Determining the effectiveness of a sticky Light trap for collecting and evaluating mosquitoes for insecticide resistance

Annika Avery[1], Miguel Barretto[1], Dereje Alemayehu[1], John Busam[1], Babak Ebrahimi[2], Noor Tietze[2], Eric Haas-Stapleton[1,*]

[1]Alameda County Mosquito Abatement District, Hayward, CA 94545
[2]Santa Clara County Vector Control District, San Jose, CA 95112

[*]Corresponding author email: eric.haas@mosquitoes.org

Introduction

EVS CO_2 traps are widely used by vector control workers to evaluate mosquito abundance, however, these traps are bulky and require dry ice to attract mosquitoes. *Culex pipiens* breed in street-side catch basins (CB) that contain water, but it is difficult to place large EVS CO_2 traps in CB to monitor mosquito abundance. Mosquitoes are attracted to LED lights (Bentley et al. 2009; Gonzalez et al. 2016) and *Cx. pipiens* can be highly resistant to pyrethroid insecticides (Scott et al. 2015). We evaluated the efficacy of a lighted adhesive trap that could be placed inside CB to capture mosquitoes that could be tested for pyrethroid resistance with the *knock down resistance* (*kdr*) single nucleotide polymorphism (SNP) quantitative polymerase chain reaction (QPCR) assay.

Methods

To construct a sticky light trap (SLT), a Catchmaster universal fly glue board (AP&G Co., Inc., Bayonnne NJ) was first trimmed to produce 10 cm^2 glue boards and four 18 mm circular magnets were attached to each corner on the non-sticky side of the glue board using extra strength hot glue (Figure 1A). LED lights with holder were inserted with the lit side facing the sticky side of the glue board and powered using a CR2032 coin battery (Figure 1B). Electrical tape was used to attach the coin battery to LED lights and the electronics were subsequently secured using duct tape. The magnets affixed to the SLT allowed the trap to be attached to the underside of a CB grate or any ferromagnetic surface (Figure 1C). SLT were lit continuously by the LED lights for at least 3 weeks by a single coin battery (data not shown). To evaluate the efficacy of SLT for capturing mosquitoes in the field, SLT were placed in CB throughout Alameda County (CA, USA; $n = 139$). The data on the abundance of the collected mosquitoes was mapped using ArcGIS Desktop software (version 10.6). To evaluate the amount of time that mosquitoes adhered to a SLT could be analyzed for the *kdr* SNP, wild-type *Cx. pipiens* from the pyrethroid-susceptible laboratory colony SM-S1 were placed on a SLT, collected daily for 16 days and subsequently tested for the *kdr* SNP using standard QPCR methods. The *kdr* SNP genotype was determined using ΔRn values (homozygous resistant if $\Delta Rn > 1.80$, heterozygous if $0.92 < \Delta Rn < 1.5$, homozygous susceptible if $\Delta Rn < 0.5$). The impact of time on trap to gene amplification efficiency was evaluated using Prism software (version 8.0.2; GraphPad, San Diego, CA).

Results and Discussion

A significant reduction in the efficiency of *kdr* allele amplification was observed as the time that the mosquitoes were on the SLT increased ($n = 32$, $Y = -0.1348X + 0.4396$, R square $= 0.4005$, $F = 20.05$, $Dfn = 1$, $DFd = 30$, $P = 0.0001$, Figure 1D). However, the *kdr* allele could be amplified and detected in mosquitoes that had been on the SLT for at least 16 days (Figure 1D). Consequently, for subsequent studies, SLT were placed in the field for no longer than 15 days.

More than half (56%) of the *Cx. pipiens* that were collected on SLT from 14 different locations throughout Alameda County were homozygous susceptible for the *kdr* SNP ($n = 84$, Figure 2A). Although from 2014 – 2018 vector control agencies in Alameda County applied pyrethroids only 6 times in areas less than 0.2 km^2, 17% of the *Cx. pipiens* that were collected on SLT were homozygous resistant for the *kdr* SNP and 27% were heterozygous (Figure 2A). When the same trap sites were sampled in June and again in August, the proportion of mosquitoes with the heterozygous *kdr* allele increased from 0% to 14 %, while the proportion of homozygous resistant mosquitoes did not change substantially (33% and 29%; Figure 2B). Mosquitoes that were homozygous for the susceptible and resistant allele or heterozygotes may have mated to increase the heterozygous population.

Conclusion

Both laboratory colony *Cx. pipiens* and field-caught *Cx. pipiens* could be successfully tested for the *kdr* allele after being caught on a SLT. The successful trapping of mosquitoes on the SLT, as well as the detection of the *kdr*

