Environmental Entomology, XX(XX), 2024, 1–8
https://doi.org/10.1093/ee/nvad124
Research

ENTOMOLOGICAL SOCIETY OF AMERICA
SHARING INSECT SCIENCE GLOBALLY

OXFORD

Behavioral Ecology

Dancing in the purple rain: color affinity and oviposition choices in *Aedes sierrensis* (Diptera: Culicidae)

Christopher S. Bibbs[1,2,*], Kai Casci[2], Thomas D. Widmer[1], M. Andrew Dewsnup[1], Kaia Jay[3], Kirsten D. Meredith[3], Ary Faraji[1,3], Neil J. Vickers[3]

[1]Salt Lake City Mosquito Abatement District, 2215 North 2200 West, Salt Lake City, UT 84116, USA, [2]College of Science, Science Research Initiative, University of Utah, 1390 Presidents Circle, Crocker Science Center, Rm. 310, Salt Lake City, UT 84112, USA, [3]College of Science, School of Biological Sciences, University of Utah, 257 South 1400 East, Rm. 201, Salt Lake City, UT 84112, USA
[*]Corresponding author, mail: csbibbs@outlook.com, chris@slcmad.org

Subject Editor: William Morrison

Received on 10 October 2023; revised on 29 November 2023; accepted on 12 December 2023

The western tree hole mosquito, *Aedes sierrensis* (Ludlow) (Diptera: Clucidae), is a pestiferous mosquito with a range extending over the entire pacific seaboard and into portions of the intermountain west. As a peridomestic heartworm vector, it demands at least some level of surveillance to understand its abundance. However, the species is refractory to a majority of conventional vector surveillance approaches for tracking mosquitoes. To find more options for *Aedes sierrensis* surveillance, a variety of oviposition attractants were evaluated in arena-style choice assays using colony reared adults. A range of infusion treatments (e.g., alfalfa, oak, and beetroot) were examined and then combined with investigations of liquid color as well as ovicup color and entryway position. These studies revealed that *Ae. sierrensis* have an affinity for purple coloration, plain water, and larger entryway sizes for oviposition cups. A prototype ovicup was 3D-printed using purple filament and multiple types of entryways, and used to re-test infusion waters. No particular attraction differences were detected after normalizing for purple color. Comparisons to black 3D-printed cups yielded surprising observations that male mosquitoes also aggregated on purple cups while females sheltered, but not necessarily oviposited, in black cups. Although this was only a laboratory-based assessment, these studies provide useful information for future field trials of potential oviposition traps for surveillance of *Ae. sierrensis*.

Key words: attractant, vector, ovitrap, color, infusion

Introduction

Mosquito monitoring is critical for understanding, keeping historical records on, and ultimately targeting nuisance or vector mosquito species for abatement purposes (Chen et al. 2011, Crepeau et al. 2013, Connelly et al. 2020). Ubiquitous vectors, particularly invasive species, are major focus areas for mosquito abatement programs (Crepeau et al. 2013, Connelly et al. 2020). However, when dealing with highly regionalized species, such as the western tree hole mosquito, *Aedes sierrensis* (Ludlow), there is a distinct lack of basic information. Deficiencies in basic information, in turn, make it difficult to properly implement associated research, surveillance, and interventions.

Despite a patchy distribution and cryptic morphological sister species (Arnell and Nielsen 1972), *Ae. sierrensis* manifests as a target for mosquito abatement because of aggressive nuisance biting, opportunistic blood feeding, and often hyperlocal density that can plague unwitting residents in peridomestic environments (Scoles et al. 1993). This species is of further significance because it is a putative vector of dog heartworm, *Dirofilaria immitis* (Leidy), in western states (Walters and Lavoipierre 1982, Scoles et al. 1993, Tran et al. 2022). The role of this species in heartworm epi-cycles is largely unclear (Tran et al. 2022), in part due to an eclectic biological niche (Maciá and Bradshaw 2000), making surveillance outside of landing catches (human or otherwise) largely ineffective (Walters and Lavoipierre 1982, Garcia et al. 1988, Chaves et al. 2020). Surveillance deficits for this species has resulted in routinely occurring nuisance complaints within riparian areas and edges in SLCMAD and other regions of the western United States (Scoles et al. 1993, Tran et al. 2022), while also potentially maintaining *D. immitis* circulation within urban and periurban green belts.

This not only instigates economic and veterinary damages (Colby et al. 2011), but potential conservation risks in areas such as local zoos, which house exotic animals that are susceptible to *Dirofilaria* infections (Alho et al. 2017).

Lessons learned from invasive container-inhabiting mosquitoes, such as *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), support that egg collections are a good, albeit variable, population indicator, especially when targeting small spatial resolution stemming from poor dispersal or low absolute numbers (Montgomery et al. 2017). Unfortunately, it is unclear what makes a good combination of oviposition attractants and receptacles for *Ae. sierrensis*, as working knowledge in operations requires manually checking harborage habitats (Walters and Lavoipierre 1982, Garcia et al. 1988, Scoles et al. 1993, Tran et al. 2022) or using location data derived from nuisance complaints (Scoles et al. 1993, Tran et al. 2022). What is known is that oviposition is seasonally biased towards organic matter (Maciá and Bradshaw 2000), regardless of pH level, even if unfavorable for the larvae (Mercer 1993), and there is a drive to oviposit even when not successfully mated (Hawley 1985). The larvae hatch when oxygen depletes to 2.5% dissolved oxygen (Judson et al. 1966), which can be initiated in the laboratory using a bacteria-laden nutrient broth. The low dissolved oxygen limit is also likely governing natural population seasonality based on the decay of detritus in the larval rearing sites (Maciá and Bradshaw 2000).

Superficially, the habits of *Ae. sierrensis* resemble various *Culex* species, other tree hole *Aedes* sp., and invasive *Ae. aegypti*. These, respectively, will respond to hay or grass infusions (Hazard et al. 1967), oak leaf infusion (Trexler et al. 1998), and a wide variety of other plant matter, including geosmin compounds from beetroot infusions (Melo et al. 2020). Anecdotally, the routine surveillance performed at the Salt Lake City Mosquito Abatement District (SLCMAD) in Utah struggles to maintain reliable field detections using hay infusions that have become standard when using gravid traps for *Culex* species (Hazard et al. 1967), even when paired with receptacles mimicking those shown to be effective for *Ae. aegypti* (Montgomery et al. 2017). Some measure of success was observed using CO_2-baited BGS traps (Chaves et al. 2020), but collections were not selective to *Ae. sierrensis* and generally only a handful of mixed-sex adults were collected (Chaves et al. 2020).

