*Journal of the American Mosquito Control Association*, 39(4):236–242, 2023


# PREVALENCE OF PERMETHRIN RESISTANCE IN *CULEX TARSALIS* POPULATIONS IN SOUTHERN CALIFORNIA

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ABSTRACT. In the western United States, *Culex tarsalis* is the most important vector of West Nile virus. Insecticides containing permethrin or other pyrethroid compounds are commonly used to control these mosquitoes. Because of the range of environments where *Cx. tarsalis* are found, this species is under insecticide pressure from both vector control and agricultural spraying. Mosquito populations may evolve resistance through mechanisms such as target site insensitivity, including the frequently identified knockdown resistance (*kdr*) mutations. Prevalence of permethrin resistance was determined for *Cx. tarsalis* from 5 southern California field sites representing 2 distinct valley regions (Coachella Valley and Inland Valley), which are geographically separated by the north-south-running Peninsular Mountain Ranges. These two valley regions are >100 km apart and vary considerably in their environmental and habitat characteristics. Permethrin resistance in mosquito populations was determined by the Centers for Disease Control and Prevention (CDC) bottle bioassay, using glass bottles coated with permethrin at 0.19 μg/cm$^2$ of internal surface. Permethrin resistance was evident in *Cx. tarsalis* populations from the Coachella Valley field sites with all sites showing similar mortality in the bottle bioassay, while *Cx. tarsalis* from the Inland Valley field sites were largely susceptible to permethrin, with mortality rates that were similar to a susceptible lab strain of *Cx. tarsalis*.

**KEY WORDS** Bottle bioassay, *Culex tarsalis, kdr*, permethrin resistance, southern California

## INTRODUCTION

*Culex tarsalis* Coquillett (the western encephalitis mosquito) is a species of public health concern as a vector of several viral etiological agents of encephalitis that impact humans, birds, and other animal species (Reisen 2012). While *Cx. tarsalis* prefer to feed on birds (Reisen 2012, Thiemann et al. 2012), they are opportunistic feeders and will also feed on mammals, potentially transferring several important mosquito-borne viruses from bird species (reservoir hosts) to humans or other mammals (Wekesa et al. 1997). In the western USA, *Cx. tarsalis* is an important zoonotic vector for West Nile virus (WNV), one of the most important mosquito-borne diseases of humans in the USA (Kilpatrick et al. 2005).

The geographic range of *Cx. tarsalis* extends from the West Coast to as far east as the Mississippi River, and from northern Mexico to Canada (Venkatesan 2009). Within this range, 3 genetically distinct population clusters of *Cx. tarsalis* have been identified, with separation of these populations perhaps maintained by differences in elevation, temperature, precipitation, or the presence of geographical features that limit dispersal among these population clusters (Venkatesan and Rasgon 2010).

Mosquito control programs conduct regular surveillance and control of mosquitoes to reduce the risk of mosquito-borne illness to the public and domestic animals (Richards et al. 2017). In the USA, insecticides containing pyrethroid compounds (IRAC code 3A), such as permethrin, are commonly used to control adult mosquitoes (Vincent et al. 2018). Pyrethroids are structural derivatives of naturally occurring pyrethrins in extracts from flowers of *Chrysanthemum* spp. plants (Dong 2007). Wide use of these insecticides, including agricultural and personal use, selects for mosquito resistance to permethrin in some geographic locations, thereby threatening the ability of mosquito control programs to effectively manage mosquitoes in these areas (McAllister et al. 2020).

