Short Communication

Surveillance for Immature Mosquitoes in Windshield Wash Basins at Gas Stations

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Abstract

Gas stations often provide windshield wash basins (WWBs) that customers may use to clean their windshields. Motivated by casual observations, we conducted a survey of WWBs in and around Raleigh, NC, to determine whether these WWBs also serve as larval habitats for mosquitoes. We found that 27.7% (95% CI: 12.4–43.14%) of the 36 surveyed gas stations had mosquito larvae in their WWBs, and 22.4% (95% CI: 15.07–29.1%) of the 152 WWBs surveyed were positive for mosquito larvae. Two species were identified inhabiting these containers: Aedes albopictus (Skuse) and Culex quinquefasciatus Say. Aedes albopictus was associated with clear, unturbid water, whereas Cx. quinquefasciatus did not have any significant association with water characteristics. Pupae of both species were observed, suggesting these habitats could be sources of pest mosquitoes. Gas stations may be a convenient surveillance target for vector control specialists and may provide insight into human-aided mosquito dispersal.

Key words: Aedes albopictus, Culex quinquefasciatus, Zika, dispersal, sampling

Larval mosquito surveys are an essential part of integrated vector management programs (Silver 2008, Faraji and Unlu 2016). However, larval surveys are plagued by a lack of standardization in sampling (Silver 2008). This can be a particular problem with container-dwelling mosquitoes such as the Aedes (Stegomyia) and certain Culex species. Larval container surveys for mosquitoes often describe a myriad of containers, varying in material (plastic, metal, wood, coconut), locations (near homes, rural settings, septic systems), and volumes (from a few milliliters to hundreds of liters) (Morrison et al. 2004, Morrison et al. 2006, Barrera et al. 2008). Containers can be cryptic and irregularly spaced, presenting difficulties to use as a survey tool.

Over several years of casual observation, we noticed that mosquitoes are capable of colonizing the windshield wash basins (WWBs) at gas stations (Fig. 1). From these observations, we suggest that WWBs may be a convenient and consistent larval survey target. To examine the feasibility of using WWBs for larval surveys, we conducted a survey of gas stations in and around Raleigh, NC, USA, each gas station was surveyed once over a 3-wk period (27 September to 16 October 2016), for a total of 36 gas stations surveyed. At each station, we first asked for permission to look for mosquitoes (all 36 gas station owners or workers agreed). Then, we searched for WWBs, counted the number of WWBs, and assessed whether each WWB contained liquid. Windshield wash basins that contained liquid were scored for water depth, color of water (clear, blue, green, or gray; blue and green colors are indicative of detergent use), presence of soap suds, relative turbidity (on a scale of 1–7, with 1 = clear to 7 = opaque), evidence of any insect activity (dead adults, pupal or larval exuviae, dead larvae or pupae), presence of adult mosquitoes (alive or dead), and visual confirmation of mosquito larvae. Turbidity, color, and presence of suds were visually estimated by one of two surveyors before sampling for larvae, and water was thus not disturbed when these subjective data were gathered. All liquid-filled WWBs were sampled for mosquito larvae by using a 7.62- by 7.62-cm aquarium net, swiped 12 times starting at the surface and then moving downward to the bottom of the WWB. The contents of this swiping were then washed into a small plastic tub with tap water, and if any mosquito larvae were present, they were transferred to a Whirl-Pak (NASCO), labeled, and brought back to the laboratory for species and stage identification (Darsie and Ward 2005).

Materials and Methods

Gas stations were chosen as convenience allowed, but these represented a good coverage of the city of Raleigh and surrounding areas (Fig. 1). Each gas station was surveyed once over a 3-wk period (27 September to 16 October 2016), for a total of 36 gas stations surveyed. At each station, we first asked for permission to look for mosquitoes (all 36 gas station owners or workers agreed). Then, we searched for WWBs, counted the number of WWBs, and assessed whether each WWB contained liquid. Windshield wash basins that contained liquid were scored for water depth, color of water (clear, blue, green, or gray; blue and green colors are indicative of detergent use), presence of soap suds, relative turbidity (on a scale of 1–7, with 1 = clear to 7 = opaque), evidence of any insect activity (dead adults, pupal or larval exuviae, dead larvae or pupae), presence of adult mosquitoes (alive or dead), and visual confirmation of mosquito larvae. Turbidity, color, and presence of suds were visually estimated by one of two surveyors before sampling for larvae, and water was thus not disturbed when these subjective data were gathered. All liquid-filled WWBs were sampled for mosquito larvae by using a 7.62- by 7.62-cm aquarium net, swiped 12 times starting at the surface and then moving downward to the bottom of the WWB. The contents of this swiping were then washed into a small plastic tub with tap water, and if any mosquito larvae were present, they were transferred to a Whirl-Pak (NASCO), labeled, and brought back to the laboratory for species and stage identification (Darsie and Ward 2005).