Observations by SLCMAD surveillance teams have indicated that *Ae. sierrensis* adults are frequently associated with deciduous trees such as cottonwoods (*Populus* sp.), boxelders (*Acer negundo*), and common horse chestnuts (*Aesculus hippocastanum*) throughout scattered urban and suburban riverine nexus or mountain foothills. Leaves and flowers from the aforementioned trees commonly contain anthocyanin, a plant-based flavonoid containing red, purple, and blue pigments (Park et al. 1996, Paterska et al. 2017, Lăpădat et al. 2022). Betacyanin, a less widely distributed functional equivalent with reddish to violet pigments (Stafford 1994), is expressed highly in beets such as those used in Melo et al. (2020). Perhaps anthocyanin, or other raw nutritional compounds available in some plant parts, can be influential in attracting *Ae. sierrensis* females to oviposit. Similarly, perhaps infusion recipes already attempted with various mosquito species may actually be attractive but are missing a fixable component, whether visual or nutritive. In an attempt to enhance *Ae. sierrensis* surveillance, we compared several variables ranging from infusion water types, to colorants, to receptacle entryways for attractiveness and efficacy. The ensuing details about oviposition preference in the laboratory were then used to develop a prototype oviposition cup for *Ae. sierrensis* surveillance.

Materials and Methods

Aedes sierrensis mosquitoes used in the oviposition trials were from a 2018 Sonoma County, CA, strain. Specimens were reared in laboratory colonies grown and maintained at the Salt Lake City Mosquito Abatement District and the University of Utah. Mosquito larvae were reared in collection trays loaded with nutrient broth and adults were housed in flight cages kept at consistent environmental conditions of 27 ± 1 °C temperature and 70 ± 5% relative humidity. Adults were fed 10% sucrose solution during regular maintenance. Adults were between 9 and 23 days old and blood fed using a custom membrane feeding apparatus (Dewsnup et al. 2023) loaded with citrated bovine blood (Lampire Biological Labs, Inc., Pipersville, PA). Lighting conditions were held at 12:12 light:dark with an incremental dawn/dusk cycle as the light phase shifted. The temperature. Humidity, and light conditions for rearing were consistent in the subsequent bioassays.

Binary Choice Assays

Initial binary choice experiments were conducted in small cubic flight cages either measuring 20 cm (collapsible lumite screen cage 1450ASV, BioQuip Products Inc, Rancho Dominguez, CA) or 30 cm (Bugdorm-1 #DP1000, MegaView Science Co. Ltd., Taiwan) stocked with 10–15 mated female mosquitoes aspirated from containers of mixed-sex and aged cohorts. Isolated female cohorts were given 24 h following relocation and were then offered a blood meal. The number of females that successfully acquired a blood meal was recorded. Oviposition trials were commenced 96 h post-blood feeding. Females used in oviposition assays ranged in age from 9 to 23 days old. Oviposition cups fashioned from 100 ml plastic vials (4.9 cm × 9.8 cm) were filled with 80 ml treated or untreated (distilled) water. Only 1 type of treated water was made available at a time, and always in tandem with a second vial filled only with untreated, distilled water as a control. Seed germination papers (#6512981311, Anchor Paper Co., Saint Paul, MN) were cut to a 6 cm height and inserted as a roll into the vials such that about 3–4 mm of oviposition substrate was apparent above the vial rim. Each vial was then inserted into a black, construction paper sleeve such that only the rim of the vial projected outside the sleeve. A separate supply of 8% sucrose solution in a small vial with a white, paper towel wick placed between the 2 treatement vials was available throughout the duration of the oviposition bioassay.

No eggs were observed to be oviposited on the sugar water wick during any of the experiments. Mosquitoes were given 48 h to lay eggs before oviposition substrates were removed. Females that died during the 48 h oviposition period were not included in the total *N* for that replicate. Since eggs were laid either on the substrate or the liquid surface (or dislodged onto the surface), the liquid was filtered through a Whatman No. 4 white filter paper to collect any additional eggs. Similarly, eggs deposited on the germination paper oviposition substrate were rinsed off and filtered. A digital image of the dried filter paper with eggs was then captured and eggs were counted using ImageJ (Schneider et al. 2012). Placement of treated/untreated cups was randomized in each replicate to mitigate any positional effects such as light-orientation biases that may have accrued in the bioassay room.

Treatments evaluated in this style of assay were as follows: (i) nutrient broth prepared as 0.9 g nutrient broth (70149-500G, Sigma-Aldrich, Inc., St. Louis, MO) and ~0.25 g of brewer's yeast (Cat. #903312, MP Biomedicals, Solon, OH) in 1 L of water; (ii) beetroot infusion (80 ml distilled water infused with 3 g of beetroot peel for 24 h). The peel was contained within a cheesecloth

bag with a small zip tie since fermentation activity degraded the peel creating a solution laden with organic material that was otherwise difficult to filter at the conclusion of the assay (Melo et al. 2020). In these experiments, the control, untreated vials also had an empty, zip-tied cheesecloth bag immersed in the distilled water. Both bionutrient broth and beetroot peel treatments were prepared 24 h in advance. (iii) Oak leaf infusion was prepared according to Trexler et al. (1998). Leaves were gathered from a Columnar English oak (*Quercus robu* var. fastigiata) on the University of Utah campus and soaked in tap water for 2 wk before being filtered out and the infusion stored in a 5 liter carboy. For bioassays, 20 ml of oak leaf infusion was withdrawn from the carboy and diluted with 60 ml distilled water; with distilled water as the control group. Replicates for each bioassay were as follows: nutrient broth vs. distilled water ($N = 8$): beetroot infusion vs. distilled water ($N = 9$); oak leaf infusion vs. distilled water ($N = 5$).

Arena Choice Assays

In an expansion of the smaller arena two-choice bioassays, larger arena assays were conducted using cubic flight cages measuring 45 cm (aluminum cage #E571, Australian Entomological Supplies Pty. Ltd., South Murwillumbah, NSW). Large arena assays were stocked with a 50:50 mixed-sex population of 2,000 adult mosquitoes that had been blood fed 96 h prior. Oviposition cups were initially presented as 473 ml, clear PET drinking cups (ASF Tashi LLC, Pittsfield, MA) filled with 153 ml of either treated or untreated water and then lined with 13 cm × 19 cm sheets of seed germination paper (#6512981311, Anchor Paper Co., Saint Paul, MN). Mosquitoes were allowed 96 h to oviposit before substrates were removed and eggs were counted under a light dissection scope. Placement of treated/untreated cups was randomized in each replicate to mitigate light-orientation biases that may occur.