Mosquito resistance to insecticides commonly involves decreased sensitivity of insecticide target sites due to genetic mutation of protein-encoding genes resulting in structural changes in target proteins that limit or prevent the binding of insecticides to target sites (Casida and Durkin 2013). This form of insecticide resistance is known as target-site insensitivity (Liu 2015). Permethrin targets the voltage-gated sodium channels that transport ions across cell membranes, where it binds to the sodium channel and inhibits its closure. The resulting prolonged action potential causes rapid insect paralysis or knockdown, which is generally followed by death of the insect (Busvine 1951). Knockdown-resistance (*kdr*) is due to reduced sensitivity of the sodium channel target site resulting from mutations in the insect genes responsible for sodium channel protein structure. Knockdown resistance to pyrethroids, including permethrin and other insecticides that similarly target the sodium channel, has been identified in several mosquito species and in many other insects (Soderlund and Bloomquist 1990; Knipple et al. 1994; Williamson et al. 1996; Martinez-Torres et al. 1998, 1999; Soderlund and Knipple 2003; Xu et al. 2006a, 2006b; Li et al. 2012; Xu et al. 2012).

Permethrin resistance has been reported in *Cx. tarsalis* from Colorado and South Dakota (Strong et al. 2008, Vincent et al. 2018) and more recently in *Cx. tarsalis*

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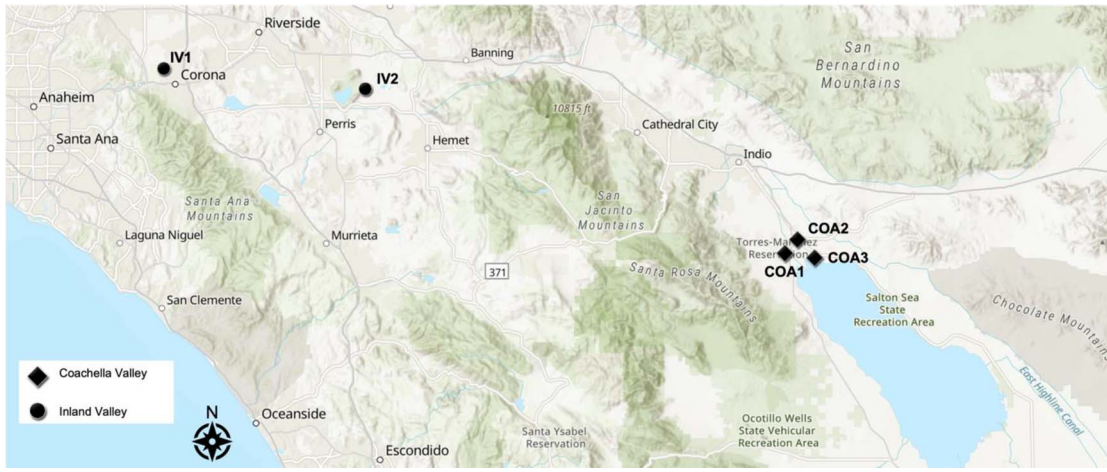


Fig. 1. Field sites in southern California where *Culex tarsalis* were captured. Inland Valley sites (IV1 and IV2) are wetland habitats in western Riverside County, while Coachella Valley sites (COA1, COA2, and COA3) are desert habitats near agricultural production north of the Salton Sea in eastern Riverside County.

from northern California (Choi 2016, Hughes 2017). In northern California, permethrin resistance in *Cx. tarsalis* was also shown to increase over a mosquito season with continued use of pyrethroid pesticides (Hughes 2017). In southern California, *Cx. tarsalis* from the Coachella Valley in eastern Riverside County was recently shown to have moderate resistance to pyrethroids, including permethrin (Hung et al. 2021b, 2021c). A related mosquito species, *Cx. quinquefasciatus* Say, also present in the Coachella Valley, was similarly shown to be resistant to pyrethroids including permethrin (Richards et al. 2017; Hung et al. 2021a, 2021d), suggesting that mosquitoes in this region of southern California may have had considerable historical exposure to pyrethroid insecticides.

The objectives of this study were to 1) determine the presence of permethrin resistance in host-seeking female *Cx. tarsalis* from 5 field sites representing 2 geographically separated regions of Riverside County in southern California during 2021 and 2) categorize individual mosquitoes from each field site by their survival time following exposure to a diagnostic dose of permethrin for use in follow-on studies evaluating the presence of genetic alleles in mosquitoes associated with each resistance category.

