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Editor: Jan Washburn, Ph.D.

Reviewer:
Jan Washburn, Ph.D.

Layout and Editorial Assistance: Todd Winslow and Audie Whitt, MVCAC

Mosquito and Vector Control Association of California
1215 K Street, Suite 940
Sacramento, California 95814
Phone: 916-440-0826 • Fax: 916-231-2141
E-mail: mvcac@mvcac.org
www.mvcac.org
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The Effect of Red Imported Fire Ant, *Solenopsis invicta* Buren, Control on Neighborhoods in Orange County, California

Kimberly J. Nelson[1,2], Brian S. Kronenfeld[1], Sean E. Walker[2] and Robert F. Cummings[1]

Orange County Vector Control District[1]; Department of Biological Science, California State University- Fullerton[2]

ABSTRACT: The Red Imported Fire Ant (RIFA), *Solenopsis invicta* Buren, threatens human health because they are aggressive, they sting, and in a few cases, they have caused fatalities. They also adversely affect humans through their ability to destroy electrical equipment and property, and as an invasive species, they impact many native organisms via direct competition and predation. The Orange County Vector Control District has been examining ways to control RIFA since their arrival in Orange County in 1999. This study looks at the effectiveness of control efforts in neighborhoods using two insecticides, Amdro Pro® and Distance®. Thirty neighborhoods were observed: ten that were treated in 2010, ten that were treated for the first time in 2011 and ten that remained untreated as a control. We compared the total number of infested homes per neighborhood pre and post treatment in the 2011 and the control groups. RIFA abundance was evaluated using baits at 6 - 8 homes per neighborhood. The number of homes infested with RIFA declined in neighborhoods treated in 2011 compared to untreated neighborhoods. One-year post treatment, neighborhoods treated in 2010 continued to have fewer homes infested with RIFA relative to control neighborhoods. RIFA abundance in neighborhoods treated in 2011 also declined relative to controls. This study shows that suppression efforts utilizing Amdro Pro® and Distance® substantially reduced RIFA abundance and, at least one year later, RIFA numbers remained significantly lower in treated areas. We are continuing to analyze data on the impact of our treatment on ant diversity.

INTRODUCTION

Biologists are continuously documenting the impact of invasive species on native biodiversity. More recently, scientists and policy makers have been focusing on the economic costs associated with invasive species and the millions of dollars spent annually on eradication efforts. Many ant species are major contributors to the environmental damage and economic loss because they can be easily transported by human trade (McGlynn 1999). Worldwide, the two most problematic invasive ant species are the Argentine ant, *Linepithema humile* Mayr, and the Red Imported Fire Ant (RIFA), *Solenopsis invicta* Buren. Originating from South America, both of these species have been transported to other countries and have become successfully established. Although both species have been responsible for rapid declines in native biodiversity, here we focus on the Red Imported Fire Ant.

RIFA are originally from parts of Argentina and Brazil and have successfully invaded New Zealand (MAF Biosecurity 2002), Australia (Moloney and Vanderwoude 2002), Taiwan (Kuo 2010), China (Zhang et al. 2007) and the United States of America (Lennartz 1973). RIFA arrived from South America into the United States in the 1930s near Mobile, Alabama and quickly became established and spread throughout the southeastern U.S., earning a reputation as a noxious pest that has impacted biological diversity and caused economic strife over a large area of the southern United States (Gotelli and Arnett 2000, Holway et al. 2002, MacGown and Brown 2006). As of August, 2012, RIFA have officially been reported in Alabama, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia and Puerto Rico (USDA 2012).

The introduction of RIFA into California has been traced to a shipment of beehives from Texas in 1997 after they had colonized almond orchards in Kern County (CDFA 2000). Two years later, RIFA were discovered in a nursery in Las Vegas, NV, and the source of those ants was from a production nursery in Orange County, CA (CDFA 2000). Once Orange County had a verified RIFA infestation, a survey was

conducted in areas surrounding the nursery and several residential areas were heavily infested (Spitzer 2000). RIFA in Orange County have been found primarily infesting north-central portions of the county and residential neighborhoods in the south (Figure 1).

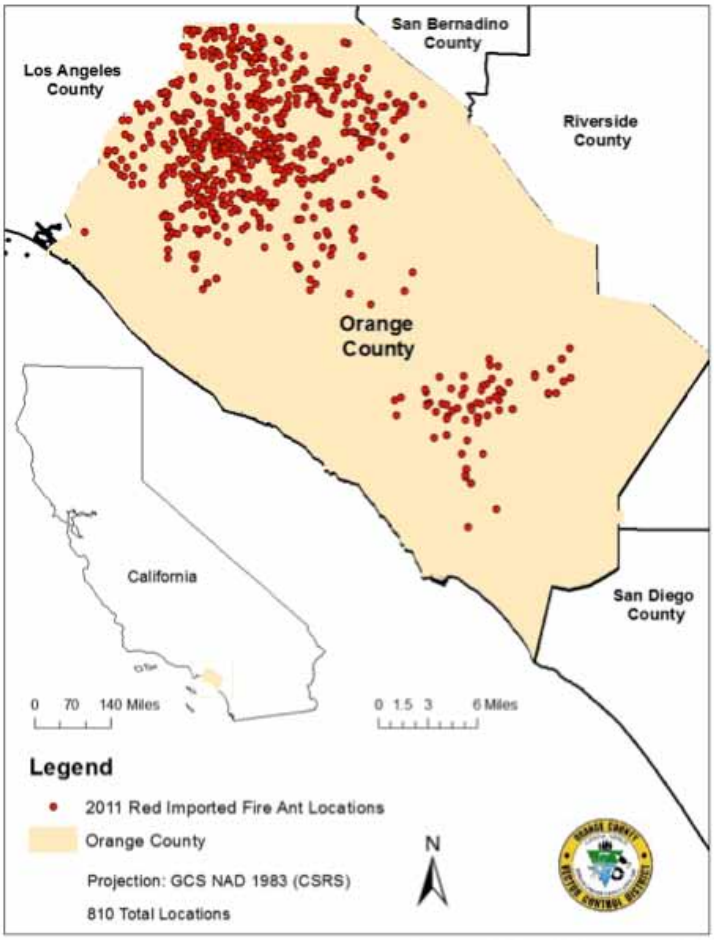

Figure 1. Map of Red Imported Fire Ants locations in Orange County. Red circles indicates areas positive for Red Imported Fire Ants.

Invasive RIFA have detrimental effects on many organisms via direct predation and exploitation in areas where they have been introduced and are considered generalist predators that will feed on any available resource, even larger organisms like birds and reptiles. RIFA have been found feeding on eggs and hatchlings of the barn swallow, *Hirundo rustica*, the eastern blue bird, *Sialia sialis* (Ligon et al. 2011), the least tern, *Sterna antillarum* (Lockley 1995), loggerhead turtles, *Caretta caretta*, green sea turtles, *Chelonia mydas* (Moulis 1997, Parris et al. 2002, Allen et al. 2011) and the Texas horned lizard, *Phrynosoma cornutum* (Donaldson et al. 2004).

Native ant diversity decreases immediately after RIFA invasion while RIFA niche expansion and exploitation is occurring (Morrison 2002). In California and throughout the southern United States, RIFA have aided in the displacement of the native southern fire ant, *Solenopsis xyloni* McCook (Wilson and Brown 1958, Glancey et al. 1976, Hung and Vinson 1978) and have been known to displace other native ant species (Greenberg 2011). In some instances, however, native ant species have been found to co-exist with RIFA years after invasion. Not only can some ant species co-exist, but when RIFA numbers are low, some of them can overtake a RIFA mound and cause death to newly-started colonies. The primary species known to destroy small colonies of RIFA are *Monomorium minimum* Buckley, *Pheidole dentate* Mayr, *Solenopsis molesta* Say, *Tetramorium bicarinatum* Nylander (Helms and Vinson 2005, Calixto et al. 2007), *Dorymyrmex insanus* Buckley (Whitcomb et al. 1973) and *L. humile*, which is an invasive species in southern California (Kabashima et al. 2007, Greenberg 2011). Although these studies were done in Texas and Florida, many of these ant species are widespread and may play a role in limiting the expansion of RIFA elsewhere in the United States.

Infestations of RIFA can directly pose threats to humans, as there have been 83 deaths in the U.S. attributed to RIFA stings (Hedges 1998). A more common response to RIFA contact is pustules on the skin as a result of stings. People can also be allergic to RIFA venom leading to more serious conditions. In 1998 an estimated $17 million was spent on medical costs for humans and pets that had interacted with RIFA (Salin et al. 2000). RIFA also indirectly impact humans through their ability to destroy electrical equipment and property when they build mounds inside and around electrical equipment, causing malfunctions and outages (Vinson and MacKay 1990). Pereira (2003) reported that RIFA damage and control costs an estimated $6 billion a year. Because of the major discomfort and injury to humans associated with RIFA, the California Department of Public Health recognizes RIFA as an important vector "… capable of causing human discomfort and injury."

Since RIFA invaded the U.S., researchers have tried different ways to eradicate them, most notably with insecticides. Eleven major chemicals have been used, beginning with calcium cyanide in the 1930s, followed

by chlordane, heptachlor and dieldrin (Tschinkel 2006). Their usage ended in 1962 after the publication of Rachel Carson's Silent Spring which questioned the safety of these chemicals. Mirex® was the next pesticide used to control RIFA in the United States, but it was discontinued in 1978 (Tschinkel 2006), again because of safety concerns. Since the 1980s, Amdro Pro® (hydramethylnon), a metabolic inhibitor, has been used to destroy RIFA mounds. The mode of action comes from a direct attack on the ants' metabolic systems, causing decreased energy, feeding and grooming. Worker death results within 24 - 72 hours after exposure, followed by queen mortality in about a week (Amdro Pro 2012). Other methods of control utilize insect growth regulators such as Distance® (pyriproxyfen), Extinguish® (methoprene), Logic® (fenoxycarb) and Clinch® (abemectin) (Hearst, 2000). These insect growth regulators prevent the immature workers from becoming adults, causing colony mortality in about eight weeks (Distance 2012).

The Orange County Vector Control District (OCVCD) currently uses Amdro® and Distance® to treat RIFA in Orange County communities because these chemicals are effective, yet degrade rapidly in the field once control is achieved. Initially OCVCD treated only individual properties that were positive for RIFA, but this sporadic treatment was found to be ineffective. Since 2001, OCVCD has been treating entire neighborhoods in an attempt to better control RIFA in the county. This study looks at how OCVCD's RIFA control strategies have impacted RIFA populations and affected native ant numbers in treated Orange County neighborhoods.

METHODS

This study was conducted in Orange County, California, from July 2011– April 2012. This large urbanized region consists of 34 cities and several unincorporated communities and has an estimated population of 3,010,232 people inhabiting 2,455.3 km² of land (U.S. Census Bureau 2012). The suburban and urban landscapes are made up of hardscape and varied turf grasses with limited plantings of mostly non-native ornamental trees and shrubs. Orange County has a Mediterranean climate with warm, dry weather from May-October and precipitation occurring primarily from November through April. The average temperature is 20ºC (www.weatherforyou.com) with an average of 330 ml of precipitation annually. Temperatures ranged from 16.7 - 33.9ºC with a total of 62.2 ml of rain during this study (weatherforyou.com). Because of the region's small amount of rainfall, most properties require artificial irrigation.

Neighborhoods were selected for treatment based on the frequency of resident calls complaining of RIFA (5 calls/ month/ square mile) received by OCVCD in 2010 and 2011. After RIFA calls were received and infestations verified, individual properties were treated. The increased frequency of calls received by any individual neighborhood was used as an indicator of greater need for neighborhood treatment. For study purposes, each neighborhood consisted of approximately 20-25 acres with at least six randomly selected properties infested with RIFA.

Three treatment groups were used in this study in Orange County, CA (Figures 2, 3). The first group consisted of ten randomly chosen neighborhoods that were treated for RIFA in 2010; the second treatment group consisted of ten randomly chosen RIFA infested neighborhoods that were treated for the first time in 2011. The third group consisted of ten RIFA infested neighborhoods that remained untreated and were used as a control. The 2011 and the control neighborhoods underwent an initial visual survey to obtain indication of the presence of RIFA, and the number of homes positive with RIFA were counted.

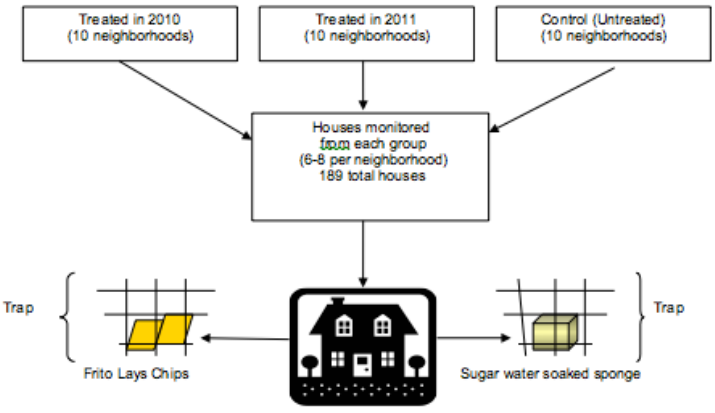

Figure 2a. Sampling Protocol. Three neighborhood treatment groups: treated in 2010, treated in 2011 and untreated control. In each neighborhood, properties containing RIFA were monitored using six bait baskets, with three containing Frito Lays chips and three containing a small sponge soaked in sugar water.

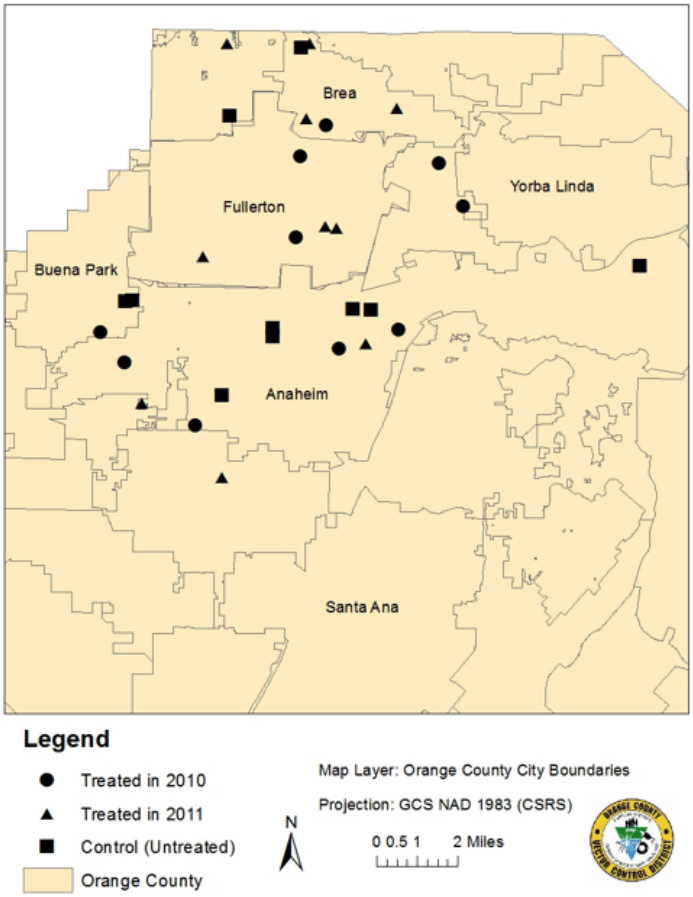

Figure 2b. Neighborhoods in Orange County. The squares indicate neighborhoods used as untreated controls; triangles indicate neighborhoods treated in 2011; circles represent neighborhoods treated in 2010.