In light of findings from the binary choice assays, beetroot infusions were carried forward to be tested alongside an alfalfa infusion (Fig. 1A) prepared using 1 g/liter of dried alfalfa cubes (animal feed) in water, then fermented at 27 ± 1 °C for 96 h. To isolate pigmentation effects from the beet infusion, an anthocyanin (Bilberry Extract 36% anthocyanin, Nature's Way Brands, LLC, Green Bay, WI) treatment was made using 3.2 g/liter in water. Evaluations for Trial 1 were conducted by simultaneously presenting untreated water, water + anthocyanin, beetroot infusion, alfalfa infusion, and alfalfa infusion + anthocyanin. In the subsequent Trial 2, color was tested by itself with varying mixes of blue or red (Culinary grade, McCormick & Co., Inc., Hunt Valley, MD) dye in otherwise untreated water (Fig. 1B). Mixtures of either 100% blue, 1:2 red:blue (violet), 4:3 red:blue (purple), 2:1 red:blue (magenta), or 100% red dye were added at 3 drops per 153 ml of water in a cup. The combination of color and infusion were then compared in Trial 3 by offering water, water + purple, water + anthocyanin, alfalfa infusion, alfalfa infusion + purple, and alfalfa infusion + anthocyanin. Follow-up in Trial 4 used water, water + purple, beetroot infusion, and beetroot infusion + purple. The final Trial 5 in this series examined receptacle color (Fig. 1C) by using either black or purple sleeves cut from colored cardstock in arenas containing water, water + purple dye, water + black sleeve, water + purple sleeve, water + purple dye + black sleeve, water + purple dye + purple sleeve. All aforementioned trials were replicated 3 times with independent cohorts of adult mosquitoes.

The progression of variables resulted in a custom oviposition cup being 3D-printed using either purple (1.75 mm purple, ESun Industrial Co., Ltd., Shenzhen, China) or black (1.75 mm black, ESun Industrial Co., Ltd., Shenzhen, China) polylactic acid filament extruded through a 0.6 mm nozzle with set temperature at 98.89 °C (210 °F) and 14.44 °C (58 °F) bed temperature. Varying lids were fabricated with either top entry or side entry into the cup. In either case, 3 entryway sizes were created, 3.8 cm diameter, 2.5 cm diameter, or 1.3 cm diameter. Comparisons of entryway preferences (Fig. 1D) were conducted with only purple ovicups by simultaneously

Fig. 1. A) Infusion additives choice assessments; B) Red-to-blue spectrum tests; C) Pairings for purple versus black cups and purple versus clear water; D) 3D-printed ovicup prototype with top or side entry for small, medium, and large holes.

presenting all 6 entryway styles as small, medium, or large for either of top entry or side entry (Trial 6). Finally, comparisons revisited the infusion water types by pairing either black or purple cups with only the large top entryway with water, beet root infusion, or alfalfa infusion. Five replicates were conducted for all trials using 3D-printed ovicups (Trial 7).

Data Analysis

Analysis was conducted using R statistical software (v.4.2.1, The R Foundation for Statistical Computing, Vienna, Austria) via RStudio (v. 3.3.0, RStudio PBC, Boston, MA) using the additional loaded packages tidyverse, ggplot2, lme4, and emmeans. Binary choice assays were analyzed by first adjusting egg collections by treatment to the female sample size in the assay (n = 8–15) and then using paired 2-sample t-tests between the egg/n counts from selected treatment (nutrient broth, beetroot, oak leaf) and corresponding untreated water pairing. Multi-treatment arena trials were using generalized linear mixed models (GLMM), with treatment factors and position as an interaction term. Replicate number was used as a random effect.

Results

In initial screenings in the binary choice assays (Fig. 2), egg deposition was higher in plain water over nutrient broth ($t_{8,17}$ = 2.8829, P = 0.0204) and beetroot infusion over plain water ($t_{8,17}$ = 4.8271, P = 0.0013). No differences were observed between oak leaf infusion and plain water ($t_{5,9}$ = 0.3466, P = 0.7464). In Trial 1 of the arena assays, water, alfalfa infusion, and beetroot infusion were all inferior to water dosed with extra anthocyanin (Fig. 3A; $Z_{5,17}$ = 2.537, P = 0.0112). In Trial 2 assessing color gradient, blue generally trended to collect fewer eggs than shade of red ($Z_{5,17}$ = 2.108, P = 0.0351). Although there was overlapping significances between the blended colors, trends generally favoring red hues in the tested spectrum, with the 4:3 red:blue dye ratio visually trending the highest (Fig. 3B). For simplicity, we elected to carry forward the 4:3 red:blue dye ratio, labeled "purple", in comparison to anthocyanin when combined with either plain water or alfalfa infusion. Trial 3 egg deposition in purple water (30.841% of eggs) and anthocyanin water (37.121%) was distinct from the other groups (Fig. 3C; $Z_{5,17}$ = 3.945, P < 0.0001). Attempting again in Trial 4 with beetroot infusion or normal water with additional purple dye yielded purple water (52.088% of eggs) and beetroot infusion with purple dye as significant (Fig. 3D; $Z_{5,11}$ = 3.926, P < 0.0001) over plain water or undyed infusion.

Modifying cup colors in Trial 5 using either clear, black, or purple containers resulted in strong affinity for purple cups containing additional purple water (70.233% of eggs; Fig. 4A; $Z_{5,17}$ = 5.352, P < 0.0001). Trial 6 used 3D-printed, purple, prototype cups (Figs. 1D and 4B and C) of varying entryway sizes and 2 orientations, of which the topside big, topside medium, and sideways medium entryways collected the most eggs (Fig. 4B; $Z_{5,29}$ = 2.441, P = 0.0146). A visual trend implied that the big topside hole may have been superior to the sideways medium hole with more replication, but for simplicity the final laboratory evaluation was conducted with only the big topside hole. Trial 7 reevaluated infusion waters in the new prototype ovicup