## MATERIALS AND METHODS

*Mosquito populations tested:* A permethrin-susceptible strain of *Cx. tarsalis* (Bakersfield lab strain; Hung et al. 2021b) originally collected from California was used to determine the diagnostic time for this species when exposed to permethrin applied to glass bottles at the diagnostic dose recommended by the Centers for Disease Control and Prevention (CDC; McAllister et al. 2020). This susceptible strain was acquired from the Coachella Valley Mosquito and Vector Control District (CVMVCD) and subsequently maintained at University of California Riverside (UCR) in an insectary room at 26°C, 50% RH, and 14:8 L:D with an additional 1 h of dimmed light before and after the light period to simulate sunrise and sunset, and otherwise following standard rearing protocols described by Kauffman et al. (2017).

Field strains of *Cx. tarsalis* were collected from 5 southern California field sites with varying environmental and habitat characteristics (Fig. 1). Two field sites in the Inland Valley of western Riverside County included natural wetlands bordering the Santa Ana River (IV1) and managed wetlands in the San Jacinto Wildlife Area that are periodically flooded to provide waterfowl habitat (IV2). Three additional field sites were located >100 km to the east of the Inland Valley field sites near the north shore of the Salton Sea in the Coachella Valley (COA1, COA2, COA3), an arid rift valley of the Colorado Desert in eastern southern California. The 3 COA sites varied in habitat characteristics including relative distance to the Salton Sea and nearby presence of urban or agricultural habitat.

Mosquitoes were collected from field sites using CDC-style $CO_2$-baited suction traps (model 512; John W. Hock Co., Gainesville, FL, USA) hung beneath insulated containers holding 1.36 kg dry ice each. Traps were attached to a stand made from PVC pipe and rebar, placing the trap entrance 1–1.2 m above ground. Mosquitoes were captured live into a container with mesh netting for ventilation and provisioned with a 10% sucrose solution to aid in mosquito survival overnight. At each field site, trapping was conducted from 1 to 2 h before sunset through sunrise the following morning. Trapping effort (traps/night) varied by field site according to abundance of *Cx. tarsalis* at each site. At IV1 and IV2, 12 traps were utilized each night, while at COA1, COA2, and COA3 only 1 trap per night was needed. After retrieving traps, captured mosquitoes were transported to UCR, where they were placed into

metal holding cages (Bioquip, Gardena, CA) labeled with field site and collection date. Cotton balls soaked in 10% sucrose solution were placed on top of each cage to increase mosquito survival. Mosquitoes were held until the following day under the same conditions described above for susceptible mosquitoes before testing for insecticide susceptibility. Field trapping was performed at each site on multiple dates in fall 2021 until at least 125 mosquitoes representing a minimum of 2 separate collection dates for each field site were tested for permethrin susceptibility as described below.

*Permethrin susceptibility bioassay:* Mosquito susceptibility to permethrin was determined using the CDC bottle bioassay procedure with a diagnostic time of 20 min based on 100% mortality of the permethrin-susceptible Bakersfield lab strain of *Cx. tarsalis* occurring via ca. 20 min of permethrin exposure (McAllister et al. 2020). Glass bottles (236 ml) (model no. S-23397, ULINE, Ontario, California) were coated internally with 40.85 µg permethrin dissolved in 1 ml of acetone to give a dose of 0.19 µg/cm$^2$ of internal surface of the glass bottle per standard CDC bottle bioassay procedures (McAllister et al. 2020). Control bottles received only 1 ml of acetone. Bottles were hand rolled within a chemical fume hood until the acetone had evaporated to evenly coat the internal surface of the bottle with permethrin. Bottles were further dried overnight in the fume hood before use the next day. Bottle bioassays were performed in a well-lit insectary lab room at 24°C and 50% RH.