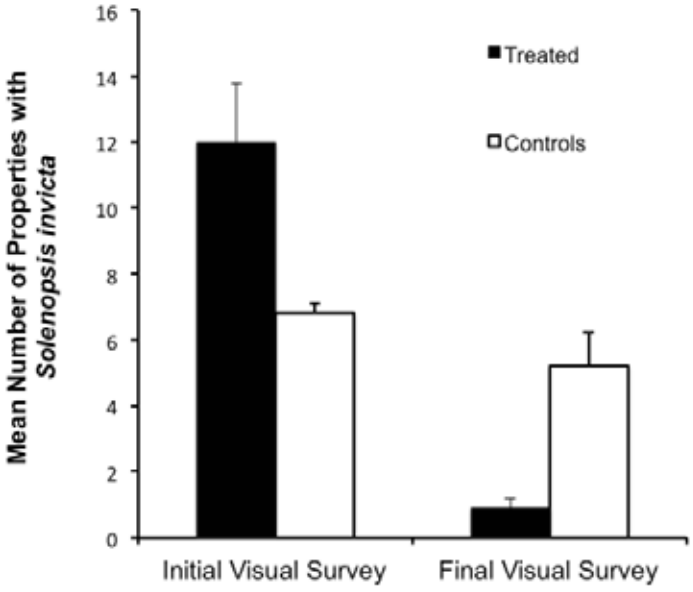

Figure 3. Mean number of properties with *Solenopsis invicta*. Treatment of neighborhoods decreased the total number of properties with RIFA in neighborhoods treated in 2011. No difference was seen in untreated control neighborhoods.

In the 2010 neighborhoods, 6 - 8 randomly chosen residential properties were monitored following treatment in 2010 to evaluate RIFA abundance one-year post treatment. In the 2011 and control neighborhoods, 6 - 8 randomly chosen properties in each neighborhood were monitored for four subsequent months (following the initial sampling and treatment of the 2011 treatment groups) using bait traps (4 cm diameter x 4 cm high plastic mesh baskets). At each individual residence, three bait baskets filled with Frito Lay™ chips and three bait baskets containing a 2 cm x 2 cm sponge that had been soaked in a solution of 25% sugar in water were spread across the front yards. Baits were left for 3 hours in the morning and then picked up to identify and count the total number of RIFA and other ants at these monitored properties. A final visual survey of the 2011 and control neighborhoods were then conducted to identify how many properties were still infested with RIFA 6 months later.

Ants were collected from bait traps for 4 consecutive months following initial sampling, and the baits were transported in coolers (4 - 8ºC) to OCVCD and then killed with dry ice (-70ºC). Using taxonomic keys (Creighton 1950, Fisher and Cover 2007), ants were sorted by species, enumerated, transferred into vials containing 70% ethanol and labeled with their unique site number, property number and sampling period. Data were analyzed using ANOVA and t-tests where appropriate. All analyses were carried out using JMP v 10.0 (http://www.jmp.com).

RESULTS

In total 3,897 properties in 30 neighborhoods were surveyed, and 76,556 ants were collected, counted and identified at bait traps (Table 1). Ninety-two percent (70,598) of these ants were non-natives, with 79% (60,800) being RIFA. The next highest percentage of

ants found in the neighborhoods was comprised of the invasive Argentine ant which consisted of about 12% (8,895) of the total ants collected. The remainder of the non-native ants made up less than 1% (903) of the total ants collected. Native ants were represented in only 8% (5,958) of the total, with the most of these consisting of *Nylanderia* spp. and *Solenopsis molesta* [4% (2,900) and 2% (1,773), respectively]. Six other native ant species found at bait traps made up the remaining 2% (1,285) of the total species found.

Treatment of neighborhoods with chemical control caused a highly significant (n = 120, -92.5%, P<0.001) decline in the number of RIFA-positive properties in the neighborhoods (Table 2, Figure 3). During the initial visual survey of entire neighborhoods treated in 2011 or left untreated, at least six properties contained mounds. About six months post treatment, the final visual survey showed a decrease in the number of properties with RIFA mounds. No difference was seen between the initial and final visual surveys of the untreated control neighborhoods (n = 67, -22%, P > 0.05).

Treatment of neighborhoods caused a significant decline (n = 1,240, -78%, P < 0.05) in RIFA abundance at baits within the first month (Table 2, Figure 4). The abundance of RIFA rapidly declined from an average of approximately 1,200 RIFA at baits during initial sampling to ~300 RIFA at baits in each neighborhood. In contrast, the control groups did not show a difference (n = 1,176, +9%, P > 0.05) between the initial sampling

Species (Common Name)	Total no. Captured	Proportion
Non-Native Ants		
Solenopsis invicta Buren (Red Imported Fire Ant)	60,800	0.79
Linepithema humile Mayr (Argentine Ant)	8,895	0.12
Pheidole moerens Wheeler (Big Headed Ant)	362	< 0.01
Brachiomyrmex patagonicus Mayr (Rover Ant)	293	< 0.01
Cardiocondyla mauritanica Forel	244	< 0.01
Cardiocondyla nuda Forel	4	< 0.01
Sub-total	*70,598*	*0.92*
Native Ants		
Nylanderia spp.	2,900	0.04
Solenopsis molesta Say (Thief Ant)	1,773	0.02
Monomorium ergatogyna Wheeler (Minute Black Ant)	804	0.01
Tapinoma sessile Say (Maladorous House Ant)	403	0.01
Dorymyrmex insanus Buckley (Pyramid Ant)	63	<0.01
Paratrichina sp.	11	<0.01
Prenolepis impairs Say (Winter Ant)	3	<0.01
Camponotus clarithorax Creighton	1	<0.01
Sub-total	*5,958*	*0.08*
Total of All Ants	**76,556**	**1.00**

Table 1. Ant species at traps. Ants collected at bait traps, including California native ant species.

Treatment	Neighborhood number	# houses RIFA+ Initial Survey	# houses RIFA+ Final Survey	Total # Properties	# of RIFA at Baits 0 mo	1 mo	2 mo	3 mo	4 mo	% Change (0 mo - 4 mo)
2010	1	-	-	131	176	136	92	33	2	
	2	-	-	135	51	55	279	316	272	
	3	-	-	102	117	5	52	0	0	
	4	-	-	153	0	0	0	0	1	
	5	-	-	119	1	2	86	83	103	
	6	-	-	103	4	0	0	0	0	
	7	-	-	123	83	42	0	0	0	
	8	-	-	165	0	33	0	0	0	
	9	-	-	163	0	0	0	0	0	
	10	-	-	90	0	0	0	0	0	
TOTALS	10	-	-	1284	432	273	509	432	378	-12%
2011	11	25	0	178	3098	766	179	113	105	
	12	7	0	141	95	62	120	2	353	
	13	13	1	141	1009	81	74	57	36	
	14	8	2	128	2002	237	667	499	213	
	15	6	1	120	1209	283	67	112	0	
	16	13	1	126	907	184	116	201	13	
	17	7	3	128	1044	182	55	0	40	
	18	13	0	194	1164	377	99	254	139	
	19	15	1	127	1109	434	225	243	200	
	20	13	0	201	767	146	14	55	0	
TOTALS	10	120	9	1484	12404	2752	1616	1536	1099	-91%
Untreated Control	21	6	12	198	421	1229	282	202	1073	
	22	9	8	89	3491	833	420	298	240	
	23	5	1	135	306	1380	223	57	609	
	24	7	3	78	898	704	569	120	149	
	25	8	4	108	1356	519	231	180	59	
	26	6	2	121	816	3860	550	1455	702	
	27	6	6	101	627	2498	351	308	1017	
	28	7	4	66	1010	675	535	575	467	
	29	7	7	135	1896	1053	734	659	719	
	30	6	5	98	943	86	1146	683	155	
TOTALS	10	67	52	1129	11764	12837	5041	4537	5190	-55%

Table 2. RIFA data.

period and the one month mark in which the average number of RIFA at baits remained approximately 1,200 RIFA per neighborhood.

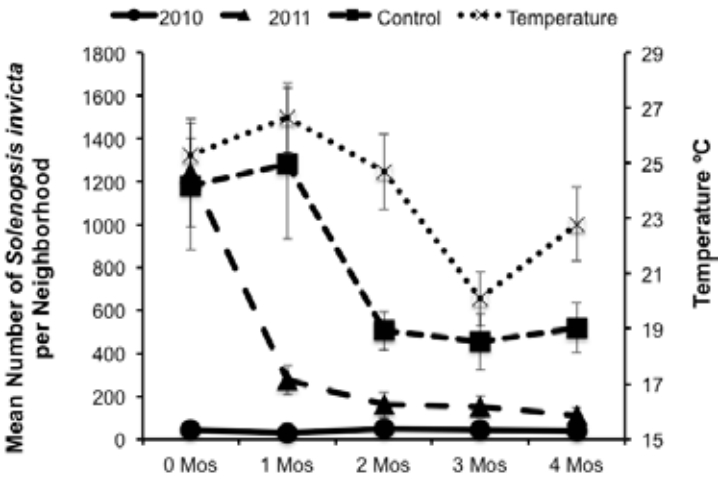

Figure 4. Mean number of RIFA per neighborhood. Treatment in 2011 caused a decline in abundance of ants in neighborhoods. Control neighborhoods showed a decline in abundance that was correlated with declining temperature. Neighborhoods treated in 2010 did not show a temporal difference. (Weather data source: John Wayne Airport Weather Station, Santa Ana, CA, http//www.weatherfromyou.com).

Although a difference in RIFA abundance was seen between the 2011 treatment and the untreated control groups, ant numbers in the untreated control group also declined during the two month sampling period, coincident with a drop in temperature. RIFA activity decreases with declining temperature (Porter and Tschinkel 1987). As for the 6 - 8 houses per neighborhood that were treated in 2010 for RIFA and monitored during the 2011 study period, no increase was seen in RIFA numbers and abundance. This shows that one year after undergoing treatment, the study areas' RIFA population appeared to have stabilized at a low level.

DISCUSSION

This study demonstrated that OCVCD's chemical control efforts were effective in reducing the number of mounds and the total number of *Solenopsis invicta* (RIFA) present in neighborhoods in Orange County. Although complete eradication was not successful, control was achieved using the insecticides and the number of RIFA complaints from treated areas fell accordingly. The initial drop within the first month at baits in neighborhoods treated in 2011 showed the effectiveness of Amdro Pro®. The active ingredient, 0.73% hydramethylnon, has been shown to be an effective fast-acting treatment for RIFA (Amdro Pro 2012) since it became FDA approved in 1980 (Thompson et al. 2009). Distance® is a slow-acting, yet highly effective product (Distance 2012), which resulted in declining abundance over time. Abundance increased at the 4th month as temperatures rose unexpectedly in the untreated control neighborhood at the end of the field evaluations, but no corresponding increase occurred in the treated areas.

Although chemical control has been shown to be effective, the untreated neighborhoods also had a decline in abundance of RIFA at baits that appeared during the 2 month sampling period. This decline appears to follow the reduction in ambient temperature. In Florida, Porter and Tschinkel (1987) reported RIFA to decrease above ground foraging at temperatures below 20ºC. Maximum RIFA foraging rates were between 22 and 36ºC, with 30ºC having the highest number of workers foraging. Lu et al. (2012) in China, also found maximum foraging activity to be similar between 25 and 33ºC.

We are continuing to analyze the impact of RIFA treatment on ant diversity. The monthly bait sampling allowed us to not only look at the effectiveness of Amdro Pro® and Distance® on the properties that initially were infested with RIFA, but allowed us to follow ant succession and view ant biodiversity that re-colonize any given area once RIFA are eradicated. Because the native ants *M. minimum*, *D. insanus* and *S. molesta* are commonly found co-occurring with RIFA, with control efforts by humans, these native species may aid in successful eradication of RIFA. As for other invasive ant species in California, the Argentine ant may also help to control RIFA expansion (Whitcomb et al. 1973, Kabashima et al. 2007). Since Argentine ants do not cause harm to humans, they are not considered a public health problem, and replacing RIFA with Argentine ants would be more beneficial to human health. Kabashima et al. (2007) showed that Argentine ants quickly overcome a dying colony of RIFA and prevent small colonies from establishing in areas they

occupy. With *L. humile* expanding across California, and specifically Orange County, once human control efforts cause reductions in RIFA, areas occupied by *L. humile* may aid in successful eradication of RIFA.

As of this writing, data on species interactions are still being analyzed and will be included in the MVCAC presentation and a summary will be published in the upcoming MVCAC proceedings. However, we intend to publish the results of this study in a peer-reviewed journal in the near future.

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West Nile virus cluster analysis and vertical transmission in Culex Pipiens complex mosquitoes in Sacramento and Yolo Counties, California, 2011*

E. D. Fechter-Leggett, B. M. Nelms, C. M. Barker and W. K. Reisen

Center for Vectorborne Disease University of California, VetMed 3A, One Shield Ave., Davis, CA 95616, ethan.fechter@cdph.ca.gov

ABSTRACT: West Nile virus (WNV) is now endemic in California, with annual transmission documented by the statewide surveillance system. Although much is known about the horizontal avian-mosquito transmission cycle, less is known about vertical transmission (passage of the virus from parent to offspring) under field conditions, which may supplement virus amplification during summer and provide a mechanism to infect overwintering female mosquitoes during fall. The current study identified clusters of WNV-infected mosquitoes in Sacramento and Yolo Counties, CA, during late summer 2011 and tested field-captured egg-laying female mosquitoes and their progeny for WNV RNA to estimate the frequency of vertical transmission. Space-time clustering of WNV-positive *Culex pipiens* complex pools was detected in the northern Elk Grove area of Sacramento County between July 18 and September 18, 2011 (5.22 km radius; $p < 0.001$ and RR = 7.80). Vertical transmission by WNV-infected females to egg rafts was 50% and to larvae was 40%. The estimated minimal filial infection rate from WNV-positive, egg-laying females was 2.0 infected females/1,000. The potential contribution of vertical transmission to WNV maintenance and amplification are discussed.

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SUMMARY OF WORK

West Nile virus (WNV) is transmitted horizontally between ornithophilic *Culex* mosquitoes and passeriform birds, occasionally causing disease in "dead-end" hosts such as humans and horses (Komar 2003, Weaver and Reisen 2010). Mosquitoes in the genus *Culex* are the primary WNV vectors (Turell et al. 2000), and several have been shown to be competent laboratory vectors, including *Cx. pipiens* and *Cx. quinquefasciatus* (Turell et al. 2001, Goddard et al. 2002, Reisen et al. 2008), two members of the *Cx. pipiens* complex that exhibit extensive hybridization in the Sacramento area (Urbanelli et al. 1997). Several flaviviruses, including WNV, have been shown to be vertically transmitted from an infected female mosquito to its progeny by *Culex* and other mosquito species both in the laboratory (Baqar et al. 1993, Turell et al. 2001, Dohm et al. 2002, Goddard et al. 2003, Reisen et al. 2006a, Anderson et al. 2008, Anderson et al. 2012) and the field (Miller et al. 2000, Anderson et al. 2006, Anderson and Main 2006, Phillips and Christensen 2006, Reisen et al. 2006a, McAbee et al. 2008, Unlu et al. 2010); however, the contribution of vertical transmission to WNV amplification during summer is poorly understood. The objectives of Fechter-Leggett, Nelms, Barker and Reisen (2012) were:

(1) To identify clusters of WNV-infected mosquitoes in space and time in Sacramento and Yolo Counties, California using surveillance data from pools of adult female *Cx. pipiens* complex mosquitoes collected using gravid traps during 2011, and

(2) To estimate the frequency of vertical transmission of WNV through testing the progeny of ovipositing *Cx. pipiens* complex females trapped within these clusters during the summer months of 2011.