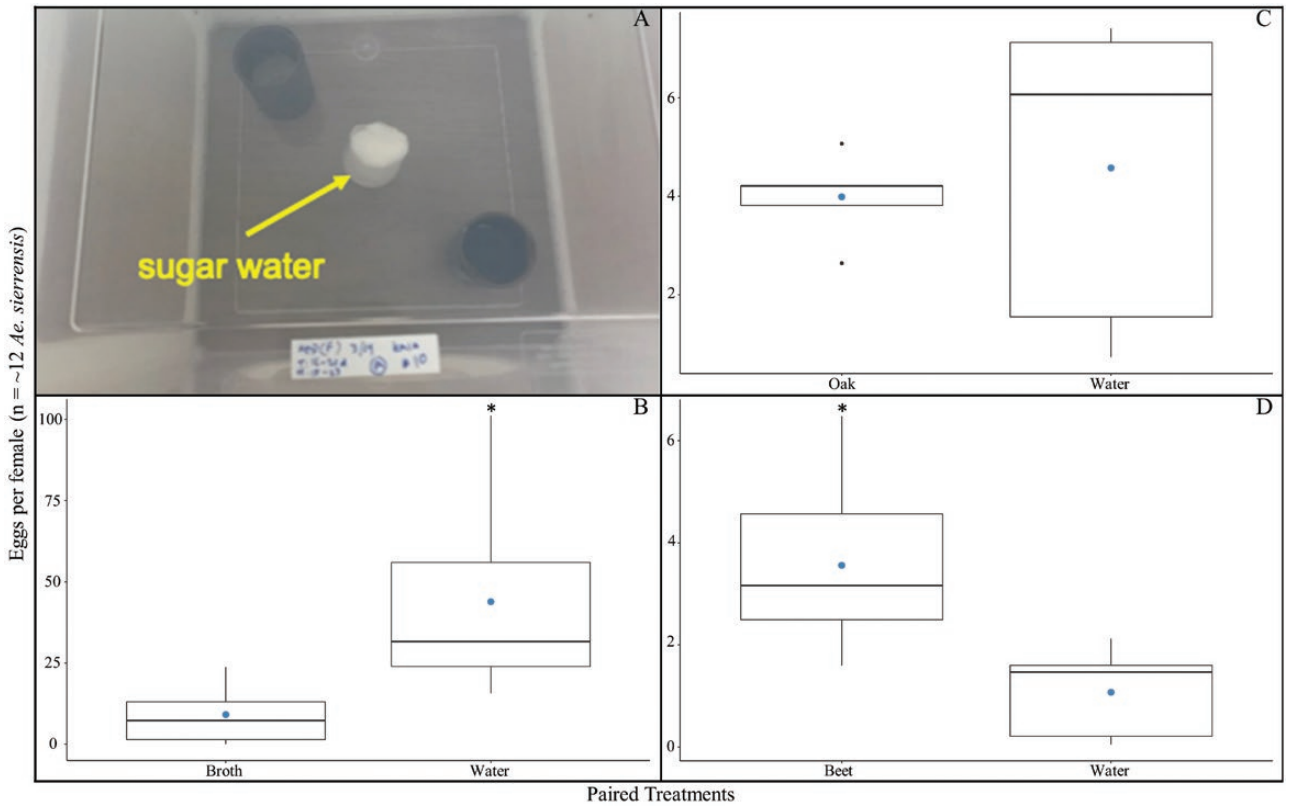

Fig. 2. A) Binary assay layout in 30 cm, cubic flight cage; B) egg collections, adjusted by number of females, for nutrient broth (left) versus untreated water (right) ($t_{8,17}$ = 2.8829, P = 0.0204); C) oak leaf infusion (left) versus water ($t_{5,9}$ = 0.3466, P = 0.7464); D) beetroot infusion (left) versus water (right) ($t_{8,17}$ = 4.8271, P = 0.0013). For all box plots, the central shaded point denotes the mean, black points falling outside the box and whisker are outliers, and asterisks denote statistical significant difference from the paired treatment.