Results

Of the 36 gas stations surveyed, 3 did not possess any WWBs, and 3 had WWBs but no liquid. Overall, gas stations had a mean of 4.22 WWBs (n = 36, SD = 2.80), and a mean of 3.69 (n = 36, SD = 2.89) WWBs that contained liquid. Of the 30 gas stations that had wet WWBs, 10 gas stations had at least one WWB with either larvae or pupae (10/30 or 30% of all gas stations). We surveyed a total of 133 liquid-containing WWBs (Table 1). Further, 25.64% of liquid-filled
WWBs contained either *Aedes albopictus* (Skuse) (18.0%) or *Culex quinquefasciatus* Say (9.0%) or both species (1.5%). Gas stations positive for *Ae. albopictus* yielded a mean of 102.8 (n = 5, SD = 126.86) larvae or pupae per gas station, and those positive for *Cx. quinquefasciatus* yielded a mean of 80.33 (n = 3, SD = 119.39) larvae or pupae per gas station. All immature life history stages were found, from first-instar larvae through pupae. There was a significant association between water scored as “clear” and presence of immature mosquitoes, relative to water scored as other categories (clear: 24/51; blue: 7/52; gray: 5/30; Pearson $\chi^2_{3} = 16.85, P = 0.0002$; no water was scored as “green”). Likewise, the presence of immature mosquitoes was associated with less turbid water (Fig. 2; Pearson $\chi^2_{3} = 28.79, P < 0.0001$). *Aedes albopictus* presence showed a significant association with clear water, whereas *Cx. quinquefasciatus* did not show any association with water color (*Ae. albopictus*: Pearson $\chi^2_{3} = 25.17, P < 0.0001$; *Cx. quinquefasciatus*: Pearson $\chi^2_{3} = 1.17, P = 0.36$). Similarly, *Ae. albopictus* presence was associated with unturbid water, whereas *Cx. quinquefasciatus* presence was not (Fig. 2; *Ae. albopictus*: Pearson $\chi^2_{3} = 49.21, P < 0.0001$; *Cx. quinquefasciatus*: Pearson $\chi^2_{3} = 4.55, P = 0.60$).

### Discussion

Windshield wash basins may be an effective and convenient larval sampling habitat. We found larvae at a high enough rate to suggest that regular collections of gas stations may provide a sufficient survey of two important pathogen vectors in an area. The central role of gas stations in human movement also suggests that gas station surveillance may be useful in monitoring dispersal. Although we did not detect any *Ae. aegypti*, the similar habitat usage between *Ae. albopictus* and *Ae. aegypti* suggests that this species might also use...
WWBs. Given the association between both *Ae. albopictus* and *Ae. aegypti* movement and human movement (Guagliardo et al. 2015, Medley et al. 2015), WWBs in gas stations may provide a convenient sampling unit to capture dispersal of these species, particularly along major corridors of vehicular traffic. Sampling gas stations, coupled with genotyping of mosquitoes, may provide direct evidence of movement routes (Medley et al. 2015). Furthermore, effective management of WWBs by gas stations may reduce the suitability of these container habitats for mosquitoes, and may be an easy target for control.

There are some important caveats in interpreting our findings. First, we did not randomly sample gas stations, but surveyed every station we encountered while driving through the area. We made an effort to survey different parts of the Raleigh area, but it is possible our sample of gas stations contained biases. Likewise, we only surveyed around Raleigh, and it is possible that other locations have different practices, different climates, and different mosquito faunas that could affect how we generalize these results. We were not able to measure the total volume of each WWB, which might help explain larval occupancy or differences between the two species we found. Chemicals in WWB water were not assessed in a quantitative manner, yet likely affect mosquito production. Some of our measurements, particularly turbidity, were subjective, requiring the WWB surveyors to make a judgment. Although this is a limitation of the study, when surveyors’ data were analyzed separately, the results related to turbidity remained significant. To improve turbidity measurements, we recommend collecting water samples for spectrophotometry analysis, or the use of a portable turbidity sensor or a small turbidity tube. Likewise, there are a number of other water quality tests that could be done to help further characterize the parameters associated with the presence of either mosquito. Finally, we conducted this survey late in the mosquito season, and it is possible that we would find different patterns or different species earlier in the warm months in North Carolina.

Management practices at gas stations did have an impact on mosquito activity. Water that was scored as clear and having low turbidity was more likely to have immature mosquitoes, due primarily to *Ae. albopictus*. We believe this represents the situation in which the WWBs are filled regularly with only water or very little detergent (which is usually blue). This is consistent with studies of *Cx. quinquefasciatus* larval ecology, which show that these mosquitoes are more tolerant of a variety of conditions (Kling et al. 2007, Yee et al. 2010). However, the relatively small numbers of *Cx. quinquefasciatus* make any conclusions from this study tentative. Despite this association, some mosquitoes were found in water scored “blue,” including pupae, showing that even treated WWBs could be a source of adult mosquitoes (see Supp. Video 1 [online only]). We believe WWBs could be effective, convenient, and useful survey tools for monitoring container mosquitoes, and may also be more likely to collect individuals from distant populations (Moore and Mitchell 1997).

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