A space-time cluster of WNV-positive *Cx. pipiens* complex mosquito pools was delineated in northern Elk Grove, Sacramento County, California and surrounding areas between 18 July and 18 September 2011 ($p < 0.001$; Figure1 1). The cluster radius was 5.22 km and consisted of 45 positive mosquito pools against an expected value of 10.22 pools, and the relative risk (RR) was 7.80. Weekly prevalence of WNV infection within the space-time cluster ranged from 15.7 to 79.5 per 1,000 *Cx. pipiens* complex mosquitoes tested (Figure 2). The actual prevalence of infection during the sampling period fell within these MLE infection limit estimates, with 11 positive of 304 individual females tested, giving a true prevalence of 36 per 1,000 gravid female *Cx. pipiens* complex mosquitoes tested.

Evidence of vertical transmission occurred in four females from two of the four trap sites where WNV-positive females were caught; the overall vertical transmission rate to eggs was 50% (5 WNV-positive egg rafts from 10 positive females) and to larvae was 40% (4 WNV-positive families of 1st instars). Interestingly, the 5 ovipositing females with negative egg rafts and larvae had Ct scores >30, indicating an infection of <100 plaque forming units (pfu) based on standard curves derived from WNV grown in Vero cell culture. Conversely, the 5 egg-laying females that passed virus vertically to either their eggs or larvae had Ct scores <20, indicating an infection of $>10^6$ pfu.

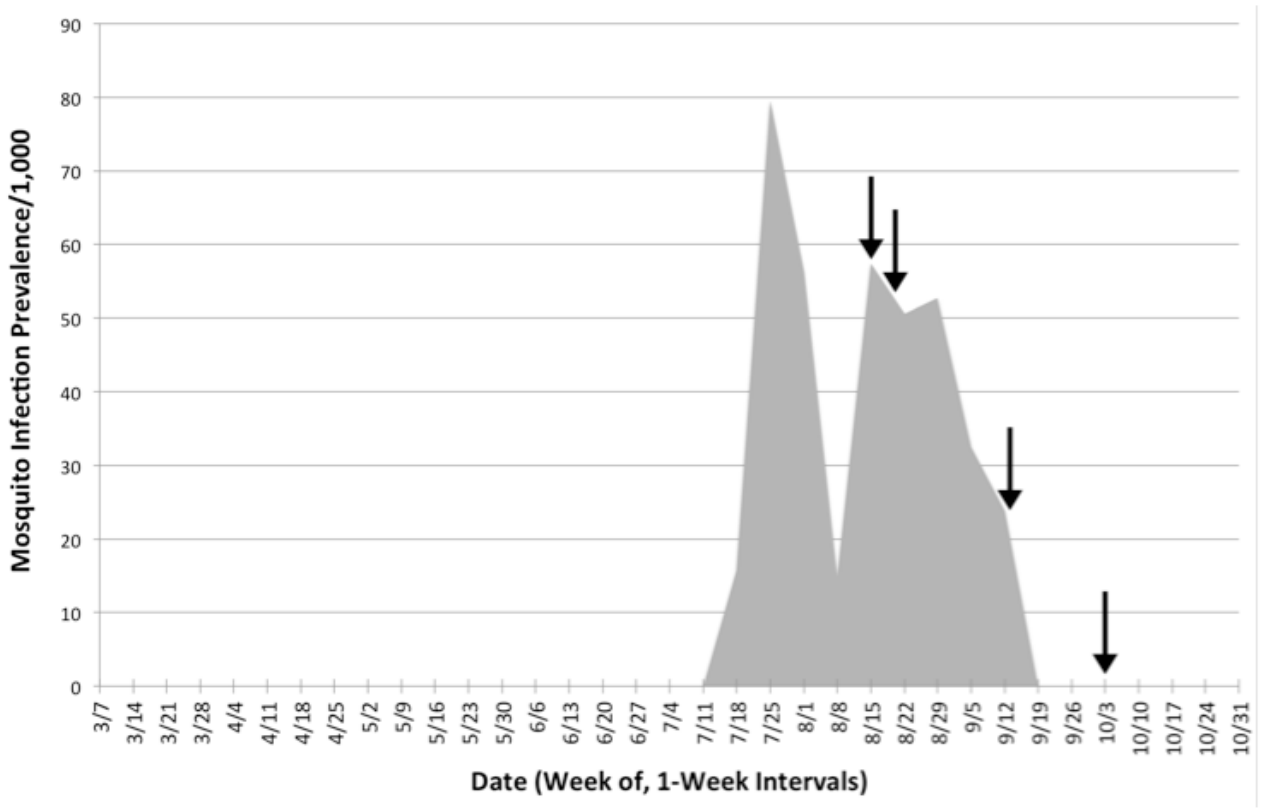

Figure 1. Sacramento-Yolo Mosquito and Vector Control District gravid trap locations and space-time cluster of West Nile virus-positive gravid *Culex pipiens* complex pools in Sacramento and Yolo Counties, California (7/18/2011–9/18/2011, 5.22 km radius, $p < 0.001$) and the location of the vertical transmission study trap sites. Points indicate trap sites were positive on one or more occasions (orange) or always negative (gray).

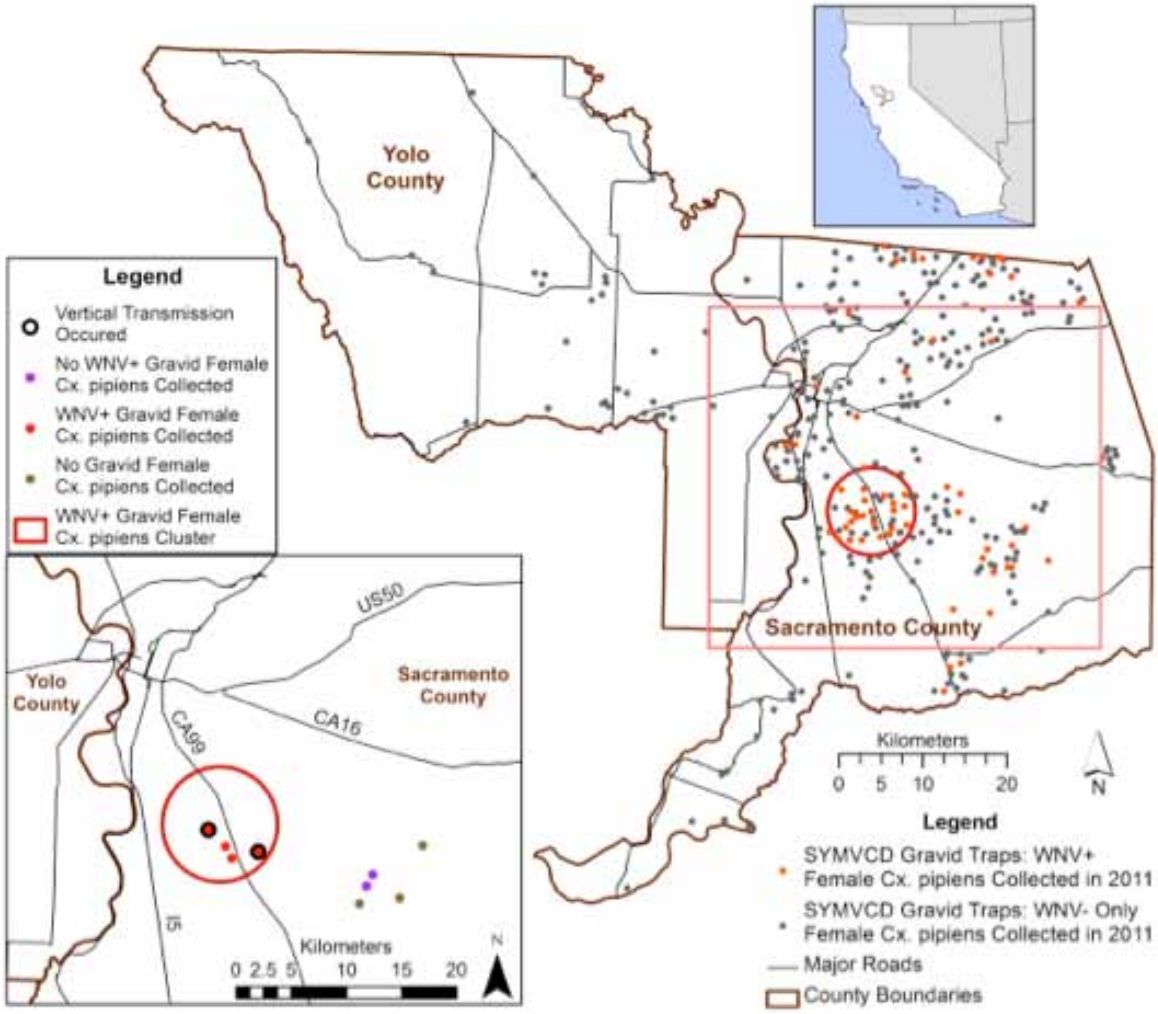

Figure 2. Weekly estimates of WNV infection prevalence in female egg-laying *Culex pipiens* complex mosquitoes within the identified space-time infection cluster. Arrows indicate trapping dates for vertical transmission investigation.

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Fine-Scale Genetic Structure of the House Finch (Carpodacus mexicanus) along an Urban Gradient: Implications for Local Movement of West Nile Virus

Timothy J. Morgan1,2, Robert Cummings1, Kiet Nguyen1, and James Archie2

1 Orange County Vector Control District, 13001 Garden Grove Blvd., Garden Grove, CA 92843, USA
2 California State University, Long Beach, CA, USA

Experimental and field studies suggest that the house finch (Carpodacus mexicanus) is an important host in the transmission cycle of West Nile virus (WNV) in many parts of California. However, the species' role in the movement of the virus through an urbanized landscape is not well known. In this study a population genetic analysis of the house finch was conducted to determine if genetic structuring and inferred dispersal patterns could provide insight to the emergence, spread, and maintenance of WNV in Orange County, California. WNV sero-prevalence data collected from 2003 to 2004 from eight sites within the urban-rural gradient were used to examine virus emergence and spread during the invasion of WNV in the county. Microsatellite analysis of six polymorphic loci revealed low, but significant, structure due to allele frequency differences between several pairs of sites. Some less-urban sites with low WNV seroprevalence and large local population sizes, estimated from the genetic analysis, may contribute high numbers of WNV-naive birds to the urbanized area of the county each generation. These potential "source" populations may play an important role in reducing the local herd immunity at distant sites, setting the stage for future WNV-outbreaks.

INTRODUCTION

The mechanisms contributing to the movement of West Nile virus (WNV) through disease-naïve host populations inhabiting heterogeneous landscapes are complex (Magori et al. 2011) and confounded in part by interactions between a multitude of vertebrate hosts and a wide range of vector species, all of which are variably abundant, distributed, mobile and WNV-competent (Komar et al. 2003; Rappole et al. 2006; Molaei et al. 2010; Venkatesan and Rasgon 2010). By examining the emergence and spread of a virus like WNV at a fine scale across heterogeneous landscapes, important processes that drive virus movement may be revealed; whereas, these processes may go undetected on a broad scale approach. Advances in molecular ecology have provided a relatively quick way to investigate aspects of vector and host population structures that would not be possible or be very difficult for some species using traditional observational methods. The value of these tools in studying the spread of vector-borne diseases has become more important as new diseases emerge across varied landscapes. For example, a recent population genetic study using microsatellite analysis of an important mosquito vector of WNV, Culex tarsalis Coquillett, revealed high levels of gene flow among distant populations, suggesting the species could facilitate long distance movement of the virus during dispersal events (Venkatesan and Rasgon 2010).

Although urbanized landscapes may seem homogeneous, they have a high degree of spatial heterogeneity. At a scale that covers an entire urban to wilderness gradient, a typical pattern emerges consisting of a highly urbanized core surrounded by a radiating landscape of decreasingly human-altered habitat extending to a wildland periphery, resulting in four major patch types: wildland, rural, suburban, and urban (Marzluff et al. 2001). However, on a finer scale (1–10 km2), the urban matrix can be marked with patches of wilderness, large parks, and other low human impact areas. Variation in the level of urbanization among the four patch types should have differential effects on a species that inhabits the entire gradient, but tolerates or benefits from each patch type to different degrees.

The house finch, Carpodacus mexicanus Say, has emerged as an important species in the WNV transmission cycle in southern California because

of its competence as an amplifying-host (Reisen et al., 2005; Kilpatrick et al. 2007; Reisen et al. 2008; Molaei et al. 2010), frequent use as a blood source by important vector species (Molaei et al. 2010), high abundance and wide distribution (Bolger et al. 1997), and dispersal capabilities (Veit and Lewis 1996; Able and Belthoff 1998). Other avian species shown to be important WNV-amplifying hosts (Komar et al. 2003) must also be considered in the movement of the virus; however this study focused on the house finch because of the species' wide distribution and high abundance across the rural-urban gradient of southern California and the ease of capture in urban settings compared to other common species, such as the elusive American Crow (Corvus brachyrhynchos Brehm).

In 2003, along the southern California coast, the Orange County Vector Control District (OCVCD) detected the county's first WNV-antibody-positive wild birds (all house finches). Within one year, eight well-established wild bird surveillance traps placed throughout the 2046km2 of Orange County (U. S. Census Bureau 2011) were regularly detecting antibody-positive birds, primarily house finches.

Studies comparing western and eastern United States house finch populations have detected significant genetic structuring (Wang et al. 2003; Hawley et al. 2006), but surprisingly, comparisons among populations within the western range alone revealed limited genetic structuring despite anecdotal reports of sedentary behavior (Hawley et al. 2006). To determine whether or not genetic population structure existed, a molecular ecological approach was used to evaluate fine-scale genetic structure and dispersal patterns by analyzing microsatellite genetic variation among local house finch populations inhabiting patches with varying degrees of urbanization. Through genetic analysis of local house finch populations, insights may reveal patterns of host movement and shed light on the role this species played in the emergence, spread, and maintenance of WNV virus in Orange County, California.

METHODS

Previously collected serological data was compiled for free-ranging house finches trapped at eight sites (Modjeska, Craig Park, OCVCD, SJWS, Villa, Seal Beach, Laguna, and Bolsa) distributed across Orange County from 2003 to 2004 (Figure 1).

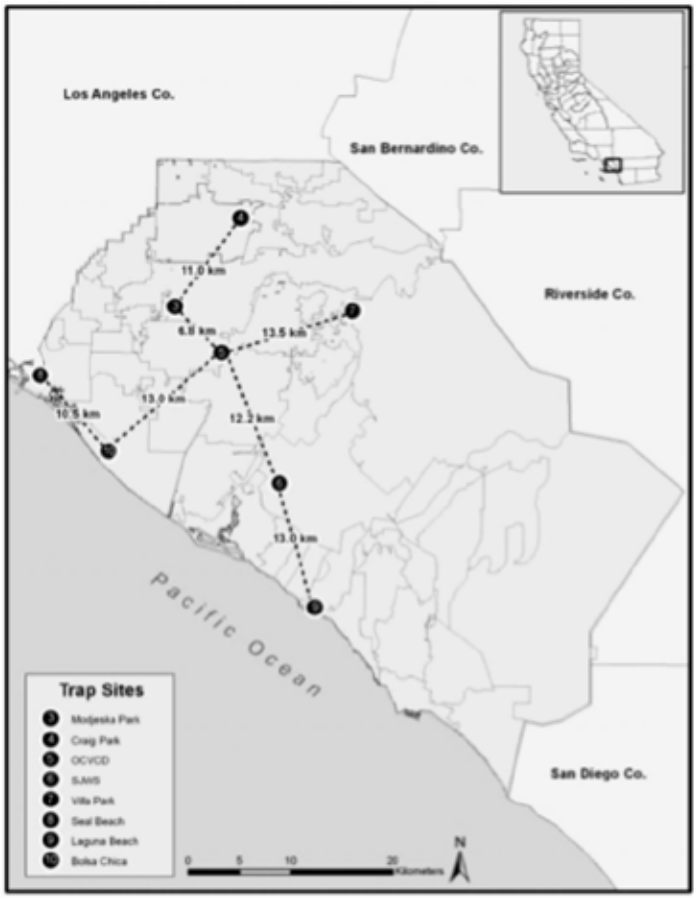

FIGURE 1. Map of eight trap sites within the urbanized landscape of Orange County, California. Distances between nearest populations are shown for spatial reference.