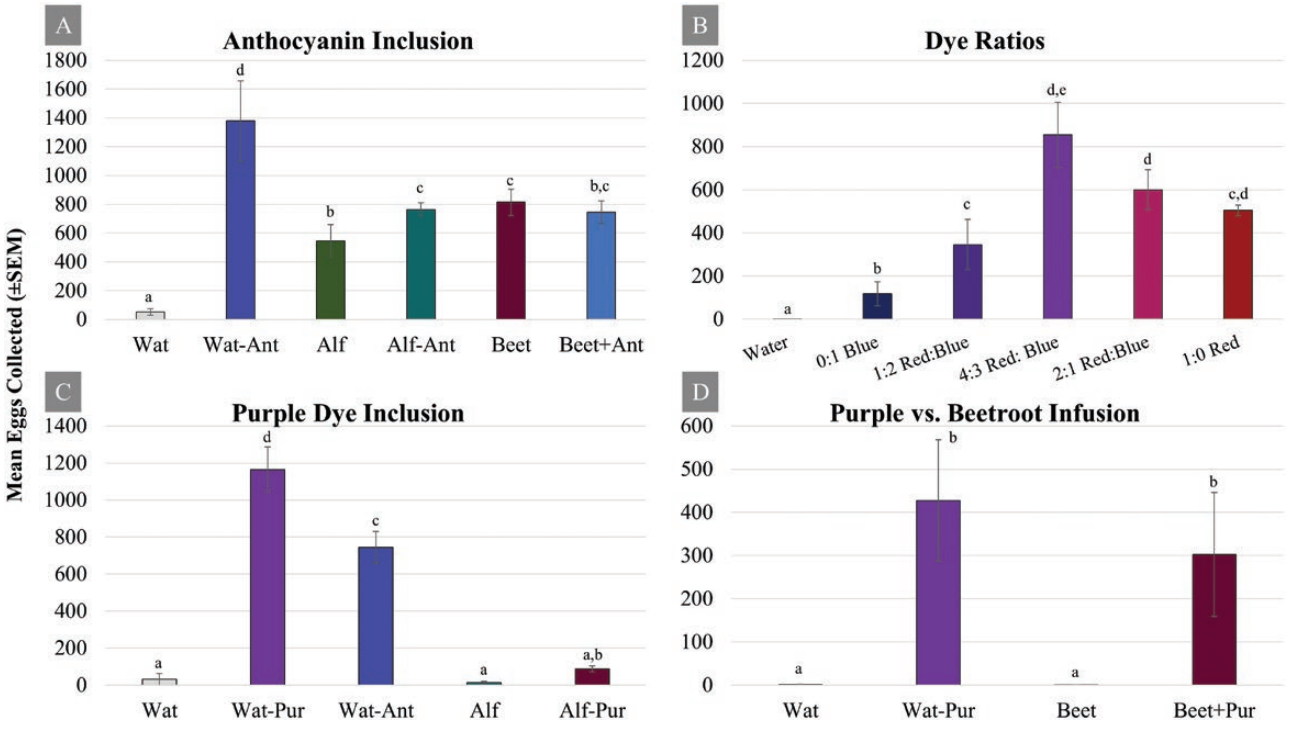

Fig. 3. Arena choice assay for water color preference. A) Trial 1 results for water (Wat), alfalfa infusion (Alf), or beetroot infusion (Beet) having supplemental anthocyanin (Ant) ($Z_{5,17} = 2.537$, $P = 0.0112$). B) Trial 2 results for dye ratios ranging between blue and red, as compared to undyed water ($Z_{5,11} = 2.108$, $P = 0.0351$). C) Trial 3 results for purple dye (Pur) or anthocyanin (Ant) in either plain water (Wat) or alfalfa infusion (Alf) ($Z_{5,17} = 3.945$, $P < 0.0001$). D) Trial 4 results for water (Wat) or beetroot infusion (Beet) having supplemental purple dye (Pur) ($Z_{6,11} = 3.926$, $P < 0.0001$). Graphs annotated for significance and shown with standard error of the mean as I-bars.

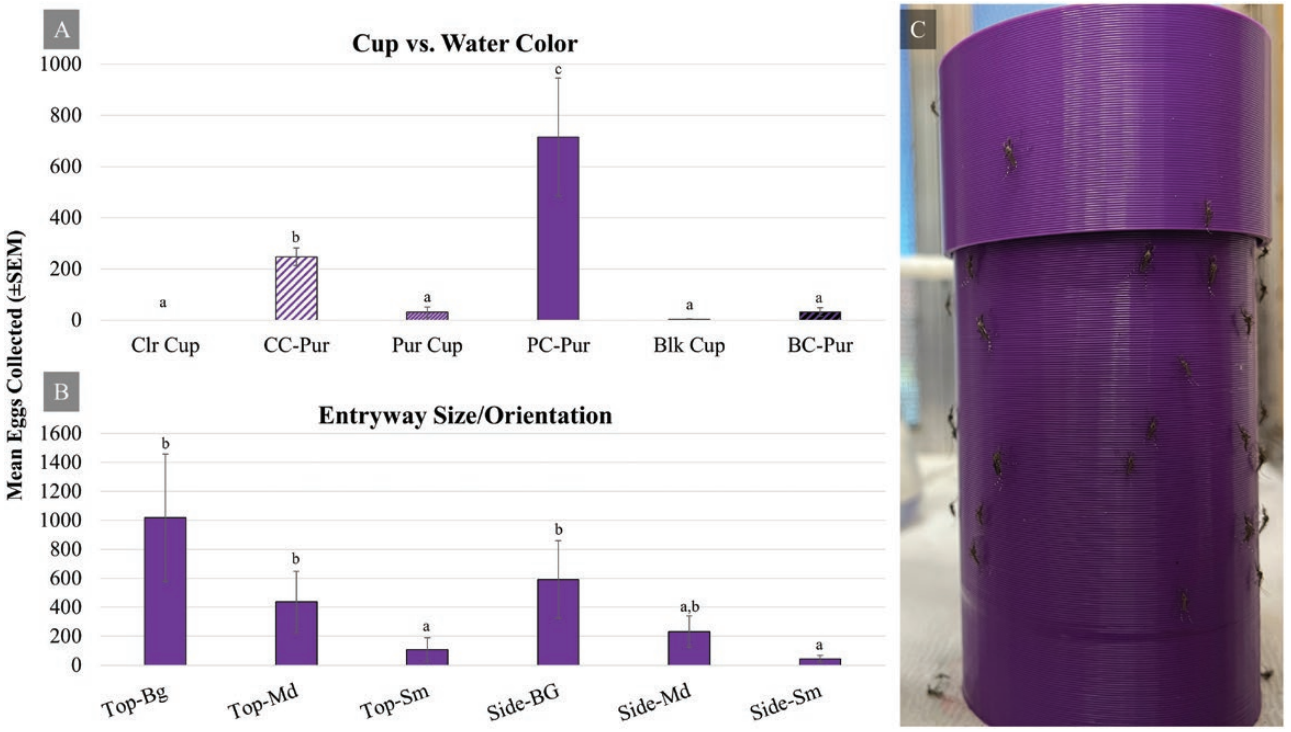

Fig. 4. Arena choice assays for ovicup changes. A) Trial 5 results for clear cups (Clr or CC), purple cups (Pur or PC), and black cups (Blk or BC) with either plain water (-Cup) or purple-dyed water (-Pur) ($Z_{5,17} = 5.352$, $P < 0.0001$). B) Trial 6 results for entryway size as small (sm), medium (md), and big (bg) with either top or side orientations for a 3D-printed oviposition cup ($Z_{5,29} = 2.441$, $P = 0.0146$). Graph annotated for significance and shown with standard error of the mean as I-bars. C) Example of a purple ovicup showing male aggregation on exterior.

