2010, Harbison et al. 2010). That all monitored basins in this study were observed to hold immature stages at least once during monitoring further supports the importance of these structures as mosquito sources and the need to find pragmatic measures to adequately address these sources. As more evidence is found associating vector mosquitoes with stormwater structures, it is important to not only utilize effective larvicides but also develop better maintenance protocols that meet the needs of both mosquito abatement and stormwater agencies.

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