Briefly, WNV-antibody detection was performed using a blocking ELISA targeting an immunodominant epitope on the West Nile Virus NS1 protein, detailed methods of which can be found in Hall et al. (1995) and Jozan et al. (2003). To assess genetic structuring, jugular venipuncture was performed to collect ~ 50µl of blood from 279 adults from the same eight sero-surveillance locations during the breeding season (March–August) of 2007. Samples were stored in 150µl of genomic lysis buffer (Zymo Research, Orange, CA) at -70°C. Collection, identification (Pyle et al. 1987), banding (McClure 1984), and bleeding of birds were done under State of California Department of Fish and Game Scientific Collecting Permit No. 009927 and USGS Master Station Banding Permit No. 23547.

DNA extractions were performed using a Genomic DNA Kit™ (Zymo Research) following the standard whole blood protocol. Allele sizes were quantified at six highly polymorphic microsatellite loci (Hofi3, Hofi17, Hofi20, Hofi23, Hofi24, and Hofi52) that were previously developed for house finches (Hawley 2005). All samples from each location were analyzed together for all analyses.

The program FSTAT 2.9 (Goudet 1995) was used to calculate Weir and Cockerham's (1984) estimator of Wright's fixation index, FST, for all pairwise combinations of sites (Goudet et al. 1996). Isolation by geographic distance was explored using the web-based program IBDWS 3.22 (Jensen et al. 2005). Six different dispersal scenarios were modeled using the program MIGRATE (Beerli 2009) to estimate the population parameters Θ (mutation scaled effective population size) and M (mutation scaled migration rate).

During this study, extensive mosquito sampling was conducted in concert with wild bird testing.

RESULTS/DISCUSSION

The first positive mosquito pool in Orange County was detected in the first week of June 2004, while the first positive birds (all house finches) were detected as early as nine months prior. This study suggests the house finch was a more likely candidate for initial spread of the virus throughout Orange County than mosquitoes. A recent blood meal study conducted across the urban landscape of southern California and in concert with this study (Molaei et al. 2010) showed that Culex quinquefasciatus Say, the region's most important WNV mosquito-vector (Reisen et al. 2005; Kwan et al. 2010), feeds more often on house finches than other birds common to the area. In addition, a semi-natural experiment where four species of birds, house finch, house sparrow (Passer domesticus L.), American crow and mourning dove (Zenaida macroura L.) were exposed simultaneously to natural populations of mosquitoes in different human altered landscapes in southern California, showed the house finch was the preferred host species for Cx. quinquefasciatus in urban areas (Lura et al. 2012). Given both Cx. quinquefasciatus and the house finch's high abundance, overlapping distributions and high vector-host affinity in urbanized Orange County, we suggest that early establishment in naïve areas and subsequent maintenance were heavily influenced by interactions between these two species.

Other avian hosts are important to consider and inevitably play a role in the transmission cycle in Orange County, but none reach the abundance levels and distribution as the house finch, with the exception of the house sparrow; however, this species is not as widely distributed as the house finch (Blair 2004) and is highly sedentary (Vangestel et al. 2010; Vangestel et al. 2011), making it a less effective mode of virus dissemination.

Overall, results indicate that genetic structure is present among some local house finch populations over a fine geographic scale within the urbanized landscape of the county (Table 1). Isolation by distance analysis showed no significant relationship between distance

Table 1. Pairwise F_{ST} Values Between All Sampling Locations.

	OCVCD	Modjeska	Craig	Bolsa	Laguna	SJWS	Seal	Villa
OCVCD		0.0100	0.0194	0.0252	0.0149*	0.0390*	0.0030	0.0186
Modjeska	0.15643		0.0151	0.0196	0.0086	0.0162	0.0102	0.0128
Craig	0.09107	0.0861		0.0211	0.0169	0.0223	0.0151	0.0107
Bolsa	0.00357	0.0036	0.1407		0.0028	0.0005	0.0160	0.0165
Laguna	0.00071	0.0143	0.0118	0.2461		0.0140	0.0010	0.015*
SJWS	0.00036	0.0125	0.0021	0.5757	0.0025		0.0208*	0.0161*
Seal	0.21357	0.05571	0.00571	0.00893	0.09429	0.0014		0.0143*
Villa	0.00357	0.00357	0.07786	0.00929	0.00107	0.00036	0.00036	

Note: The fixation index F_{ST} is shown above the diagonal and p-values are shown below for each pair of sampling sites. Asterisks indicate significant differences after correction for multiple comparisons (adjusted $\alpha = 0.00125$). Sites are arranged from most urban to least urban.

and genetic structure among local populations in Orange County. This suggests that if the observed genetic structure was caused by restriction of gene flow among populations it was not related to the distance between any two populations.

In a WNV enzootic area, antibody-positive birds begin to accumulate over time, resulting in the suppression of viral transmission through herd immunity. However, an influx of unexposed migrants from a WNV naïve or refractory location would provide many newly available reservoirs, causing a reduction in herd immunity that could set the stage for a potential increase in WNV activity. Based on patterns of gene flow, peripheral less-urban sites, such as Seal Beach and Craig Park (Sites 8 and 4, respectively, Figure 2) in north Orange County, contribute the highest level of migrants to the urban locations, acting as important source populations. This relatively high movement of birds from large source populations (inferred from effective population size, Θ) could act to reduce herd immunity by flooding sink-like urban populations. Temporal shifts in abundance and disappearance of house finches at trap sites within the urbanized matrix of Orange County (House 2000) suggest a possible metapopulation structure, which supports the hypothesis of local source/sink populations.

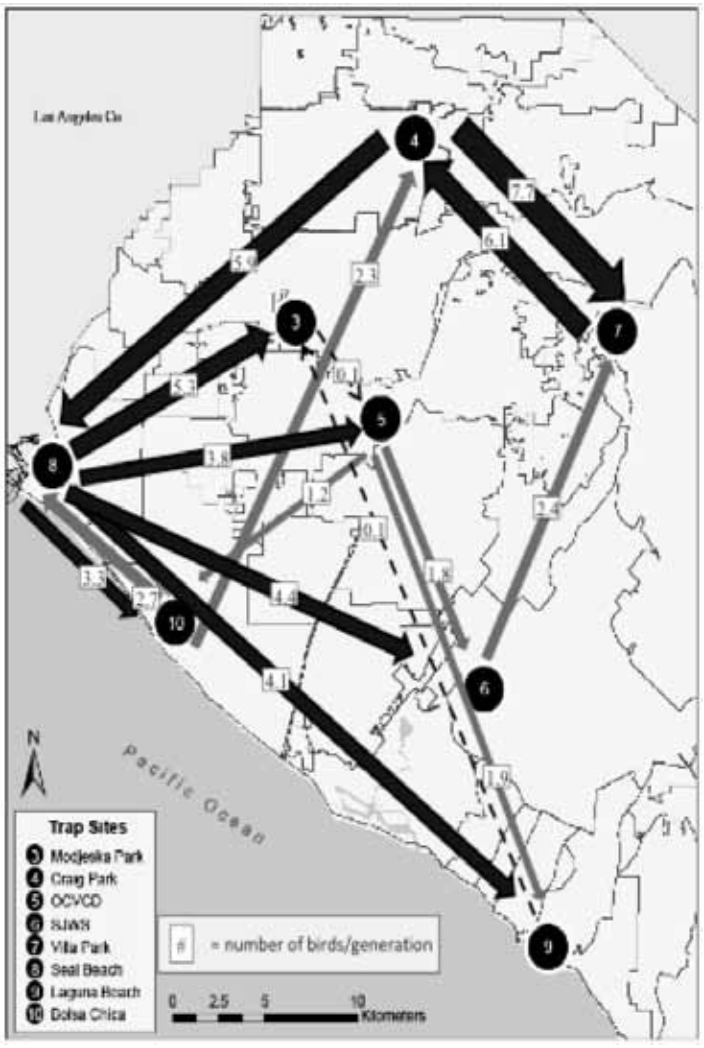

FIGURE 2. Map showing the highest and lowest number of migrants entering each population per generation based on MIGRATE analysis. Arrows represent the magnitude and direction of dispersal and the width of each is proportional to number of migrants (1 migrant = 0.07 cm). Only the highest and lowest dispersal estimates for each population are shown out of the 56 possible pairs for clarity. Dashed arrows indicate < 1 migrant per generation.

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Introduction to the Symposium: Arbovirus Research at the Center for Vectorborne Diseases

William K. Reisen

Center for Vectorborne Diseases, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616

The current review of mosquito and arbovirus research at the Center for Vectorborne Diseases [CVEC] is the 9th symposium summarizing collaborative projects by staff, students and faculty affiliated with CVEC and collaborating with MVCAC agencies. Topics this year include an overview of the impact of demography and temperature on the epidemiology of West Nile Virus [WNV] focusing on events during 2012 [Reisen: ' Summer in the City']. Because WNV probably will not be the last exotic virus to be introduced into North America, including California, CVEC has been conducting research to develop new assays for surveillance [Theimann: 'New Assays to Detect Arboviruses'] and on the vector competence of California mosquitoes, including the invading strain of *Aedes albopictus* now established in Los Angeles, for exotic viruses [Armijos: 'Emerging Viruses: Vector Competence Studies with California Mosquitoes']. We also have been conducting research to understand and improve methods for better tracking of WNV. This year we again revisited the detection and importance of chronic infections among dead birds submitted by the public to the Dead Bird program and how different assays affected the quantitation of results using real-time PT-PCR assays on kidney and oral swab data [Chouicha: 'Cycle Threshold Scores for Dead Birds in California: What Does This Mean for Mosquito Control?']. Research on the genetics of the *Culex pipiens* complex in California (Kothera et al. 2012) was extended to include more information on phenotypes related to vector competence and overwintering biology [Nelms: 'The *Culex pipiens* Complex: Genotype Meets Phenotype']. All *Culex* take sugar meals throughout their lifetime, perhaps even more frequently than bloodmeals (Reisen et al. 1986). We have been following up on an Australian study attempting to use this behavior for arbovirus surveillance (Hall-Mendelin et al. 2010) and found that females readily expectorate WNV while sugar feeding [Wheeler: 'Sucking Sugar and Spitting Virus: a Laboratory Study'] and that WNV can be found deposited on sugar wicks positioned in arid areas of California [Lothrop: 'Field Evaluation of Sugar Baits to Detect West Nile Virus]. Other projects presented reviewed new approaches attempting to predict *Culex* abundance from climate data [Danforth: 'Impacts of Climate Change on *Culex tarsalis* in California between 1966 and 2001'] and studies on the evolution of WNV in California using a novel competitive fitness assay in model avian and mosquito hosts [Worwa: 'West Nile Virus in California Evolves Towards Increased Avian Replicative Fitness and Reduced Vector Infection'].

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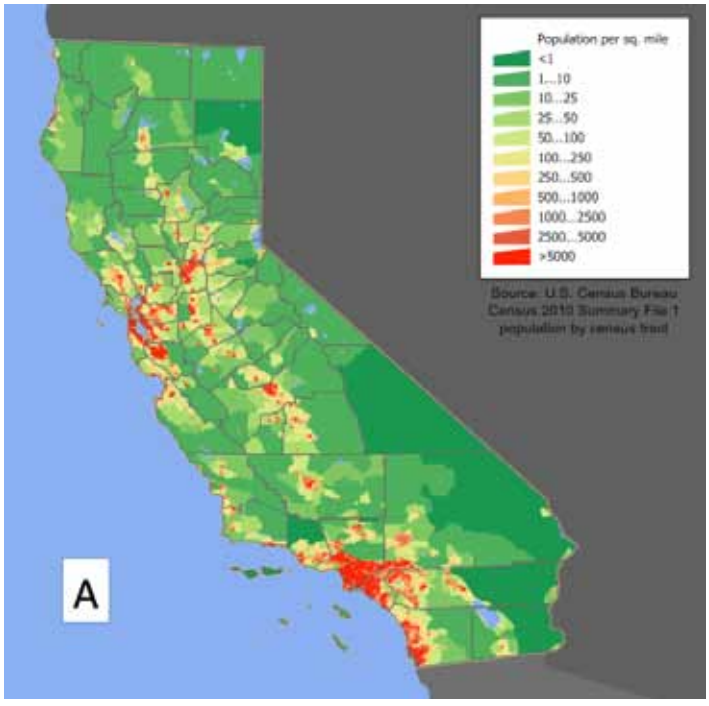
"Summer in the City" - Thoughts on West Nile Virus Ecology

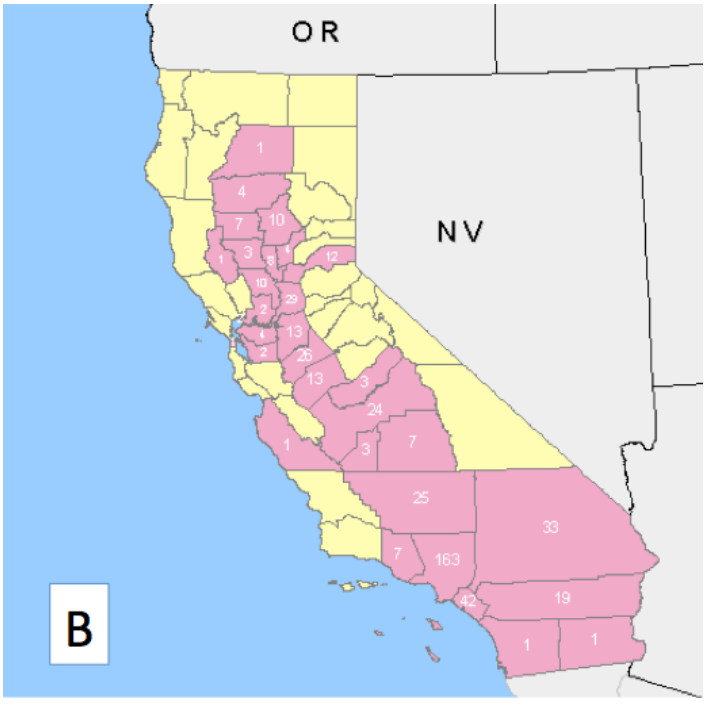
William K. Reisen

Center for Vectorborne Diseases, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616

The current paper summarizes some thoughts on the ecology of West Nile Virus [WNV] in California, relevant to its distribution in time and space. These comments seem timely considering the nationwide resurgence of WNV during 2012, after some researchers thought the epidemic was subsiding (Snapinn et al. 2007). Extended versions of this synthesis will be published this coming summer in Outlooks on Pest Management and as an invited review article in a special issue of Viruses (http://www.mdpi.com/journal/viruses/special_issues/west-nile-virus).