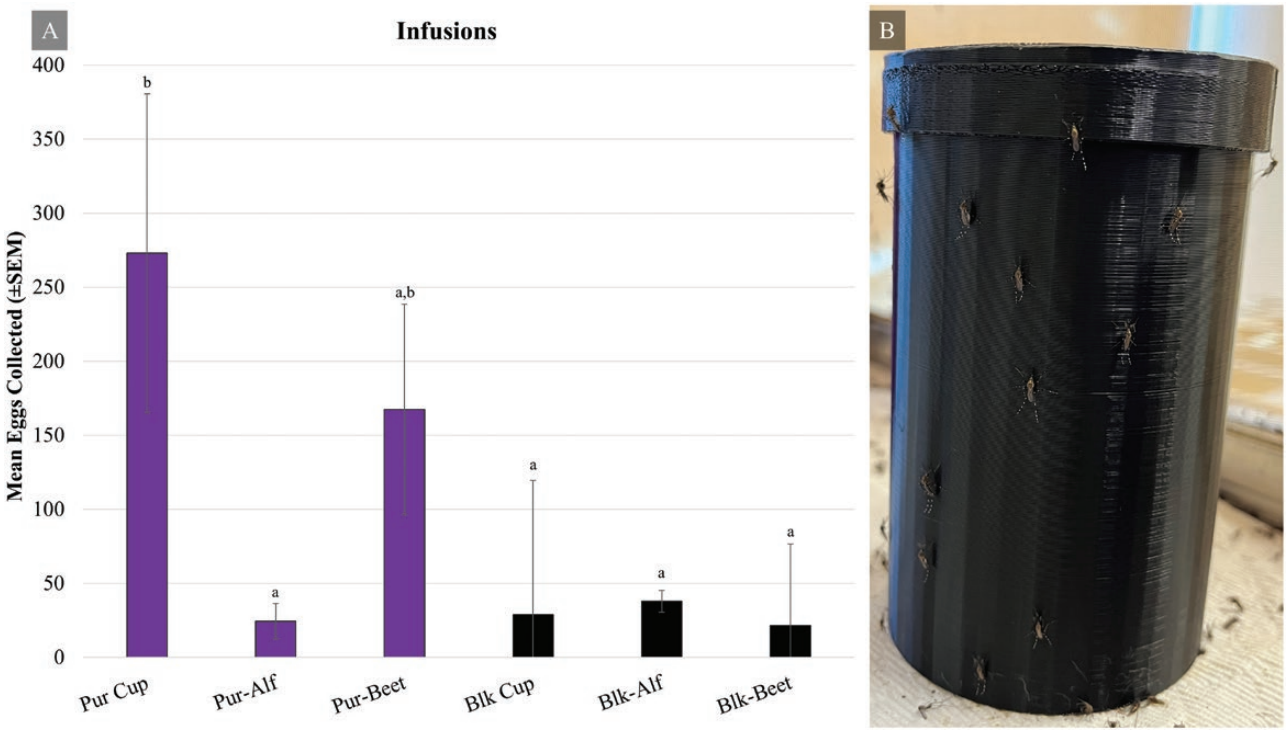

Fig. 5. A) Trial 7 arena choice assay results for water (Cup), alfalfa infusion (Alf), or beetroot infusion (Beet) with either purple (Pur) or black (Blk) 3D-printed oviposition cups ($Z_{5,29} = 5.632$, $P < 0.0001$). Graph annotated for significance and shown with standard error of the mean as I-bars. B) Example of a black ovicup showing mixed-sex aggregation on exterior.

and found significant differences by color, but not by infusion type (Fig. 5; $Z_{5,29} = 5.632$, $P < 0.0001$). A majority of eggs were collected in the purple ovicups with water (51.9%) or beet infusion (32.7%) (Fig. 5A). As an aside, it was observed that almost entirely male *Ae. sierrensis* superficially clustered on purple cups (Fig. 4C) while a mix of males and females clustered on black cups (Fig. 5B).

Discussion

In an unexpected shift in focus from infusion treatments to color, it appears *Ae. sierrensis* exhibited a strong affinity for purple oviposition containers when tested under laboratory conditions. Despite a life cycle requiring low dissolved oxygen to stimulate larval hatch (Judson et al. 1966), it seems gravid females do not seek out analogous conditions for oviposition (Fig. 2B). Surprisingly, *Ae. sierrensis* were indifferent to oak leaf infusion over plain water, despite an attraction to beetroot over plain water. This characterized beetroot as the benchmark for trying to optimize an attractive oviposition lure. Although merely a suspicion because of local association of *Ae. sierrensis* to suspected anthocyanin contaminated tree holes (Park et al. 1996, Paterska et al. 2017, Lăpădat et al. 2022), it was evident that the anthocyanin did in fact encourage greater egg deposition even over the beetroot infusion. To then find that pigmentation was the primary driver rather than any particular type of organic matter-laden water is perplexing given that *Ae. sierrensis* is reported to require certain vegetative decay states in larval rearing water (Maciá and Bradshaw 2000).

Although uncommonly reported, it is known from other species that coloration and silhouette can be key foci for oviposition. Particularly, *Wyeomyia* sp., also known for their eclectic biology (epiphytic plants in trees as larval habitats), were studied in a similar fashion using various colors of bromeliad-mimicking ovicups (Frank 1986). In an unintentionally clandestine mirroring of Frank's own work, both the *Wyeomeyia* research using *Tillandsia* bromeliad leaf extract and this current study with *Ae. sierrensis* using beetroot found an unexpected departure from the nutritive infusion as visual cues came to light as a stronger driver of behavior. Furthermore, Frank (1986) found that color/saturation may not specifically be representative of pigment so much as being a substitute for shade and sun exposure (Prokopy and Owens 1983, Frank 1986).

This hypothesis has been linked, at least for *Wyeomyia* sp., to their respective distribution and plant habitats, since there is no remarkable association with specific plants (Frank and Curtis 1982), despite clear fragmentation between species in preferred oviposition sites (Frank and O'Meara 1985). We believe the aforementioned logic and observations explaining *Wyeomyia* ecology is applicable to *Ae. sierrensis* as well. Our hypothesis is reinforced by detecting more eggs in anthocyanin or purple-dyed infusions regardless of pre-existing colorants. This finding was most notable with the beetroot, which already possess colorants (betacyanins), but was more attractive with additional added colorants. This echoes the same color saturation ideas expressed by others (Frank 1986, Isoe et al. 1995). Further, saturation preferences in plants could be correlated to UV attraction (Peach et al. 2019), given that beta- and anthocyanins are also UV filtering pigments (Stafford 1994, Park et al. 1996, Paterska et al. 2017, Lăpădat et al. 2022).

Our original intention in looking at different hole sizes and a top versus side orientation was to be expressive of the local tree holes already monitored by SLCMAD for *Ae. sierrensis* management. After all, it has been documented in other mosquitoes that an unfavorable entry will deter oviposition (Strickman and Kittayapong 1993), even in the field (Harbison et al. 2008). However, prior work in California has challenged whether *Ae. sierrensis* really have any sort of preference to the orientation of the entrance (Woodward et al. 1996),

even though horizontal entry is preferred in the eastern tree hole mosquito, *Aedes triseriatus* (Say) (Lang 1990). Regardless, to our knowledge the entryway size for the ovicup has not been a previous target of study. It still may be that color attraction is not the primary mechanism for choice in oviposition sites.

Work by Woodward et al. (1996) experimented with nesting ovicups in a sheltered structure ("oviboxes") to improve interest by mosquitoes. Woodward et al. (1996) inferred that *Ae. sierrensis* may seek shelter before ovisites, and that ovisites need to be sheltered in order to function well (Woodward et al. 1996). We may have observed this in our studies when females sheltered, but not necessarily oviposited, in black cups. Although not specifically about attraction, other investigations have found California-based *Ae. sierrensis* to deposit eggs primarily during daylight hours closer to sunset, even though visitation to the ovisite was scattered through day and night hours (Woodward et al. 2008). This may possibly link back to the need for shelter, rather than strictly an ovisite. It was not an objective of this study, but observations of male aggregations on the purple prototype ovitrap were an exciting finding. Males are undoubtedly also layered in their preferences, whether time of day, color, need for shelter, or other features.