To perform the bottle bioassay, approximately 25 female *Cx. tarsalis* captured from a field site on the previous day were aspirated from their holding cages using a Brigii Mini Vacuum (ASIN B07WN6D3TK, Amazon.com) fitted with a boba straw with a netting plug near the vacuum housing to prevent mosquitoes from reaching the vacuum fan. Aspirated mosquitoes were then blown into a labeled (Treatment or Control) glass bottle to start the bioassay period. Each bottle had a "cap barrier" constructed of a plastic cap with a mesh netting top containing a small slit allowing the straw to be inserted to blow mosquitoes into the bottle but preventing mosquitoes from escaping after removal of the straw. On each testing date, a bioassay was performed using 1–5 permethrin-treated bottles and 1–2 control bottles depending upon the number of female *Cx. tarsalis* that were captured at a field site on the previous day, and that were still alive in the holding cages the following morning when the bioassay was initiated. Mosquito mortality within each bottle was recorded every 5 min for the first 30 min, and then every 15 min thereafter for up to 180 min or until all mosquitoes in the bottle were dead. Mosquitoes were recorded as dead if they could not stand upright or fly. At the end of the 20 min diagnostic time, dead mosquitoes were removed from the permethrin treated bottles by flipping each bottle upside down to allow dead mosquitoes to collect in the bottle cap while live mosquitoes moved upward within the bottle away from the bottle cap. The bottle cap was then briefly removed and dead mosquitoes were separated into a labeled container. Mosquitoes that died within the 20 min diagnostic time were recorded as "susceptible" (S). Mosquitoes that died between 20 and 180 min were similarly removed at 180 min and recorded as "semiresistant" (SR). Mosquitoes surviving >180 min (the full bioassay period used in this study) were killed by placing the bottle into a lab freezer before mosquitoes were removed and recorded as "resistant" (R). This categorization allowed for separation of individual mosquitoes into 3 groups (susceptible, semiresistant, resistant) for future analyses using molecular methods to evaluate the association of each group with specific alleles known to confer mosquito resistance to pyrethroids. Because mosquitoes were captured at field sites, all mosquitoes were confirmed to species and sex at the end of each bioassay.

*Statistical analysis:* All data were analyzed in R (R Core Team 2022). Mortality at each observational time point was assessed as the number of *Cx. tarsalis* that were dead as a proportion of the total tested within each bioassay bottle. To conform with CDC guidelines for assessment of insecticide resistance variation among mosquito populations, mortality data were right censored at 120 min with Abbott's correction applied to any tested mosquito group with a control mortality of 3–10% (occurred on one testing date; McAllister et al. 2020). The mortality rate of mosquito populations from the different field sites and the laboratory strain was visualized with Kaplan–Meier survival analysis using the R package "survival" version 3.3-1 (Therneau 2023), with further analysis to compare differences in the mortality rate among field populations with the Cox proportional hazards model using the R package "coxph" (Therneau 2023) with data clustered by bioassay bottle and with statistical significance among field populations confirmed with the Wald test.

Differences in the prevalence of permethrin-resistant mosquitoes among the 5 field sites were determined by chi-squared test of independence, with pairwise comparisons between individual field sites using Fisher's exact test. Due to the very low number of fully resistant (R) individuals across all field sites, all mosquitoes from a single field site that survived the 20 min diagnostic exposure time, whether classified as semiresistant (SR) or fully resistant (R), were combined into a single "permethrin-resistant" group for these analyses. Abbott's correction was applied to adjust the number of mosquitoes in each phenotype category for any assay when control mortality was 3–10% (1 assay date for the COA2 population).

## RESULTS

Mosquitoes were captured from each field site on 2–4 separate collection dates with mosquitoes from each field site tested in 7–10 replicate permethrin-treated bottles ($n$ = 143–325 mosquitoes per field site; Table 1). Mosquito mortality in permethrin-treated bottles varied by geographic region ($z$ = 5.7, $P$ = 1.17e-08) with mosquitoes from COA1, COA2, and COA3 surviving longer than mosquitoes from IV1 and

Table 1. Bottle bioassays performed by field site. Mosquitoes from the same field site were combined for all collection dates and bioassay bottles (by treatment group) for analyses.