Population growth, urbanization and climate change seem to be altering the landscape of California in ways that facilitate the establishment, persistence and amplification of WNV. At present, most human cases are reported from cities, especially Los Angeles and Sacramento, during late summer, although the highest incidence and risk of infection remains in rural areas such as Glenn County. Human cases occur where people live and in California most live in cities (Figure 1). The primary vectors here, members of the *Culex pipiens* complex, have become urbanized, exploiting peridomestic breeding sources as well as municipal waste water systems. In addition, the downturn in the economy and the associated housing crisis has dotted the urban landscape with unmaintained homes and swimming pools (Kim et al. 2011, Reisen et al. 2008), producing productive habitat for rural mosquitoes such as *Culex tarsalis* as well as several urban species. Urbanization also reduces avian species diversity (Loss et al. 2009), leaving abundant populations of successful peridomestic species such as House Finches, House Sparrows and American Robins that are competent maintenance hosts and American Crows and Western Scrub-Jays that are high susceptible and important amplification hosts (Komar et al. 2003; Reisen et al. 2005). Urbanization therefore has produced an environment conducive to WNV transmission (Ezenwa et al. 2006), due to vector abundance, reduced avian diversity, and the summer formation of communal roosts by American Crows (Reisen et al. 2006a).





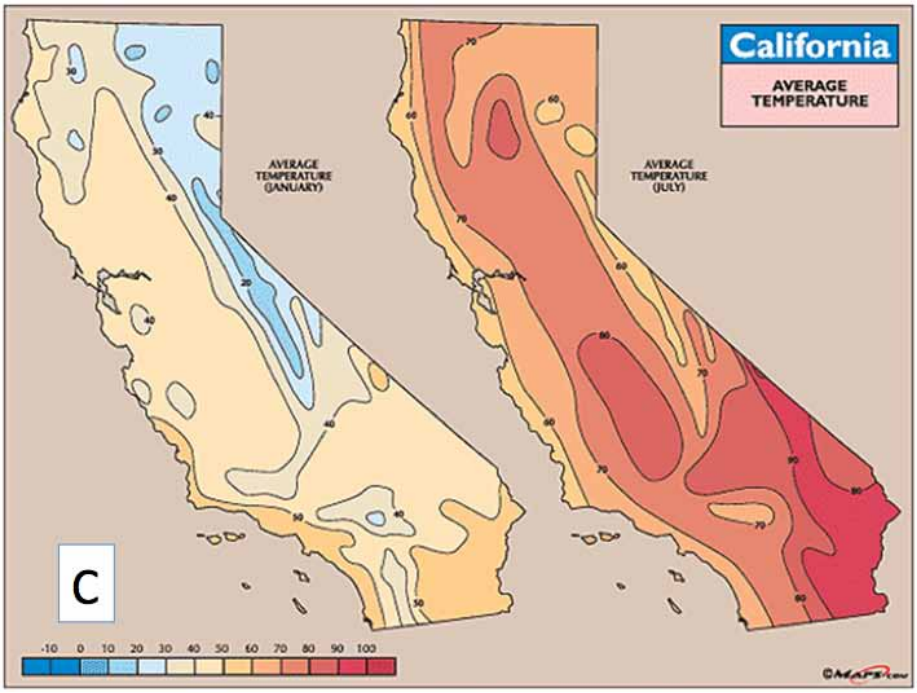


Figure 1. Patterns of (A) human population density, (B) West Nile virus human disease and (C) summer and winter temperature. Data from the US Census Bureau, the USGS, and www.maps.com.

Although there has been general loss of California residents moving to other states, they have more than been replaced by foreign immigration, mostly from Latin America and Asia, and by 2050 the California Department of Finance estimates the state population will approach 60 million. Although Los Angeles may increase numerically, the greatest percentage growth, and therefore landscape change ,will be realized in the Central Valley from Kern through Butte counties (Figure 2). This increase will occur in areas historically endemic for western equine encephalomyelitis and St Louis encephalitis viruses and now supporting consistent tangential transmission of WNV (Figure1). This increased human population in combination with the current relocation and expansion of the dairy industry is anticipated to increase the abundance of mosquitoes in proximity to humans and therefore the risk of tangential transmission of these zoonotic viruses

WNV transmission to humans occurs mostly during mid to late summer. Although the magnitude of transmission varies markedly among years, the timing of amplification is always associated with summer and driven by the thermodynamics of virus replication within the mosquito host. Although mosquitoes can modify their temperature by behavior (Meyer et al. 1990), mosquito temperature correlates well with ambient conditions, being hottest in summer. Being poikilotherms, physiological processes are faster at warm temperatures, so larval development, blood digestion and ovarian development are more rapid, decreasing generation times, increasing abundance and increasing the frequency of blood feeding (Reisen

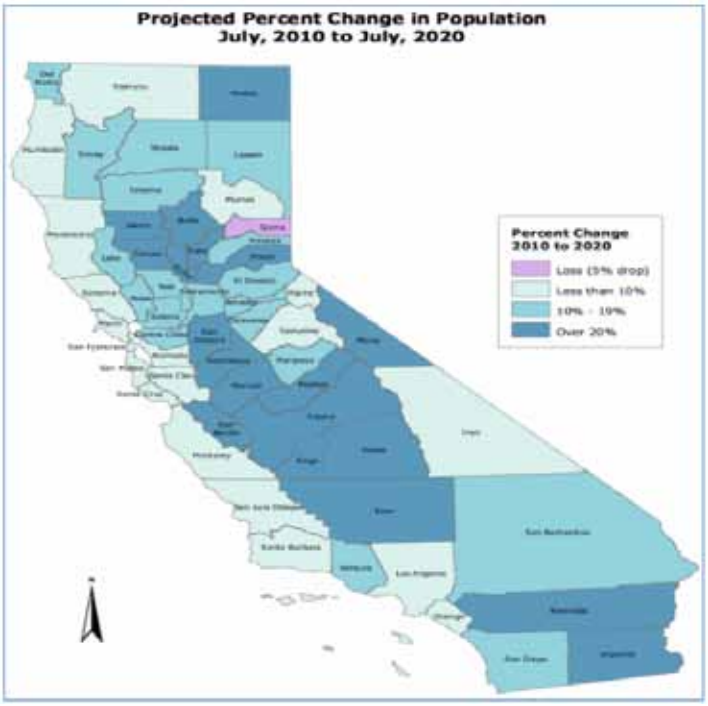


Figure 2. Projected percent change in human population in California by county from 2010 to 2020. Map from California Department of Finance.

1995). Frequent blood feeding increases contact with avian hosts during the brief viremia period and therefore the chances of vector infection. In addition, the rate of virus replication within the mosquito increases, reducing the duration of the extrinsic incubation period [EIP] and allowing transmission to occur earlier in life (Reisen et al. 2006b). The impact of temperature on the virus is greater than on the mosquito, resulting in transmission occurring earlier in female reproductive life. This is exemplified with representative data from Kern County taken during the summer of 2004 (Figure 3) when enzootic WNV activity, as measured by mosquito and sentinel chicken infections, did not commence until after the EIP was completed in less than 2 gonotrophic cycles. Although there was a lag period, enzootic activity subsided in the fall after the duration of the EIP increased beyond 2 gonotrophic cycles [GC]. This relationship was described recently and plotted as a risk surface over California as bites per transmission or BT = GC/EIP (Hartley et al. 2012) and was validated by sentinel chicken seroconversions. BT clearly explained the geographic distribution of enzootic virus activity.

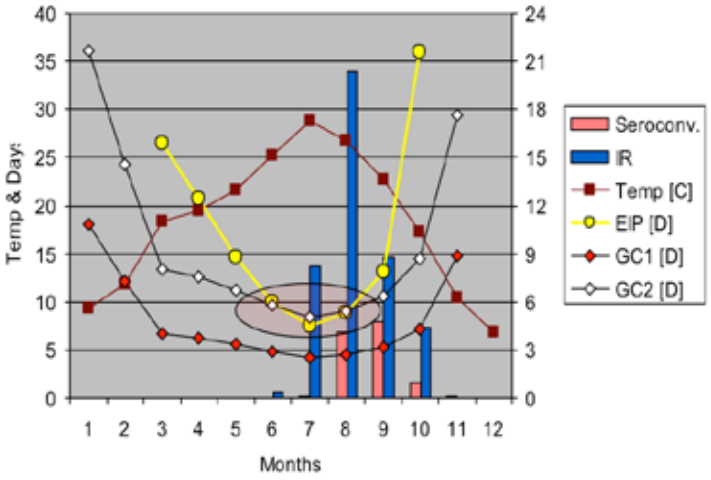


Figure 3. Mean monthly ambient temperature at Bakersfield airport (Temp C) and the duration of the extrinsic incubation period [EIP D] and the first two gonotrophic cycles (GC1 and GC2 D) in days plotted as a function of time in months. Also shown on the right axis are monthly *Cx. tarsalis* infection rates per 1,000 (IR, n = 475 pools) and mean sentinel chicken seroconversion rate per flock of 10 hens (Seroconv.) (redrawn from Reisen et al. 2006b).

Anthropogenic changes will alter these enzootic transmission patterns further by urbanization and climate change. Because of the extensive heat absorbing impervious surfaces, cities generate their own weather and create heat islands that can range from 1 to 5 C warmer than their surrounding areas (Oke 1982). Therefore, the expanding urbanization of the Central Valley will be expected to increase temperatures and thereby facilitate transmission near where people live. Climate change also has and will continue to modify annual temperature profiles for California. Although these changes may not markedly alter the duration and efficiency of transmission in the warmer portions of the state such as the southeastern deserts, areas of coastal California including the San Francisco Bay area may become more receptive, placing this large metropolitan population at risk. Human behavior may also be modified to enhance transmission during hot periods. This would seem especially relevant to communities where warm evenings are spent sitting outside and socializing. In addition, farm and other work, as well as outdoor sport activity, frequently are scheduled for periods during evening when night active *Culex* blood feed thereby enhancing transmission risk. People tend to wear less clothes (short sleeves, short pants) during summer exposing more skin surface to host-seeking mosquitoes when they are outdoors, especially if physically active.

Avian flock immunity (Kwan et al. 2012) and depopulation (LaDeau et al. 2007, Wheeler et al. 2009) determine the intensity of amplification. Frequently, large outbreaks of WNV are followed by one or more years of decreased activity, until flock immunity declines due to dilution and bird numbers increase by recruitment. House Sparrow and House Finch populations have rapid population turnover rates and rebound rapidly, whereas corvid populations may take longer due to longer life and slower reproduction. If left unchecked, this seems to result in a 3 to 4 year cycle of resurgent outbreaks with spillover transmission to humans. Effective adult control seems to interrupt this cycle, protect avian populations, preclude elevated flock seroconversion and immunity, and therefore facilitate annual amplification to emergency planning or epidemic rick levels. So although adulticide applications effectively interrupt transmission and prevent human infection, they also may protect the avian population and allow more seronegative birds to persist through the transmission season and into the following critical spring amplification period.

These scenarios provide a continuing challenge to mosquito control and health planners, but point out the importance of surveillance in intervention decision support.

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Emerging Viruses: Vector Competence Studies with California Mosquitoes

Veronica Armijos, Brittany Nelms, Tara Thiemann, Sarah Wheeler and William Reisen

Center for Vectorborne Disease and Department of Pathology, Microbiology and Immunology School of Veterinary Medicine, University of California, Davis, CA 95616

BACKGROUND AND METHODS

Emerging diseases have been defined as infections that are increasing in incidence or spreading rapidly in a geographic region. Many infectious diseases, in particular those of vector borne origin, were thought to be under control but have spread to places where they were absent just years ago (Gratz 1999). This resurgence is the result of many factors, including an increase of international travel to and from parts of the world where infectious diseases are circulating (Gubler 1998). This, along with ecological and environmental changes and an increase in international trade, are thought to be the main reasons for this resurgence (Jones et al. 2008). Gratz (1999) presented a list of re-emerging infectious diseases, and these included arboviruses such as dengue (DEN), chikungunya (CHIKV), Barhma Forest (BFV) and Ross River (RRV).

These pathogens are of particular interest in California. Each year travelers from many parts of the world enter the State through ports of entry such as airports, seaports and the Mexican-American border. Imported cases of dengue and chikungunya have been reported to the California Department of Public Health, and frequent travelers from areas of Australia where BFV and RRV are endemic makes it necessary to study the vector competence of California mosquitoes for some of these viruses (USGS 2012; Lanciotti et al. 2007; Lindsay et al. 1995; Harley et al. 2001). In addition, *Ae albopictus* is now found in the southern part of the state and *Ae. aegypti* in neighboring Arizona (Madon et al. 2002; Fujioka et al. 2012; Engelthaler et al. 1997). The overall objective of the current research was to begin to evaluate the vector competence of California mosquitoes for exotic arboviruses with a high probability for introduction.

Vector Competence Studies. Field-collected adult female *Ae. dorsalis* from Morro Bay and *Ae. melanimon* from Sutter county were infected with Ross River and Barmah Forest viruses. Additionally, the F1 progeny of field-collected immature *Ae. aegypti* from Arizona and *Ae. albopictus* from Los Angeles County were orally infected with DENV serotype I and CHIKV. For our vector competence studies, we used the following virus isolates: BFV (K10521 TVP-4119) and RRV (SW38457 TVP-4120) from Australia, DENV serotype I from Mexico (WHO collection) and CHIKV (DHS-4263) isolated in California from an infected traveler.

Adult female mosquitoes that were 4 - 5 days old were fed an infectious blood meal prepared with 50% defibrinated sheep blood mixed with 45% virus supernatant harvested from cell culture at different days post inoculation (day 10 for DENV-I; day 2 for CHIV, BFV and RRV) and 2.5% sucrose dilution (1:4 dilution). Viruses were serially diluted and mosquitoes fed through a membrane feeder. After blood feeding, engorged females were transferred to separate cartons and held at 26°C for 10 to 14 d after which bodies, legs and expectorate were tested for virus by Vero cell plaque assay [BFV, RRV] or qRT-PCR [DENV, CHIKV].

Sample Collection. Mosquitos were anesthetized using triethylamine (TEA) and allowed to expectorate for 10 min into a capillary tube filled with a mixture of fetal bovine serum (FBS) and 10% sucrose; these samples were used to determine their ability for transmission. Legs were separated from the body to determine dissemination, and samples were stored in transport media at -80°C for future testing. Additionally, whole bodies (without separating heads) of dead females were collected daily for testing of their infectious status. All infections were done within the J1 Biosafety Level 3 facility.

Testing of Samples. Total RNA was extracted from mosquito samples using an ABI MagMax system. RRV and BFV samples were tested by Vero cell plaque assay. DENV and CHIKV viral RNA were detected by real-time-RT-PCR using TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems, Carlsbad, USA) and set of primers and a probe previously described (Lanciotti et al. 2007, Johnson et al. 2005).

RESULTS

Aedes dorsalis and *Ae. melanimon* were susceptible to high, but not to moderate, doses of RRV and BFV, respectively, as shown by the proportion of infected bodies (Table 1). However, because few expectorate samples tested positive for virus, females of both species were poor vectors for the two viruses. *Aedes melanimon* seemed to have a midgut escape barrier that prevented dissemination of RRV, because most legs tested negative. In contrast, *Ae. dorsalis* seemed to have a salivary gland barrier for BFV, because virus legs, but not expectorate samples, were frequently positive (Table 1). Both *Ae. aegypti* and *Ae. albopictus* were not competent vectors for either CHIKV or DENV serotype I in this first infection study (data not shown).