Given that surveillance tools have to compete with resource options in the field, the prototype ovicups still need to be vetted in the field before deciding if they have a chance at encouraging oviposition in spite of nearby trees. This likely requires expanding the use of the ovicup to include accessory shelters (Woodward et al. 1996) and timing data collection with respect to evening oviposition trends (Woodward et al. 2008). If field validation succeeds, then expansion of *Ae. sierrensis* surveillance using custom ovicups may improve the otherwise dismal sample sizes typically collected of this species (Walters and Lavoipierre 1982, Garcia et al. 1988, Scoles et al. 1993, Tran et al. 2022). Future study could include more targeted *Dirofilaria* investigations (Tran et al. 2022) by having better hotspot maps, helping to forecast or prevent *Dirofilaria* incursion into sensitive areas (ex: zoos). Phylogenetic work is another potential gain, given this species is within a scattered complex (Arnell and Nielsen 1972). The greatest immediate gain is a means of diagnosing active areas with *Ae. sierrensis*, ideally streamlining labor and interventions so as not to waste limited resources by reactively following up on backyard nuisance calls (Garcia et al. 1988, Scoles et al. 1993, Tran et al. 2022) instead of preemptively curtailing the population. These are only optimistic suggestions, but even if ovicups end up not being competitive to natural tree holes in field testing, there may be other ways to exploit the color preference, such as in resting boxes or colored adult mosquito traps. These investigations will ultimately lead to enhanced surveillance of *Ae. sierrensis* throughout much of its western range.

Acknowledgments

Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

Funding

Support for K.C. was provided by the Science Research Initiative at the University of Utah. Part of this study (N.J.V. and K.M.) was supported by cooperative agreements U01CK000516 and U01CK000649 funded by the U.S. Centers for Disease Control and Prevention. Support for K.J. was provided through the College of Science ACCESS program at the University of Utah.

Author Contributions

Christopher Bibbs (Conceptualization [Equal], Data curation [Lead], Formal analysis [Lead], Investigation [Supporting], Methodology [Equal], Project administration [Lead], Supervision [Lead], Validation [Equal], Visualization [Equal], Writing—original draft [Lead], Writing—review & editing [Equal]), Kai Casci (Investigation [Equal], Methodology [Equal], Validation [Equal], Writing—original draft [Supporting]), Thomas Widmer (Conceptualization [Equal], Investigation [Supporting], Methodology [Supporting], Validation [Supporting], Writing—review & editing [Supporting]), Andrew Dewsnup (Methodology [Equal], Resources [Equal], Software [Lead], Validation [Supporting], Visualization [Supporting]), Kaia Jay (Investigation [Supporting], Methodology [Supporting]), Kirsten Meredith (Data curation [Supporting], Investigation [Supporting], Methodology [Supporting], Project administration [Supporting], Validation [Supporting]), Ary Faraji (Resources [Equal], Writing—review & editing [Equal]), and Neil Vickers (Conceptualization [Equal], Data curation [Equal], Formal analysis [Equal], Investigation [Equal], Methodology [Equal], Project administration [Equal], Supervision [Equal], Visualization [Supporting], Writing—original draft [Equal], Writing—review & editing [Equal])

References

Alho AM, Marcelino I, Colella V, Flanagan C, Silva N, Correia JJ, Latrofa MS, Otranto D, Maderia de Varvalho L. *Dirofilaria immitis* in pinnipeds and a new host record. Parasites Vectors. 2017:10:142. https://doi.org/10.1186/s13071-017-2073-0

Arnell JH, Nielsen LT. Mosquito studies (Diptera, Culicidae) XXVII. The *varipalpus* group of *Aedes* (*Ochlerotatus*). Contrib Am Entomol Inst. 1972:8:1–48.

Chaves LF, Reissen N, White GS, Gordon S, Faraji A. Trap comparison for surveillance of the western tree hole mosquito, *Aedes sierrensis* (Diptera: Culicidae). J Insect Sci. 2020:20(1):3. https://doi.org/10.1093/jisesa/iez131

Chen YC, Wang CY, Teng HJ, Chen CF, Chang MC, Lu LC, Lin C, Jian SW, Wu HS. Comparison of the efficacy of CO_2-baited and unbaited light traps, gravid traps, backpack aspirators, and sweep net collections for sampling mosquitoes infected with Japanese encephalitis virus. J Vector Ecol. 2011:36(1):68–74. https://doi.org/10.1111/j.1948-7134.2011.00142.x

Colby KN, Levy JK, Dunn KF, Michaud RI. Diagnostic, treatment, and prevention protocols for canine heartworm infection in animal sheltering agencies. Vet Parasitol. 2011:176(4):333–341. https://doi.org/10.1016/j.vetpar.2011.01.018

Connelly CR, Gerding JA, Jennings SM, Ruiz A, Barrera R, Partridge S, Beard CB. Continuation of mosquito surveillance and control during public health emergencies and natural disasters. MMWR Morb Mortal Wkly Rep. 2020:69:938–940. http://dx.doi.org/10.15585/mmwr.mm6928a6

Crepeau TN, Healy SP, Farajollahi A, Fonseca DM. Experiences with the large-scale operation of the Biogents sentinel trap. J Am Mosq Control Assoc. 2013:29:177–180. https://doi.org/10.2987/12-6277r.1

Dewsnup MA, Faraji A, White GS, Bibbs CS. Do it yourself: fabricating and evaluating a mosquito (Diptera: Culicidae) blood feeding device to replace a commercial option. J Insect Sci. 2023:23(4):18. https://doi.org/10.1093/jisesa/iead072

Frank JH. Bromeliads as ovipositional sites for *Wyeomyia* mosquitoes: form and color influence behavior. Fl Entomol. 1986:69(4):728–742. https://doi.org/10.2307/3495221

Frank JH, Curtis GA. Bionomics of the bromeliad-inhabiting mosquito *Wyeomyia vanduzeei* and its nursery plant *Tillandsia utriculata*. Fl Entomol. 1982:64(4):491–506. https://doi.org/10.2307/3494406

Frank JH, O'Meara GF. Influence of micro- and macrohabitat on distribution of some bromeliad-inhabiting mosquitoes. Entomol Exp Appl. 1985:37:169–174.