| Field site | Treatment | No. collection dates | No. bottles | No. mosquitoes tested |
|---|---|---|---|---|
| IV1 | Permethrin | 3 | 9 | 162 |
| | control | 3 | 3 | 42 |
| IV2 | Permethrin | 4 | 7 | 143 |
| | control | 4 | 4 | 56 |
| COA1 | Permethrin | 2 | 10 | 199 |
| | control | 2 | 4 | 67 |
| COA2 | Permethrin | 2 | 8 | 23 |
| | control | 2 | 3 | 66 |
| COA3 | Permethrin | 2 | 10 | 325 |
| | control | 2 | 4 | 103 |

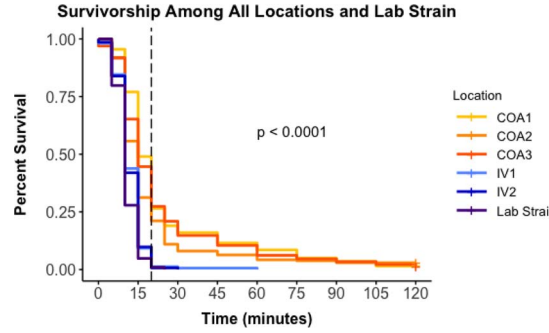


Fig. 2. Mosquito survival rate by Kaplan–Meier survival analysis. Vertical dashed line at 20 min indicates the diagnostic exposure time with mosquitoes surviving beyond this time considered resistant to permethrin according to CDC bottle bioassay procedures.

IV2 ($z \leq -2.5$, $P \leq 0.02$; Table 2, Fig. 2). There was no difference in mortality rate of mosquitoes from among the 3 COA sites ($P > 0.05$), with 21–27% survival of mosquitoes at the 20 min diagnostic time for each COA site. Mosquitoes from IV1 and IV2 had similar mortality rates to each other and to the susceptible laboratory strain ($P > 0.05$).

Permethrin-resistant mosquitoes (based on survival time) were recorded from all 5 field sites, with prevalence of permethrin resistance being similar among field sites within a geographic region (Inland Valley or Coachella Valley) but varying greatly between the 2 geographic regions (Table 3). There was very little permethrin resistance in mosquitoes from the Inland Valley, with only 1 mosquito each from IV1 and IV2 classified as semiresistant (SR) (0.6% and 0.7% of mosquitoes, respectively) and no mosquitoes surviving the full 180 min bioassay period to be classified as resistant (R) in this study. Furthermore, the 2 SR mosquitoes survived only between 30 and 45 min (IV1) or 20 and 25 min (IV2) of permethrin exposure suggesting limited or no resistance as these survival times were similar to the susceptible lab strain (Table 2). In

Table 2. Proportional survival of mosquitoes by collection site and exposure time within permethrin-treated glass bottles. Proportional survival was determined using Kaplan–Meier Survival Analysis with Abbott's correction applied when control mortality was 3–10% (occurred on one date for COA2).