CONCLUSIONS

Our results indicate that although *Ae. melanimon* and *Ae.dorsalis* were susceptible

Ross River	Infection titer (log_{10} pfu/mL)	Days post infection	Number positive			
			Total	Body	Legs	Expectorant
Ae. melmanimon	6.7	14	30	19	7	1
Ae. melmanimon	4.2	14	30	3	1	0
Ae. dorsalis	7.7	10	11	4	4	0
Ae. dorsalis	5.7	11	18	1	1	0
Barmah Forrest	**Infection titer (log_{10} pfu/mL)**	**Days post infection**	**Number positive**			
			Total	**Body**	**Legs**	**Expectorant**
Ae. melanimon	6.3	15	25	3	1	0
Ae. melanimon	4.2	15	30	0	0	0
Ae. dorsalis	6.7	10	26	20	18	2
Ae. dorsalis	4.7	11	7	1	0	0

Table 1. Results for infection and dissemination of RRV and BFV in *Ae. melanimon* and *Ae. dorsalis.*

Chikungunya Virus	Infection titer (log_{10} pfu/mL)	Days post infection	Total	Number positive		
				Body	Legs	Expectorant
Ae. aegypti	7	13	12	1	0	0
Ae. aegypti	7	18	15	1	1	0
Ae. aegypti	7	23	14	0	0	0
Ae. albopictus	7	13	21	3	0	0
Ae. albopictus	7	18	17	1	1	0
Ae. albopictus	7	23	23	2	0	0
Dengue I	**Infection titer (log_{10} pfu/mL)**	**Days post infection**	**Total**	**Number positive**		
				Body	**Legs**	**Expectorant**
Ae. aegypti	4	7	40	0	0	0
Ae. aegypti	4	15	37	0	0	0
Ae. aegypti	4	22	38	0	0	0
Ae. albopictus	4	7	32	0	0	0
Ae. albopictus	4	15	26	0	0	0
Ae. albopictus	4	22	29	0	0	0

Table 2. Results for infection and dissemination of CHIKV and DENV I in *Ae. aegypti* and *Ae. albopictus.*

to infection with RRV and BFV, *Ae. melanimon* seemed to have a midgut escape barrier that prevented dissemination, even when infected with a high dose of virus. Conversely, *Ae. dorsalis* that were infected with BFV frequently had positive legs, but not expectorant, indicating a salivary gland barrier. Wild-caught *Ae. dorsalis* survivorship was low, so therefore transmission experiments were done at day 10 post infection rather than at day 14; this may have contributed to the low transmission rates.

Our CHIKV results are surprising because the viral isolate and titers used for our experiments reflect viremia levels seen in patients during the recent epidemic. Furthermore, genetic analyses have shown that this sample contains a mutation in the viral envelope that increases its ability to infect *Ae. albopictus* (Tsetsarkin et al. 2007).

Our negative DENV I results were not as surprising as those with CHIKV. These could be the result of a low viral dose in the infectious blood meal or low vector competence, at least for *Ae. albopictus*. Several studies have documented a low oral susceptibility of this species in comparison to *Ae. aegypti* (Vazeille et al. 2003). Furthermore, studies of *Ae aegypti* by Ricco-Hesse et. al (2001) showed more efficient replication of DENV strains belonging to the Asian serotype rather than the American serotype used for this experiment (Armstrong et al. 2001, Anderson et al. 2006, Cox et al. 2011).

Future experiments using higher infectious doses and additional DENV serotypes and genotypes may help us understand the vector competence of local *Ae. aegypti* and *Ae. albopictus* and the risk for invasion of California by these viruses.

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Cycle Threshold Scores for Dead Birds in California: What Does this Mean for Mosquito Control?

Nadira Chouicha, Sandra Garcia[1], Amy Mikula, Leslie Foss, Ying Fang1 and William K. Reisen[1]

[1]*Center for Vectorborne Diseases, Department of Pathology, Microbiology and Immunology School of Veterinary Medicine, University of California, Davis, CA 95616;* [2]*Californiai Animal Health and Safety Laboratory, University of California, Davis, CA 95616;* [3]*WNV Dead Bird Surveillance Program, California Department of Public Health, Division of Communicable Disease Control, Vector Borne Disease Section, 850 Marina Bay Parkway, Richmond, CA 94804*

INTRODUCTION

The prevalence of West Nile virus (WNV) infection in dead birds submitted by the public is an important surveillance tool useful in tracking WNV in time and space and forms one of five metrics of risk within the California State Mosquito-Borne Virus Surveillance and Response Plan (Kramer 2012). Recent experimental infection studies (Wheeler et al. 2012a, 2012b) as well as an evaluation of Ct scores among dead birds submitted by the public (Reisen et al. 2013) have indicated that birds frequently survive acute infection and develop long-lasting chronic infections that can be detected at necropsy. These latter viral infections characteristically exhibit elevated cycle threshold (Ct) scores indicating the presence of few RNA copies and are difficult to confirm using qRT-PCR or isolation in cell culture. The bimodal frequency distribution of Ct scores has led to the separation of birds into 'recent' infections with low scores indicating they most likely died during acute infection and 'chronic' infections with very high scores indicating they survived acute infection sometime in the past. The current paper provides additional insight on how to interpret current surveillance test results.

METHODS AND RESULTS

Frequency Distribution of Results from 2012. Kidney snips and oral swabs from dead birds submitted for necropsy were collected at the California Animal Health and Food Safety (CAHFS) laboratory, immediately inactived by lysis buffer, frozen at -80C, and then tested at the Center for Vectorborne Diseases (CVEC) by qRT-PCR using previously published methods (Lanciotti et al. 2000). The frequency distribution of the number of positive birds plotted as a function of Ct score is shown in Figure 1. These data

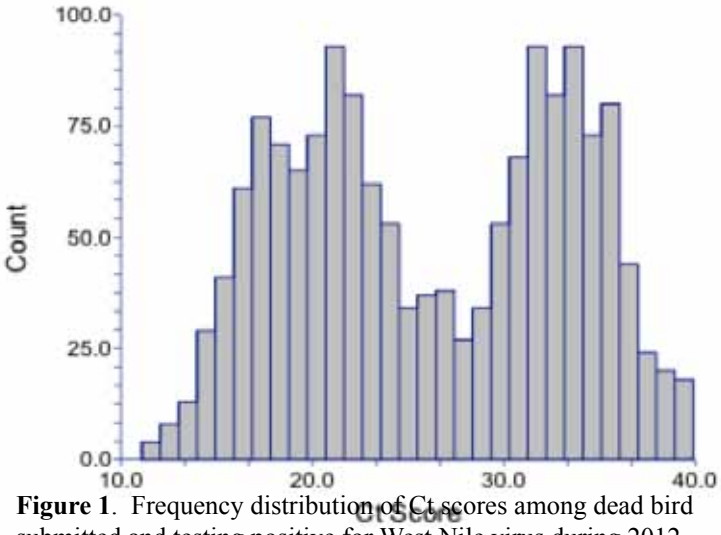

Figure 1. Frequency distribution of Ct scores among dead bird submitted and testing positive for West Nile virus during 2012.

show the expected bimodal distribution among birds that died during acute infection [left peak with high amounts of virus] or after surviving acute infection and most likely dying of other causes or perhaps sequellae from the acute infection [right peak with little virus]. The relationship between the amount virus in Vero cell culture and qRT-PCR Ct score from proficiency panel data is shown in Figure 2. These data indicate that very few RNA copies would be expected in samples with Ct scores >30 characterizing 'chronic' infection.

Temporal Distribution during 2012. To place necropsy data into a season context, the percentages of birds positive for recent and chronic infections were plotted with the percentage of WNV positive mosquito pools as a function of time in months during 2012 (Figure 3). As expected most of birds positive during February were scored as chronic and most likely were infected during the previous transmission

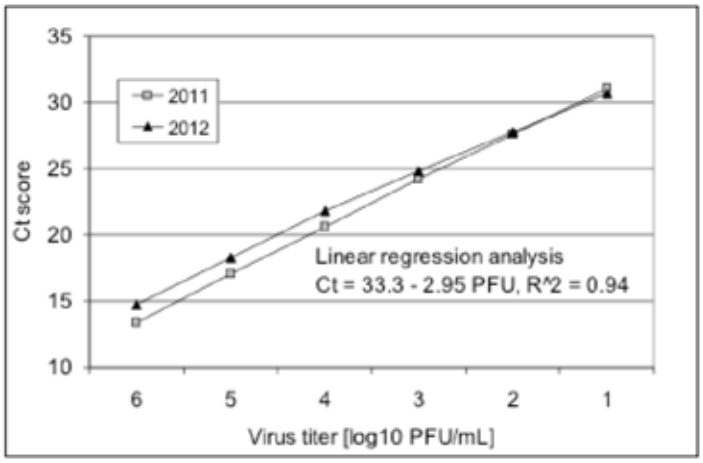

Figure 2. Relationship between Ct score and virus titer determined for samples taken from Vero cell culture and used for proficiency panel testing during 2011 and 2012.

season. Unexpectedly, the number of chronic birds peaked during July-August, somewhat earlier than the recent birds that peaked during August – October or the percent positive mosquito pools that peaked in August-September. The late season increase in recent infections was comprised mostly of American Crows that began to gather at communal roosts at this time, perhaps facilitating bird-bird as well as mosquito-bird transmission.

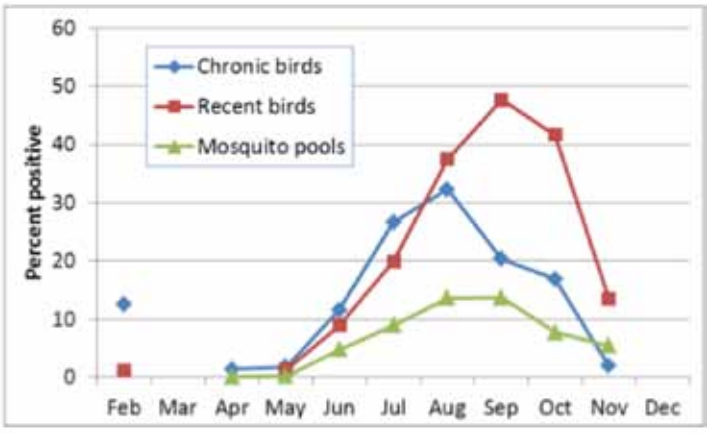

Figure 3. Percent of monthly samples of dead birds scored as recent or chronic infections and mosquito pools positive for West Nile virus during 2012.

Sensitivity of Oral Swabs. Previous investigation showed that the oral swabs from American Crows provided a sensitive and accurate measure of infection and saved the time and cost of doing necropsies to gather kidney tissues for surveillance. Swabs from other species, including other corvid species ,were generally less reliable and were not recommended as a replacement for kidney snips (Padgett et al. 2006). Considering our recent quantitative use of Ct scores to divide samples into recent and chronic, we felt it was useful to compare Ct scores between swab and kidney samples taken from the same birds during 2012. Of 187 American Crows with both oral swabs and kidney snips tested, 122 (65%) were positive by kidney snip – the 'gold standard'. Of these 122 positives, 97 were positive by both kidney snip and oral swab (sensitivity = 78.7%). Examining the Ct scores of these samples, all 25 false negatives were from birds with a kidney snip Ct score >29.1. In addition, although the Ct scores among paired swab and kidney snips were significantly correlated ($r = 0.76$, $n = 95$, $P < 0.01$), the mean ± SD value for kidney snips averaged 19.4 ± 5.2 Ct, 4.9 Ct less than the mean value for oral swabs (24.3 ± 4.7, $t = 13.8$, $df = 95$, $P < 0.001$). There were four birds with a positive oral swab, but negative kidney sample; their Ct values ranged from 29.6 to 38.3, demonstrating the difficulty in testing birds with chronic infections.

Finders Technology Associate Cards (FTA). FTA cards were developed to preserve RNA/DNA in dried samples for up to five months and have been used to collect avian oral and cloacal swabs for tracking influenza virus (Abdelwhat et al. 2011). In the current study, oral swabs from American Crows were smeared onto FTA cards at CAHFS, held overnight at room temperature, punch samples removed, eluted into lysis buffer and then tested for WNV RNA as described above. Concurrently collected oral swabs and kidney snips were collected as above, and all were tested by qRT-PCR at CVEC as described above. Overall, kidney snips from 31 (57%) of 54 corvids (20 Western Scrub-Jays, 24 American Crows, 10 Yellow-billed Magpies) tested positive. Considering the kidney samples as the 'gold standard,' there were 25 positive by both kidney snip and FTA card samples, 6 false negatives (FTA cards negative, sensitivity = 81%) and 4 false positives (FTA cards positive, but kidney negative). As above, all false negatives were chronic infections with Ct scores ranging from 31.3 to 37.4. The false positives were more problematic, with FTA scores of 20.7, 26.1, 29.6 and 29.7; retest results were similar for all but one Magpie sample (FTA = 26.1 Ct) that was positive

for FTA card (Ct = 26.3) as well as kidney (Ct = 31.2) on retest. If just the 25 American Crow samples were considered, the results were similar with 9 positive by both samples, 2 false negatives (sensitivity = 82%), 1 false positive and an overall specificity of 93%. Comparing Ct scores among specimens positive by both assays, kidneys averaged 17.9 Ct whereas FTA cards averaged 26.0 Ct and this difference was relatively consistent among samples (Figure 4); for 9 birds positive by oral swab and FTA card, oral swabs averaged 1.6 Ct lower than FTA cards.

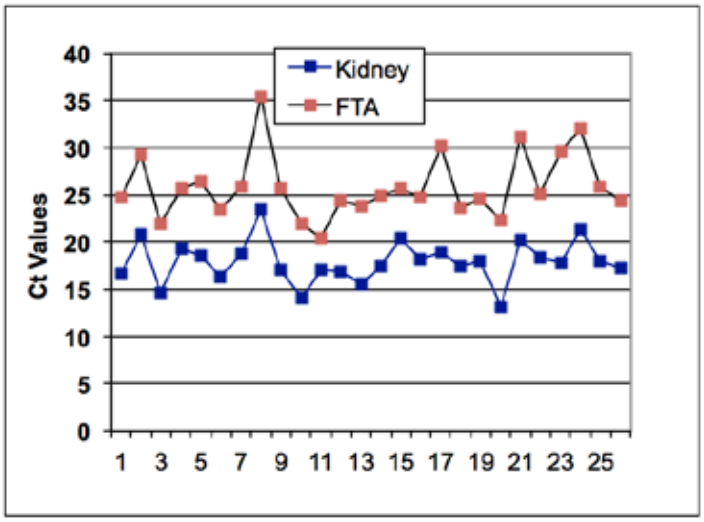

Figure 4. Ct scores for 25 birds with positive kidney samples and oral swabs collected on FTA cards.

DISCUSSION

Despite sampling limitations and cost, the dead bird program has provided a valuable extension of the arbovirus surveillance program because virus activity frequently is detected in areas not routinely selected for sampling by mosquito traps or sentinel chickens. Previously, birds were considered positive regardless of qRT-PCR Ct score or testing method (also RAMP, VecTest); however, this frequently resulted in the misinterpretation of results producing early season news releases of WNV transmission activity, early surveillance and even control. To place results into a correct contact, we used quantitative results from qRT-PCR to divide samples into recent and chronic infections. This was based upon equal sensitivity of test results. The current results indicate that the use of oral swab samples from American Crows may have produced a large number of false negatives among birds that were mostly chronically infected. Although this may not have altered the control response, it did provide an under appreciation of the ensuing resistance to WNV infection evolving within American Crow populations. FTA cards were shown herein to be even less sensitive than oral swabs placed directly in lysis buffer. Collectively, these results document how changing testing protocols has compromised sensitivity resulting in false negatives.

Interpretation and how to use chronic infection data has been problematic because the time from infection to positive chronic infection test results is unknown. It also is unknown if the bird died from sequellae related to WNV infection or from other causes. Based on data from tissues taken at necropsy from experimentally infected birds, these Ct values could represent birds infected from 2 - 3 weeks to 6 – 8 months previously (Wheeler et al. 2012a, 2012b). The current field data showed that there was an increase in the percentage of chronic infections concurrent with recent infections and positive mosquito pools (Figure 3). These data indicate that during the transmission season agencies should respond to chronic infection results with additional surveillance to determine if these data indicate relatively recent WNV amplification requiring enhanced control or if the birds were infected sometime in the past.