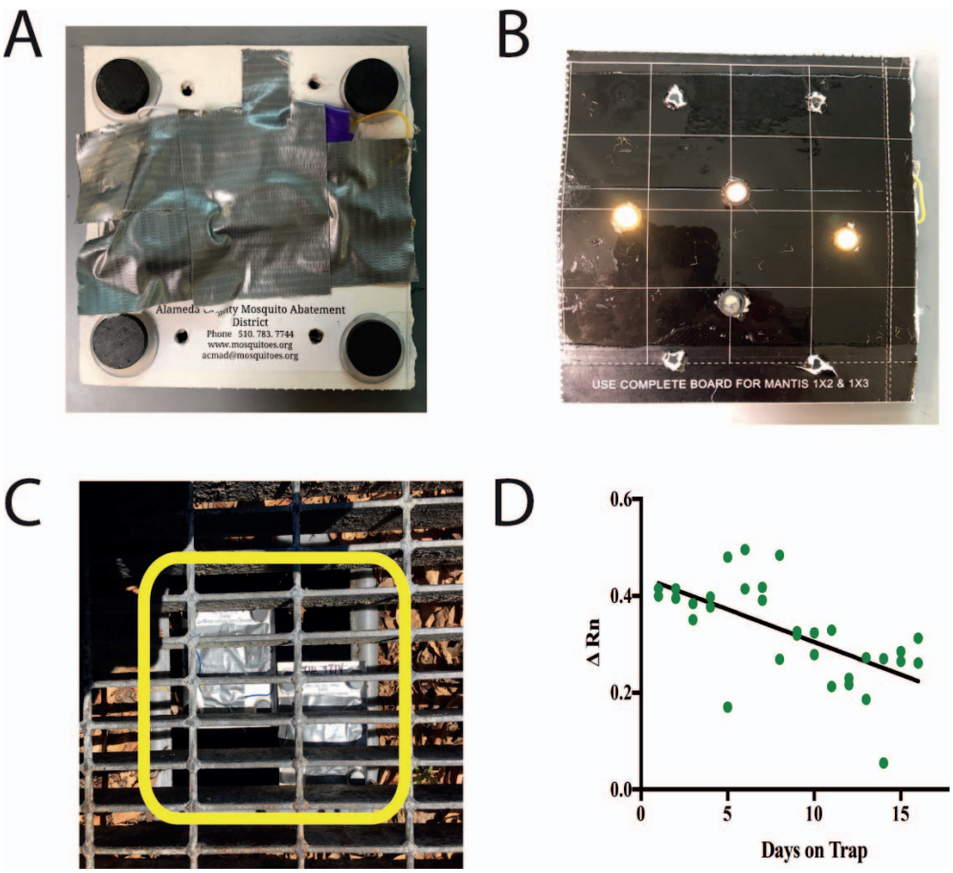

Figure 1.—Preparing and evaluating the SLT. (A) Rear side of SLT that attaches to ferromagnetic surfaces. (B) Front sticky side of SLT with lit LED lights. (C) Two SLT shown in yellow box attached to the metal grate that covers a catch basin. (D) Relationship of *kdr* allele amplification efficiency as measured by ΔRn value to the number of days wild-type *Cx. pipiens* from the pyrethroid-susceptible laboratory colony SM-S1 resided on the SLT.

allele in *Cx. pipiens* indicated that SLT may be a useful alternative to EVS CO_2 traps to monitor insecticide resistance using molecular genetic methods. More testing is needed to determine whether the mosquitoes collected on SLT can be successfully tested for arbovirus infection.

References Cited

Bentley M.T., Kaufman P.E., Kline D.L., and J.A. Hogsette. 2009. Response of adult mosquitoes to light-emitting diodes placed in resting boxes and in the field. J.Am. Mosq. Contr. Assoc. 25:285–291.

González, M., P. M. Alarcón-Elbal, J. Valle-Mora, and A. Goldarazena. 2016. Comparison of different light sources for trapping *Culicoides* biting midges, mosquitoes and other dipterans. Vet. Parasitol. 226:44–49.

Scott, J. G., M. H. Yoshimizu, and S. Kasai. 2015. Pyrethroid resistance in *Culex pipiens* mosquitoes. Pesticide Biochem. Physiol. 120:68–76.

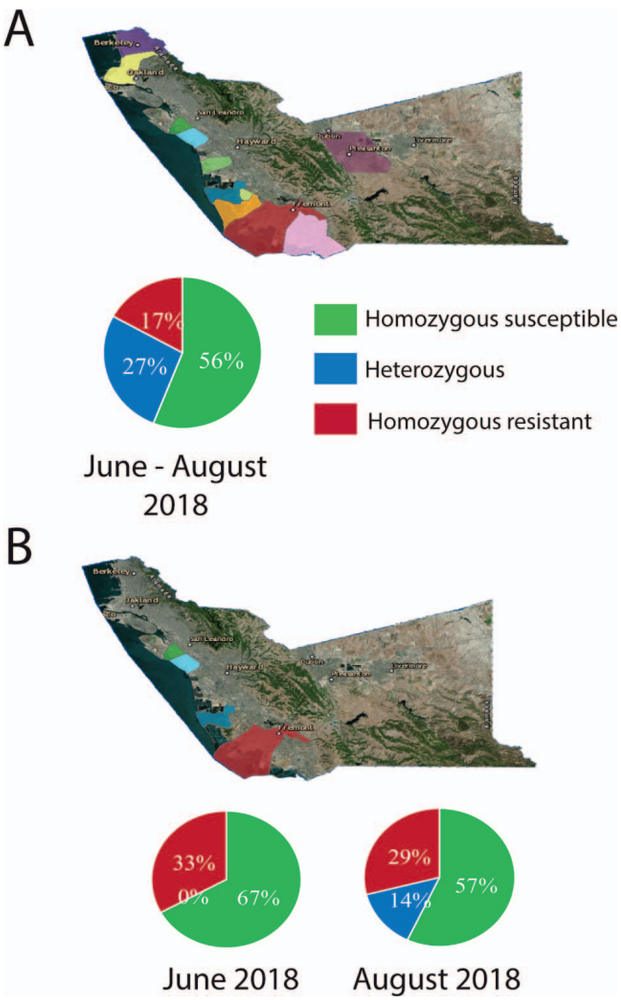

Figure 2.—Geographic distribution of the *kdr* allele *for Cx. pipiens* collected on SLT in Alameda County. Polygons overlaying satellite imagery show the location of SLT placements. Circle graphs show the proportion of *Cx. pipiens* collected from the SLT with the homozygous susceptible, homozygous resistant or heterozygous *kdr* alleles. (A) Aggregate SLT placed throughout Alameda County from June – August of 2018. (B) Repeated sampling of sites with SLT in June and August of 2018.