| | Survival ± SE (CI) | | | | | |
|---|---|---|---|---|---|---|
| Time (min) | Susceptible lab strain | IV1 | IV2 | COA1 | COA2 | COA3 |
| 0 | 0.99 ± 0.01 (0.98, 1.00) | 0.98 ± 0.01 (0.96, 1.00) | 0.99 ± 0.01 (0.97, 1.00) | 0.99 ± 0.01 (0.97, 1.00) | 1.00 | 0.97 ± 0.01 (0.95, 0.99) |
| 5 | 0.89 ± 0.02 (0.85, 0.93) | 0.85 ± 0.03 (0.79, 0.90) | 0.84 ± 0.03 (0.78, 0.90) | 0.96 ± 0.01 (0.93, 0.98) | 0.92 ± 0.02 (0.89, 0.96) | 0.92 ± 0.02 (0.89, 0.95) |
| 10 | 0.59 ± 0.03 (0.53, 0.65) | 0.44 ± 0.04 (0.37, 0.52) | 0.42 ± 0.04 (0.35, 0.51) | 0.77 ± 0.03 (0.71, 0.83) | 0.56 ± 0.03 (0.50, 0.62) | 0.65 ± 0.03 (0.60, 0.71) |
| 15 | 0.13 ± 0.02 (0.03, 0.09) | 0.09 ± 0.02 (0.06, 0.15) | 0.10 ± 0.02 (0.06, 0.16) | 0.50 ± 0.04 (0.43, 0.56) | 0.31 ± 0.03 (0.26,0.38) | 0.45 ± 0.03 (0.40, 0.50) |
| 20 | 0.01 ± 0.01 (0.00, 0.04) | 0.01 ± 0.01 (0.00, 0.05) | 0.01 ± 0.01 (0.00, 0.05) | 0.27 ± 0.03 (0.21, 0.33) | 0.21 ± 0.03 (0.16, 0.27) | 0.27 ± 0.02 (0.23, 0.33) |
| 25 | 0.00 | 0.01 ± 0.01 (0.00, 0.04) | 0.00 | 0.19 ± 0.03 (0.14, 0.25) | 0.11 ± 0.02 (0.08, 0.16) | 0.21 ± 0.02 (0.17, 0.26) |
| 30 | 0.00 | 0.01 ± 0.01 (0.00, 0.04) | 0.00 | 0.16 ± 0.03 (0.12,0.22) | 0.08 ± 0.02 (0.05, 0.12) | 0.15 ± 0.02 (0.11, 0.19) |
| 45 | 0.00 | 0.00 | 0.00 | 0.12 ± 0.02 (0.08, 0.17) | 0.06 ± 0.02 (0.04, 0.10) | 0.10 ± 0.02 (0.08, 0.14) |
| 60 | 0.00 | 0.00 | 0.00 | 0.09 ± 0.02 (0.05, 0.13) | 0.04 ± 0.01 (0.02,0.08) | 0.06 ± 0.01 (0.04, 0.09) |
| 75 | 0.00 | 0.00 | 0.00 | 0.05 ± 0.02 (0.03, 0.09) | 0.04 ± 0.01 (0.02. 0.07) | 0.05 ± 0.01 (0.03, 0.80) |
| 90 | 0.00 | 0.00 | 0.00 | 0.04 ± 0.01 (0.02, 0.07) | 0.03 ± 0.01 (0.01, 0.06) | 0.03 ± 0.01 (0.02, 0.06) |
| 105 | 0.00 | 0.00 | 0.00 | 0.02 ± 0.01 (0.00, 0.05) | 0.03 ± 0.01 (0.01, 0.06) | 0.02 ± 0.01 (0.01, 0.04) |
| 120 | 0.00 | 0.00 | 0.00 | 0.02 ± 0.01 (0.00, 0.05) | 0.03 ± 0.01 (0.01, 0.06) | 0.01 ± 0.01 (0.00, 0.03) |

Table 3. Number (percent) of mosquitoes tested for permethrin resistance by field site that were susceptible (survival time ≤20 min) or resistant (survival time >20 min) according to CDC bottle bioassay procedures, with resistant mosquitoes further classified as semiresistant (survival 20–180 min) or resistant (survival > 180 min) for future studies to examine resistance mechanisms.

| | Number (percent) of tested mosquitoes | | |
|---|---|---|---|
| | | Resistant | |
| Field site | Susceptible (S) | Semiresistant (SR) | Resistant (R) |
| IV1 | 161 (99%) | 1 (0.6%) | 0 (0%) |
| IV2 | 142 (99%) | 1 (0.7%) | 0 (0%) |
| COA1 | 147 (74%) | 52 (26%) | 1 (0.5%) |
| COA2 | 188 (79%) | 47 (20%) | 2 (0.8%) |
| COA3 | 236 (73%) | 89 (27%) | 0 (0) |

Table 4. Test for hypothesis of equal frequency of permethrin resistance in mosquito populations from different field sites (Fisher's exact test).