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The *Culex pipiens* complex: Genotype Meets Phenotype*

Brittany M. Nelms[1], Linda Kothera[2], Paula Macedo[3], Harry Savage[2], & William K. Reisen[1]

[1]*Center for Vectorborne Disease University of California, VetMed 3A, One Shield Ave., Davis, CA 95616, email: bnelms@wesphotos.com*
[2]*Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO 80521*
[3]*Sacramento-Yolo Vector Control District, 8631 Bond Road, Elk Grove, CA 95624*

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ABSTRACT: *Culex pipiens* form *pipiens* L. and *Cx. pipiens* f. *molestus* Forskäl, members of the *Cx. pipiens* complex that occur worldwide, exhibit important behavioral and physiological differences that may impact their role in the transmission and persistence of West Nile virus (WNV). We evaluated the vector competence and bionomics of populations of both forms from the Sacramento Valley of California. Overall, both f. *pipiens* and f. *molestus* females became infected, produced disseminated infections and were able to transmit WNV. Form *molestus* females also transmitted WNV vertically to egg rafts and F_1 progeny (minimum filial infection rate: 1.2/1,000), whereas f. *pipiens* females only transmitted vertically to egg rafts. Only infected mothers with mean cycle threshold (Ct) scores < 20 (>10^5 PFU/mL) vertically transmitted WNV to egg rafts and/or adult progeny. *Culex pipiens* complex populations from urban Sacramento blood-fed on 7 different avian species, of which 80% (32/40) were from passerine birds. Structure analysis for 22 of the 40 blood-fed *Cx. pipiens* complex females identified four (K = 4) genetically distinct ancestry clusters. Of these, 50% (11/22) of the blood meals were from f. *molestus* females, 41% (9/22) from admixed females and one female each from an f. *pipiens* female and a female of hybrid origin. Although one mammal feed was identified from an f. *molestus* female, the remaining blood meals were derived from avian hosts, especially Cedar Waxwings and House Finches. Finally, we assessed the overwintering potential and autogeny rates for *Cx. pipiens* complex females in bioenvironmental chambers set to experimental midwinter and summer conditions. Under diapause conditions, 85% (67/79) of aboveground *Cx. pipiens* complex females and 100% (n = 34) of underground f. *molestus* females from the Sacramento Valley did not enter a reproductive diapause. Ovarian follicles in the majority of anautogenous females progressed to ≥ stage I-II or degenerated, whereas follicles in f. *molestus* females progressed autogenously to stages III-V. Our results suggest that members of the *Cx. pipiens* complex in California are competent vectors for WNV, feed on WNV-competent host species, and employ multiple overwintering strategies that may enable virus persistence by multiple mechanisms.

Sucking Sugar and Spitting Virus: a Laboratory Study

Sarah S. Wheeler, Hugh D. Lothrop, Ying Fang and William K. Reisen

Center for Vectorborne Diseases and Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616

INTRODUCTION

Surveillance for West Nile virus (WNV) and other arboviruses is critical to direct intervention to prevent spillover of virus into human populations. A new method for the detection of West Nile virus transmission by mosquitoes has been implemented targeting sugar feeding behavior (Lothrop et al. 2012). Here we describe the three laboratory experiments designed to evaluate critical aspects of sugar wick deployment: (1) Impact of sugar bait on WNV RNA detection, (2) Detection of WNV RNA deposited on sugar wicks by WNV-infected mosquitoes, and (3) The potential for infection of naive mosquitoes that feed upon wicks previously fed upon by WNV-infected mosquitoes. Details of these experiments have been published previously (Lothrop et al. 2012).

METHODS

Experiment 1. To evaluate the detection threshold of WNV RNA in sugar bait, stock WNV was diluted 10-fold from 10^8 to 10^1 plaque forming units (pfu)/mL. Sugar wicks (1 cm absorbent wading) were placed into 1.5mL cryovials containing 1.0 mL sugar bait (66% sucrose scented with phenyl acetaldehyde) and spiked with 0.01 mL (approximation of mosquito expectoration volume) of diluted virus in triplicate. Wicks were then frozen to -80°C for a minimum of 24 hours to simulate the processing of field samples. RNA was extracted using a MagMax magnetic particle processor (Life Technologies, Carlsbad, CA) and a Virus Isolation kits (Life Technologies) using manufacturer protocols. West Nile Virus RNA was detected by a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) using an ABI7900 System (Life Technologies) and primers/probe specific for the envelop region of the viral genome (Lanciotti, et al. 2000).

Experiment 2. *Culex tarsalis* mosquitoes were infected with WNV by feeding on an artificial bloodmeal containing 10^8 pfu/mL. Cartons containing ≤ 22 female mosquitoes were held at 26°C for 13 days with fresh sugar wicks placed in the mosquito enclosures on days 1, 6 and 11. On day 13, the mosquitoes were anesthetized using triethylamine, and expectorant was collected using the capillary tube method (Aitken 1977). Mosquito bodies and expectorant were frozen to -80°C prior to testing. Sugar wicks, mosquito bodies and expectorant were tested for WNV RNA as described above.

Experiment 3. Sucrose (10%) solution was spiked with WNV to approximate sugar meals containing 10^7 to 10^1 pfu/mL. *Culex tarsalis* female mosquitoes were allowed to feed on sugar pads saturated with the virus/sucrose solutions for 48 hours, then the pads were removed for testing and replaced with clean sugar pads. Mosquitoes were held for 10 days at 26°C then tested for WNV RNA by qRT-PCR (methods above).

RESULTS AND DISCUSSION

Experiment 1. Overall, the detection of WNV RNA in sugar bait was successful. When sugar wicks were spiked with WNV, the overall titer of sugar baits ranged from 10^6 – $10^{\leq 1}$ pfu/mL. These titers corresponded to 10^8 – 10^1 pfu/mL of the original inoculum, respectively. WNV RNA was detected in all sugar baits containing ≥ 10^2 pfu/mL; this value is within the sensitivity range of the ABI7900 system and indicated that the WNV RNA was sufficiently stable in sugar bait.

Experiment 2. The *Culex tarsalis* WNV infection rate per carton ranged from 59-100% (Table 1), and 8 out of 9 cartons contained at least one mosquito with expectorant positive for WNV RNA. All sugar wicks from each time point, including those presented to mosquitoes between days 1 and 6, were positive for WNV RNA. The fact that all wicks, including the early time points, were positive was unexpected, and fecal material deposited by sugar-feeding mosquitoes

may have contributed to this finding. Overall, WNV RNA was readily detected on sugar wicks fed upon by WNV-infected mosquitoes.

		Mosquitoes		Sugar Wicks		
		% qRT-PCR positive		Days exposed to mosquitoes[b]		
Carton	n[a]	Body	Expecterant	1-6	6-11	11-13
1	9	100	44	+	+	+
2	11	100	55	+	+	+
3	9	78	0	+	+	+
4	4	75	50	+	+	+
5	5	100	80	+	+	+
6	22	100	NT	+	+	+
7	12	92	92	+	+	+
8	10	100	60	+	+	+
9	22	59	41	+	+	+

[a] n= number of mosquitoes that survived to day 13

[b] "+" indicated that the sugar wick was positive for WNV RNA by qRT-PCR

Table 1. Experiment 2: WNV infection of *Culex tarsalis* and sugar wicks.

Experiment 3. *Culex tarsalis* females were allowed to feed on sugar pads saturated with sucrose spiked with WNV titers ranging from 10^1 to 10^7 pfu/ml. All mosquitoes offered sugar pads containing $\leq 10^6$ pfu/mL remained negative for WNV. However, in the group offered a sugar pad containing 10^7 pfu/mL, 33% (n = 27) of the mosquitoes were found positive for WNV RNA. These results indicate that it is possible for mosquitoes to become infected with WNV during sugar feeding, but the titer necessary for infection is much higher than is normally expectorated by *Culex* mosquitoes and has been detected on sugar wicks in nature (Lothrop et al. 2012).

In summary, our experiments showed that WNV RNA was stable and could be readily detected on sugar wicks intentionally spiked with known concentrations of virus or contaminated and fed upon by mosquitoes infected with virus. Infectious virus was less stable, and we could not isolate virus from spiked sugar wicks; additionally, few females feeding upon sugar pads spiked with WNV became infected. Future studies will focus on field deployment of this system.

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Field Evaluation of Sugar Baits to Detect West Nile Virus

Hugh D. Lothrop[1], Sarah S. Wheeler[1], Ying Fang, Brian Carrol[1], Susanne Kluh[2], William K. Reisen[1].

[1]*Center for Vectorborne Disease Research, School of Veterinary Medicine, University of California, Davis, CA 95616, hdlothrop@ucdavis.edu*
[2]*Greater Los Angeles County Vector Control District, 12545 Florence Ave., Santa Fe Springs, CA 90670*

ABSTRACT: To detect West Nile Virus RNA expectorated by sugar feeding mosquitoes, vials baited with a 60% sucrose solution scented with 10 µl of phenylacetaldehyde and plugged with a 1 cm segment of dental wick were used at field sites in Southern California. Sampling was conducted at three to seven sites in the Coachella Valley from January 1 to September 19, and resulted in 14 positive samples from 930 sample-weeks with 12 positives occurring in August. These results overlapped, but did not match temporally, with the results from current surveillance methods of mosquito pools and sentinel chickens.

INTRODUCTION

Exploiting sugar feeding behavior for mosquito-borne disease surveillance and control is a growing area of research. Expectoration of Ross River Virus by sugar feeding mosquitoes collected by CO_2-baited traps was previously documented (Hall-Mendelin et al. 2010) indicating that sugar bait stations could supplement sentinel chicken flocks to detect arbovirus transmission and require far less infrastructure and maintenance. Sampling at five sites during 2011 in the Coachella Valley resulted in 27 West Nile virus (WNV) positives from 400 samples (Lothrop et al. 2012). In 2012 the bait formula was slightly modified, and two more sampling sites were added in the area where there was no indication of *Culex tarsalis* Coquillett presence. Less extensive sampling was conducted in Los Angeles and Kern counties, but results were limited to a single positive from Kern; therefore, only results for the Coachella Valley are presented in this report.

MATERIALS AND METHODS

Laboratory Protocols. A 1.5 ml microcentrifuge vial (VWR, Radnor, PA) was loaded with 1.2 ml of 60% sucrose solution. Thirty microliters of phenylacetaldehyde (PAA) (Alfa Aesar, Ward Hill, MA) solution (33% in denatured absolute ethanol) containing 10 µl of PAA was added, and the vial plugged with a 1 cm segment of dental wick. Caged colony *Culex quinquefasciatus* Say were presented with baited vials containing 0.03 g/l Tinopal® SFP (BASF, Ludwigshafen am Rhein, Germany**)** dye to test for feeding inhibition due to the denatured ethanol solvent portion of the bait. To test for RNA recovery, five baited wicks were spiked with 10 µl of a 10 fold dilution series of $10^{7.7}$ plaque forming units (PFU)/ml of the NY99 strain of WNV. The lowest titer, a 50 PFU dose, was our estimate of a single infected mosquito expectoration (Colton et al. 2005, Reisen et al. 2005). Vials were held at -80 ºC until tested for WNV RNA using qRT-PCR using previously published methods (Lanciotti et al. 2000).

Field Protocols. Vials were prepared without Tinopal following the laboratory protocol and deployed at field sites inverted in holders fabricated from PVC pipe fittings fastened to 3 foot wooden stakes; five holders with vials were placed at each field site (Lothrop et al. 2012). Samples were retrieved weekly and held at -80 ºC until shipped on dry ice to the Center for Vectorborne Diseases where they were tested using qRT-PCR. To compare sampling in rural habitats dominated by *Cx. tarsalis* with urban habitats dominated by *Cx. quinquefasciatus*, the bait stations were set at four rural sites around the margin of the Salton Sea and at three sites in the urbanized upper valley where *Cx. tarsalis* was absent. Sampling began on January 6 at three sites on the north shore of the Salton Sea where surveillance has generally detected virus activity the earliest in the season. Sampling at a fourth site started on April 12 on the west shore and at three additional on May 16: the upper Coachella Valley, Palm Desert and Cathedral City. Sampling was terminated at all sites on September 19.

RESULTS AND DISCUSSION

Laboratory Observations. No feeding inhibition was detected for caged mosquitoes offered vials baited with 30 µl of 33% phenylacetaldehyde [PAA] solution compared to approximately 10 µl of 95% PAA. West Nile Virus RNA was recovered from spiked wicks with qRT-PCR Ct scores, from highest titer to lowest, of 21.4, 26.5, 26.6, 28.7 and 34.5, respectively. This finding assured us that we could recover WNV RNA from wicks saturated with the alcohol formulation in an amount representing a single mosquito expectoration.

Field Observations. As in 2011 there appeared to be dissociation between the conventional surveillance methods (sentinel chickens and mosquito pools) and baited sugar wicks (Figure 1). Mosquito pool results are exaggerated in the figure because during weeks 25 through 33 there were only between three and nine pools tested per week. In both years most of the positive sugar wicks were recovered in August, but in 2012 WNV positive sentinel chickens and mosquito pools preceded most of the positive sugar wicks. In Contrast, in 2011 the results were reversed. Of the 12 positive sugar wicks from August 2012, six were from urbanized and six from rural sites indicating similar sensitivity for *Cx. quinquefasciatus* and *Cx. tarsalis* habitats. Continuation of this research will include comparing the current dispersed deployment of bait stations along ecotones with clustered deployment around a scent emitter station. The latter protocol may improve the sensitivity of the bait stations without the repellant effect of placing a high concentration of scent in the stations themselves.

ACKNOWLEDGMENTS

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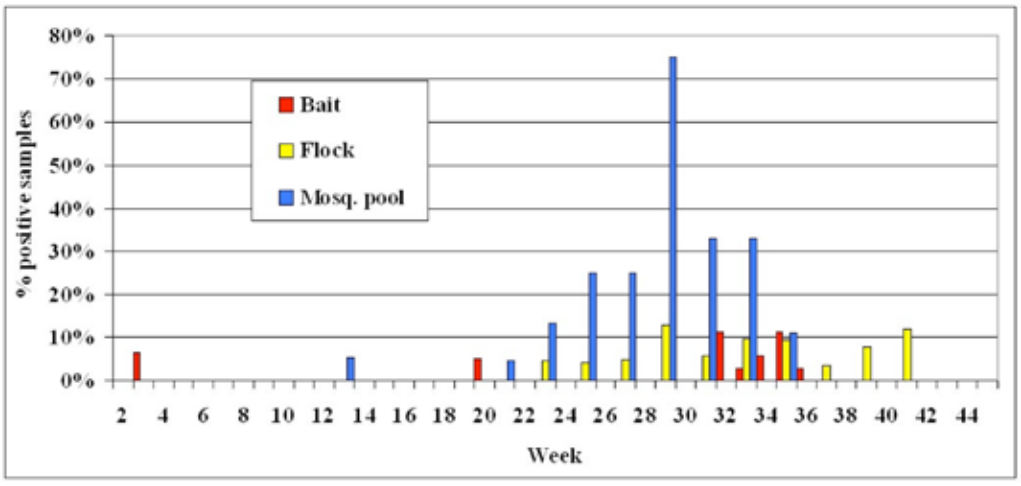


Figure 1. Percent sugar bait (Bait), sentinel chicken seroconversions (Flock) and mosquito pool (Mosq. Pool) samples testing positive for West Nile Virus at seven sites in Coachella Valley, 2012.