Garcia R, Des Rochers BS, Voigt WG. A bait/carbon dioxide trap for the collection of the western tree hole mosquito *Aedes sierrensis*. J Am Mosq Control Assoc. 1988:4(1):85–88.

Harbison JE, Metzger ME, Hu R, Fritz CL, Kramer VL. Effect of conveyance pipe dimension and orientation on mosquito oviposition in a simulated stormwater management device. J Am Mosq Control Assoc. 2008:24(1):98–104. https://doi.org/10.2987/5628.1

Hawley WA. A high-fecundity aedine: Factors affecting egg production of the western treehole mosquito, *Aedes sierrensis* (Diptera: Culicidae). J Med Entomol. 1985:22(2):220–225. https://doi.org/10.1093/jmedent/22.2.220

Hazard EI, Mayer MS, Savage KE. Attraction and oviposition stimulation of gravid female mosquitoes by bacteria isolated from hay infusions. Mosq News. 1967:27:133–136.

Isoe J, Millar JG, Beehler JW. Bioassays for *Culex* (Diptera: Culicidae) mosquito oviposition attractants and stimulants. J Med Entomol. 1995:32(4):475–483. https://doi.org/10.1093/jmedent/32.4.475

Judson CL, Hokama Y, Kliewer JW. Embryogeny and hatching of *Aedes sierrensis* eggs (Diptera: Culicidae). Ann Entomol Soc Am. 1966:59(6):1181–1184. https://doi.org/10.1093/aesa/59.6.1181

Lang J. Ovipositional response of *Aedes triseriatus* females to horizontally and vertically open ovitraps in southern Illinois. J Am Mosq Control Assoc. 1990:6:530–553l.

Lăpădat AS, Drăgucian VD, Ciobanu DG, Ianovici N, Datcu AD. Non-invasive anthocyanin investigation on some plant species from urban habitats. Res J Agric Sci. 2022:54:76–81.

Maciá A, Bradshaw WE. Seasonal availability of resources and habitat degradation for the western tre-hole mosquito, *Aedes sierrensis*. Oecologia. 2000:125(1):55–65. https://doi.org/10.1007/PL00008891

Melo N, Wolff GH, Costa-da-Silva AL, Arribas R, Triana MF, Gugger M, Riffell JA, DeGennaro A, Stensmyr MC. Geosmin attracts *Aedes aegypti* mosquitoes to oviposition sites. Curr Biol. 2020:30:127–134. https://doi.org/10.1016/j.cub.2019.11.002

Mercer D. Effect of tannic acid concentration on development of the western treehole mosquito, *Aedes sierrensis* (Diptera: Culicidae). J Chem Ecol. 1993:19:1119–1127.

Montgomery BL, Shivas MA, Hall-Mendelin S, Edwards J, Hamilton NA, Jansen CC, McMahon JL, Warrilow D, van den Hurk A. Rapid surveillance for vector presence (RSVP): Development of a novel system for detecting *Aedes aegypti* and *Aedes albopictus*. PLoS Neg Trop Dis. 2017:11:e0005505. https://doi.org/10.1371/journal.pntd.0005505

Park YG, Choi MS, Son SH. 1996. *Populus* species (Poplars): in vitro culture and the production of anthocyanins. In: Bajaj YPS, editor. Medicinal and aromatic plants IX; biotechnology in agriculture and forestry. Vol 37. Berlin, Heidelberg (Germany): Springer. https://doi.org/10.1007/978-3-662-08618-6_19

Paterska M, Bandurska H, Wysłouch J, Molińska-Glura M, Moliński K. Chemical composition of horse-chestnut (*Aesculus*) leaves and their susceptibility to chestnut leaf miner *Cameraria ohridella* Deschka and Dimić. Acta Physiol Plant. 2017:39(4):105. https://doi.org/10.1007/s11738-017-2404-y

Peach DAH, Ko E, Blake AJ, Gries G. Ultraviolet inflorescence cues enhance attractiveness of inflorescence odour to *Culex pipiens* mosquitoes. PLoS One. 2019:14(6):14. https://doi.org/10.1371/journal.pone.0217484

Prokopy RJ, Collier RH, Finch S. Leaf color used by cabbage root flies to distinguish among host plants. Science. 1983:221(4606):190–192. https://doi.org/10.1126/science.221.4606.190

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012:9(7):671–675. https://doi.org/10.1038/nmeth.2089

Scoles GA, Dickson SL, Blackmore MS. Assessment of *Aedes sierrensis* as a vector of canine heartworm in Utah using a new technique for determining the infectivity rate. J Am Mosq Control Assoc. 1993:9(1):88–90.

Stafford HA. Anthocyanins and betalains: evolution of the mutually exclusive pathways. Plant Sci. 1994:101(2):91–98. https://doi.org/10.1016/0168-9452(94)90244-5

Strickman D, Kittayapong P. Laboratory demonstration of oviposition by *Aedes aegypti* (Diptera: Culicidae) in covered water jars. J Med Entomol. 1993:30(5):947–949. https://doi.org/10.1093/jmedent/30.5.947

Tran TD, Nelms BM, Koschik ML, Scott JJ, Thiemann T. Prevalence of filarial parasites in field-caught mosquitoes in northwestern California. J Vector Ecol J Soc Vector Ecol. 2022:47(1):61–68. https://doi.org/10.52707/1081-1710-47.1.61

Trexler JD, Apperson CS, Schal C. Laboratory and field evaluations of oviposition response of *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) to oak leaf infusions. J Med Entomol. 1998:35(6):967–976. https://doi.org/10.1093/jmedent/35.6.967

Walters LL, Lavoipierre MJ. *Aedes vexans* and *Aedes sierrensis* (Diptera: Culicidae): potential vectors of *Dirofilaria immitis* in Tehama County, northern California, USA. J Med Entomol. 1982:19:15–23. https://doi.org/10.1093/jmedent/19.1.15

Woodward DL, Colwell AE, Anderson NL. Temporal and spatial distribution of *Aedes sierrensis* oviposition. Proc Mosq Vector Control Assoc CA. 1996:64:51–62.

Woodward DL, Colwell AE, Mills BM. Time of day that *Aedes sierrensis* females enter oviposition sites prior to laying eggs. J Am Mosq Control Assoc. 2008:24(1):112–114. https://doi.org/10.2987/5625.1