| Field sites | *P* value |
|---|---|
| IV1/IV2 | 1 |
| IV1/COA1 | 1.9e-14* |
| IV1/COA2 | 4.15e-11* |
| IV1/COA3 | 1.11e-16* |
| IV2/COA1 | 4.53e-13* |
| IV2/COA2 | 3.05e-10* |
| IV2/COA3 | 5.49e-15* |
| COA1/COA2 | 0.17 |
| COA1/COA3 | 0.84 |
| COA2/COA3 | 0.07 |

* *P* values ≤ 0.05.

contrast, permethrin resistance was common in mosquitoes from the Coachella Valley, with a much greater proportion of mosquitoes classified as semiresistant (SR) from COA1, COA2, and COA3 (26%, 20%, and 27%, respectively) or resistant (R) from COA1 and COA2 (0.5% and 0.8%, respectively) relative to the Inland Valley field sites. Overall, there was a significant difference in resistance phenotype among the field sites ($\chi^2 = 63.81$, df = 4, $P = 4.59e\text{-}13$), with the proportion of mosquitoes with permethrin resistance (SR + R) varying by region but not by site within a region (COA1 = COA2 = COA3 ⋙ IV1 = IV2; Table 4).

## DISCUSSION

Considerable variation in permethrin resistance was evident among mosquito populations from the 5 field sites where *Cx. tarsalis* was collected in Riverside County, with permethrin resistance being much greater in mosquito populations from the Coachella Valley relative to those from the Inland Valley. With 20–30% mosquito survival at the diagnostic time, the 3 Coachella Valley populations of *Cx. tarsalis* are permethrin-resistant based on CDC criteria of >10% survival in the CDC bottle bioassay at the diagnostic dose and time (McAllister et al. 2020). In addition to the relatively low mortality rate of mosquitoes from the Coachella field sites, the survival of some of these mosquitoes for >180 min of exposure to permethrin suggests the presence of a strong resistance mechanism in *Cx. tarsalis* within this region.

Although there were substantial differences in mosquito resistance to permethrin between the 2 geographic regions, within a region the mosquito survival and resistance categorization was similar, suggesting a similar prevalence and mechanism of pyrethroid resistance across all sites within a region. Variation in prevalence of permethrin resistance by region may relate to the relative frequency of past exposures of these mosquito populations to insecticides containing permethrin or related pyrethroids. Use of pyrethroid insecticides by mosquito control districts or other public health agencies for the control of *Cx. tarsalis* or other mosquitoes has been limited near the Inland Valley sites, while pyrethroids including permethrin have been more frequently used for mosquito control near the Coachella Valley sites (California Pesticide Information Portal 2023). In addition, use of pyrethroids in agricultural production appears to be more common in the Coachella Valley relative to the 2 Inland Valley sites (California Pesticide Information Portal 2023). Frequent use of pyrethroids in agriculture can also lead to increased insecticide resistance within mosquito populations, as was previously shown for *Anopheles arabiensis* Patton exposed to pyrethroids applied for crop production in Kenya (Orondo et al. 2021). Considering the more frequent reported use of pyrethroids overall in the Coachella Valley relative to the Inland Empire region, mosquito populations in the Coachella Valley have likely experienced stronger selection pressure for resistance development.

This study confirms earlier reports of permethrin resistance by *Cx. tarsalis* from the Coachella Valley that were exposed to commercial formulations of pyrethroids using bottle bioassays similar to those used in the current study. Resistance to permethrin (Aqua-Reslin, Bayer Environmental Science, Cary, NC) and resmethrin (Scourge 18 + 54, Bayer Environmental Science, Park, NC) were noted in 2018 (Hung et al. 2021b) and again in 2020 along with resistance to pyrethrins (Merus 3.0, Clarke Mosquito Control Products, Roselle, IL; Hung et al. 2021c), though the level of resistance varied by mosquito population tested and mosquitoes were not resistant to all commercial products tested that contained a pyrethroid as the active ingredient. It is difficult to directly compare the level of permethrin resistance determined in previous studies to resistance noted in the current study, in part because the current study utilized technical permethrin only while the previous studies utilized commercial insecticides including Aqua-Reslin, which contains both permethrin and piperonyl-butoxide (PBO). Nevertheless, it is evident that at least some populations of *Cx. tarsalis* in the Coachella Valley have developed resistance to pyrethroids including permethrin and that this resistance has persisted over several years.

Relative to *Cx. tarsalis*, resistance to commercial insecticides containing pyrethrins or pyrethroids is higher in populations of *Cx. quinquefasciatus* from the Coachella Valley (Hung et al. 2021a, 2021d). Permethrin resistance of *Cx. quinquefasciatus* from the Coachella Valley was also demonstrated using technical grade permethrin with 100% survival of *Cx. quinquefasciatus* following 30 min of exposure to permethrin at 0.07 μg/cm$^2$ (Richards et al. 2017). While survival of *Cx. tarsalis* at 30 min of exposure to technical grade permethrin in the current study is much lower than the survival of *Cx. quinquefasciatus* reported by Richards et al. (2017) at this same exposure time, the dose of permethrin used in the current study (0.19 μg/cm$^2$) was much greater than the dose used by Richards et al. and is likely responsible for at least some of the lower survival of *Cx. tarsalis* in the current study.

The Inland Valley and Coachella Valley of southern California are >100 km apart and separated by a north-south-running group of mountain ranges (Peninsular Ranges). Although *Cx. tarsalis* is reported to disperse a maximum of 35 km (Bailey et al. 1965), the distance between the 2 valleys combined with the separating mountain ranges might be expected to limit gene flow between mosquito populations in these valleys (Reisen 2010, Ridenour et al. 2021). While the prevalence of permethrin resistance in other populations of *Cx. tarsalis* throughout southern California is unknown, the southern California mountain ranges and other geographical barriers may limit spread of resistance genes among disparate mosquito populations, thereby sustaining permethrin susceptibility in mosquito populations in the Inland Valley and perhaps other locations where mosquitoes remain fully susceptible to permethrin. Coordinated monitoring for mosquito resistance to permethrin and other insecticides critical for integrated vector management programs should be conducted across a regional geographic scale to better understand resistance trends in mosquitoes, identify the potential for spread of resistance genes among mosquito populations, and perhaps to investigate how use of pyrethroids in crop production might play a role in increasing resistance levels in mosquitoes or other unintended targets.

This study reinforces the need to identify and utilize management options other than the sole use of pyrethroid insecticides. Although regular rotation of insecticidal products targeting the immature mosquito stages is common and can include products other than pyrethroids, including bacteria (*Bacillus thuringiensis var. israelensis* de Barjac [*Bti*] and *B. sphaericus* [*Bs*]), surface agents (mineral oils or monomolecular films), insect growth regulators (diflubenzuron, pyriproxyfen), or chemical larvicides (temephos), products registered in California for control of the adult mosquito stage (adulticides) are limited to products containing pyrethroids or organophosphates, thus limiting product rotation options. Yet adulticides remain an important part of an emergency response plan to quickly reduce risk of arbovirus transmission when adult mosquito populations exceed transmission thresholds (Richards et al. 2017). To counter further development of permethrin resistance in *Cx. tarsalis* and other mosquito species, new research is needed to identify insecticides with novel modes of action or to develop emergent mosquito control technology. These efforts would provide additional tools for mosquito control districts and help to reduce the burden of insecticide resistance.

## ACKNOWLEDGMENTS

Mosquitoes from Coachella Valley sites were captured by Arturo Gutierrez from the Coachella Valley Mosquito and Vector Control District (CVMVCD). Bakersfield lab strain mosquitoes were maintained at the CVMVCD by Melissa Snelling. Northwest Mosquito and Vector Control District assisted with identifying suitable field sites in the Inland Valley region and for information related to historical use of insecticides for mosquito control within the region. The authors thank Edwin (Ted) Burgess (University of Florida) for aiding with statistical analyses. Funding was provided by USDA Hatch to AG and by Moore Fellowship from the University of California to JT.

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