Impacts of Climate Change on *Culex tarsalis* in California between 1966 and 2001

Mary E. Danforth and Christopher M. Barker

Center for Vectorborne Diseases, University of California, Davis, 4206 Vet Med 3A. One Shields Avenue, Davis, CA 95616, cmbarker@ucdavis.edu

ABSTRACT: The abundance of *Culex tarsalis* is related to a number of climatic factors, such as temperature and water availability. Using Bayesian hierarchical quadratic regression models, we studied if the abundance response to those climate-based variables changed over the range of the variables. For a number of those variables, spring and summer mosquito counts increased along with increases in the predictor until it reached a point at which abundance was maximal, then began to decline in the presence of further increases in the climate variable. This effect was observed more often in temperature variables than water variables.

INTRODUCTION

Culex tarsalis is a primarily rural mosquito found in California (Reeves 1990) and is a key vector of West Nile Virus (Reisen et al. 2004). Previous correlative studies have linked *Cx. tarsalis* spring and summer abundance to a variety of climatic factors, including concurrent and prior seasonal temperatures as well as indicators of water availability such as precipitation and snow pack (Reeves 1990, Wegbreit and Reisen 2000, Pecoraro et al. 2007, Reisen et al. 2008, Chuang et al. 2011, Uejio et al. 2012). However, the impacts of these climate variables may differ over their range, and the ranges are changing due to global warming. Data from California shows that the average decadal summer temperature increased by 0.45°C between 1951-1960 and 1991-2000, while average decadal spring temperature increased by 0.6°C over the same time period (Cayan et al. 2006). Temperatures are not the only climatic factor in California to be impacted by global warming. Between 1949 and 2004, a smaller fraction of precipitation fell as snow (Knowles et al. 2006). In the current project, we are quantifying the changes in the responses of *Cx. tarsalis* over the range of a number of climate variables and characterizing the shape of those responses.

METHODS

Data Set. We examined the data from 29 vector control agencies (Table 1) that operated New Jersey light traps (NJLTs; Mulhern 1942) for more than 15 years between January 1, 1966 and December 31, 2001 and had gaps of less than 10 years in the data. There were 906 unique NJLT sites that were operated for both spring and summer trapping seasons for at least two years and were checked at least every 14 days. Female *Cx. tarsalis* counts and the total number of trap-nights for each site were aggregated by 30 day months, then averaged for the spring (April, May and June) or summer (July, August and September) seasons. Agencies were matched with the appropriate National Oceanic and Atmospheric Administration climate division (Guttman and Quayle 1996) using ArcGIS (ESRI, Redlands, CA) and if an agency overlapped two climate divisions, we treated it as being entirely within the climate division covering the majority of the agency's traps.

We matched mosquito trap counts at each site with mean seasonal nighttime low temperature (°C) and daytime high temperature (°C) for the prior and current seasons, calculated from data from collaborators at the National Aeronautics and Space Administration Ames Research Center (Nemani et al. 2007). From the same data set, we also calculated the annual water-year precipitation (cm) from October 1 to March 31 preceding the spring and summer trapping period for each site. The April 1st snow water content (in) at Donner Pass, as provided by the California Department of Water Resources (http://cdec.water.ca.gov), is representative of the water available for summer agricultural use and was applied to all sites for the concurrent year. Finally, the Palmer Drought Severity

Climate Division	Agency
North/Central Coast	Alameda County Mosquito Abatement District
North/Central Coast	Contra Costa Mosquito and Vector Control District
North/Central Coast	Marin-Sonoma Mosquito and Vector Control District
North/Central Coast	Northern Salinas Valley Mosquito Abatement District
North/Central Coast	San Mateo Mosquito Abatement District
North/Central Coast	Solano County Mosquito Abatement District
South Coast	Greater Los Angeles County Vector Control District
South Coast	Orange County Vector Control District
South Coast	San Diego Department of Environmental Health
Sacramento	Butte County Mosquito and Vector Control District
Sacramento	Burney Basin Mosquito Abatement District
Sacramento	Colusa Mosquito Abatement District
Sacramento	Glenn County Mosquito and Vector Control District
Sacramento	Lake County Vector Control District
Sacramento	Sacramento-Yolo Mosquito and Vector Control District
Sacramento	Shasta Mosquito and Vector Control District
Sacramento	Sutter-Yuba Mosquito and Vector Control District
Sacramento	Tehama County Mosquito and Vector Control District
San Joaquin	Consolidated Mosquito Abatement District
San Joaquin	Delano Mosquito Abatement District
San Joaquin	Delta Vector Control District
San Joaquin	Fresno Mosquito and Vector Control District
San Joaquin	Fresno Westside Mosquito Abatement District
San Joaquin	Kern Mosquito and Vector Control District
San Joaquin	Merced County Mosquito Abatement District
San Joaquin	San Joaquin County Mosquito and Vector Control District
San Joaquin	Tulare Mosquito Abatement District
San Joaquin	Turlock Mosquito Abatement District
Southeast Desert	Coachella Valley Mosquito and Vector Control District

Table 1. The participating Mosquito and Vector Control Association of California agencies.

Index, which is monitored by the National Oceanic and Atmospheric Administration (http://www.ncdc.noaa.gov), reflects long-term wet and drought periods for each climate division. We averaged the monthly rating across the concurrent season for each year.

Interaction and Confounding. We included human population density to adjust for primarily spatial variation in mosquito counts resulting from urban light competition (Milby and Reeves 1989). Density may also confound the effect of temperature on mosquito populations through urban heat island effects (Kim 1992) combined with limitations on *Cx. tarsalis* habitat availability (Eisen et al. 2010). We used human population density as a continuous variable measured as households per square mile * 1,000, calculated from California Fire and Resource Assessment Program records (http://frap.cdf.ca.gov).

Statistical Methods. Data were analyzed using JAGS (Plummer 2003) software through R (R Core Team 2012). Using Bayesian Poisson hierarchical quadratic regression, a model was developed with outcomes of the mean spring or summer *Cx. tarsalis* abundance per trap-month. Human population density and an adjustment for the number of trap-nights were included in all models. Each site had hierarchically structured terms for trap sites, which were centered on their respective agency means, which were in turn centered on climate division means. This captured spatial variation in trap counts due to site placement or agency trap designs or management practices, in addition to ecological differences among climate divisions. In each model, there were three site level terms: an intercept, a coefficient for a linear climate variable and a coefficient for a squared climate variable.

RESULTS

Time. Spring *Cx. tarsalis* in the Sacramento, San Joaquin and Southeast Desert climate divisions experienced a general decline in abundance over the course of the study after adjusting for changes in population density, while the two coastal climate divisions did not see a statistically significant change. Summer trends were not statistically significant in any climate division.

Climate Variables. For a number of climate models, the coefficient for the quadratic term was less than zero and statistically significant. That indicates that *Cx. tarsalis* abundance increased along with warmer temperatures and more water availability up to a certain point, then began to decrease in the presence of warmer or wetter weather. The point at which abundance was maximal is summarized in Tables 2 and 3.

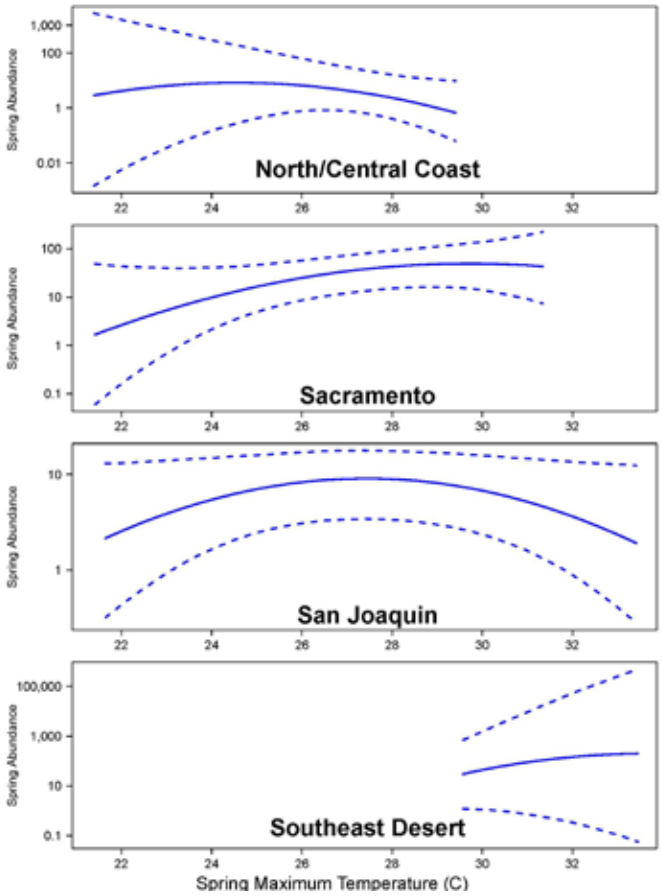
Temperature. For temperature models with a statistically significant curve, the point at which abundance was maximal was similar for most climate divisions. However, the tipping point for the Southeast Desert climate division was consistently at a warmer temperature than the rest of the state, as exemplified by spring mosquitoes when predicted by spring maximum temperatures (Figure 1). Concurrent seasonal temperatures were stronger predictors and more likely to be statistically significant for spring abundance then temperatures from the prior season, while antecedent spring temperatures were the best predictors of summer abundance. Overall, spring abundance models had narrower 95% credibility intervals than summer

	North/Central Coast	South Coast	Sacramento	San Joaquin	Southeast Desert
Winter Low Temp	7.2 (4.4, 2.4)	N/A	5.7 (4.2, 6.3)	N/A	N/A
Winter High Temp	N/A	N/A	N/A	17.0 (16.3, 17.5)	N/A
Spring Low Temp	11.2 (10.0, 12.4)	N/A	12.5 (10.6, 13.9)	11.8 (11.2, 12.5)	15.6 (13.4, 19.5)
Spring High Temp	24.8 (11.3, 34.8)	N/A	N/A	27.4 (24.0, 30.9)	33.5 (27.7)
Annual Precipitation	N/A	N/A	N/A	N/A	N/A
Snow Water	N/A	N/A	N/A	66.4 (48.0, 124.9)	N/A
PDSI	N/A	N/A	-1.0 (-0.4, 2.5)	N/A	0.5 (0.8, 2.9)

Table 2. The median estimate and its 95% credibility interval of the point of maximum spring abundance for each climate division.

	North/Central Coast	South Coast	Sacramento	San Joaquin	Southeast Desert
Spring Low Temp	10.5 (4.4, 13.8)	N/A	11.7 (10.4, 12.5)	10.6 (-2.3, 20.9)	N/A
Spring High Temp	N/A	N/A	27.5 (26.5, 28.7)	N/A	N/A
Summer Low Temp	15.2 (9.8, 18.9)	N/A	N/A	N/A	N/A
Summer High Temp	N/A	N/A	N/A	N/A	N/A
Annual Precipitation	N/A	N/A	N/A	N/a	N/A
Snow Water	N/A	N/A	42.2 (18.4, 69.8)	N/A	44.5 (37.4, 65.1)
PDSI	N/A	N/A	N/A	1.8 (1.4, 3.2)	N/A

Table 3. The median estimate and its 95% credibility interval of the point of maximum summer abundance for each climate division.



models. For both spring and summer outcomes, the South Coast showed no statistically significant changes.

Water. The results for models using water variables [annual precipitation, April 1st snow water content and PDSI] were not as strongly associated with mosquito abundance as those based on temperature variables. Models that did show changes in *Cx. tarsalis* abundance over the range of the climate variable had a similar shape to the temperature models, showing an increase until an area became too wet and mosquito counts started to decrease. Once again, models for spring abundance had narrower 95% credibility intervals and none of the models for the South Coast were significant.

Figure 1. The mean and 95% credibility interval for spring mosquitoes per 30-day trap month from the four significant climate divisions, when predicted by spring maximum temperature.

DISCUSSION

Previous research was limited to looking at the relationship between *Cx. tarsalis* and climate in California as a strictly linear relationship; that is, abundance changed at the same rate across the entire range of the climate variable. With quadratic models we can now see that the relationship changes as climate variables increase and may eventually switch from positive to negative.

When temperature variables were used as a predictor, the point at which mosquito abundance began to decrease in the presence of warmer conditions was consistent in all climate divisions except for the Southeast Desert. Female *Cx. tarsalis* abundance in this climate division continued to increase at warmer temperatures as compared to mosquitoes from the rest of the state. This agrees with prior research that found that *Cx. tarsalis* from the Coachella Valley developed more rapidly and survived longer at warmer temperatures than mosquitoes from the San Joaquin Valley (Reisen 1995) and that there are genetic differences between the two populations (Rasgon et al. 2006). When comparing the temperature models, it became clear that spring temperatures are strong predictors of both spring and summer mosquito abundances.

The point at which mosquito abundance was highest for water variables was not consistent between climate divisions. This and the fact that the credibility intervals for water predictors were wider than those for temperature variables might be due to the fact that water availability in California is largely controlled by man-made systems. Natural indicators like annual precipitation or snow water availability might not reflect the actual water availability in agricultural habitat.

The final point of note is the fact that our analysis of *Cx. tarsalis* in the South Coast did not produce any statistically significant results. This is not surprising as that area of the state is largely urbanized, the habitat for *Cx. tarsalis* is poor and the NJLTs have light competition that interfere with obtaining a representative sample of the mosquito abundance. As a result, future studies will not include this climate division.

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West Nile Virus in California Evolves towards Increased Avian Replicative Fitness and Reduced Vector Infection

Gabriella Worwa[1], Andra A. Hutton[1], Michèle C. Frey[1], Sarah S. Wheeler[1], Aaron C. Brault[2] and William K. Reisen[1]

[1] *Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Davis, CA, gworwa@ucdavis.edu*
[2]*Division of Vectorborne Diseases, Centers for Disease Control and Prevention (CDC), Fort Collins, CO*

ABSTRACT: To evaluate the spatiotemporal phenotypic evolution of West Nile virus (WNV) in California, the replicative capacity (fitness) of sixteen mosquito pool isolates was quantitatively assessed and compared to the founding California strain from 2003 using an *in vivo* fitness competition model in House Finches (*Carpodacus mexicanus*) and *Culex tarsalis* mosquitoes. Results indicated that WNV may have phenotypically evolved towards increased fitness in the avian host in Sacramento County, Kern County and the Greater Los Angeles area, but not in Coachella Valley from which three isolates showed markedly reduced fitness for birds compared to the founding California strain. Although competition in *Culex tarsalis* mosquitoes revealed reduced replication (low RNA copy count) of most of the isolates, higher infection rates were determined in infected mosquito bodies compared to the founding strain.

INTRODUCTION

The COAV997 isolate from a mosquito pool collected in Imperial County in July 2003 was the first detection of West Nile virus (WNV) in California. Following invasion, strains of the WN02 genotype have become endemic and caused recurring outbreaks throughout the State. However, it remains unclear to which extent selective pressure has impacted WNV evolution and adaptation to different regions of California. In the current study, we tested the hypothesis that WNV has evolved phenotypically since its invasion in 2003 to adapt to different vector-host systems within the differing biomes of California. Using an *in vivo* fitness competition model (Worwa et al. 2012, Worwa et al. 2011) we compared the replicative capacity, also known as the viral fitness, of sixteen WNV isolates made from 2007 and 2008 mosquito collections from Sacramento County (SAYO), Kern County (KERN), Greater Los Angeles area (GRLA) and Coachella Valley (COAV) to the invading COAV997 isolate to provide information about the spatiotemporal phenotypic evolution of WNV in California.

MATERIAL AND METHODS

Representative mosquito pools were selected from late 2007, early 2008, mid 2008 and late 2008 from each study site at SAYO, KERN, GRLA and COAV. Infectious virus was isolated after one passage in African green monkey kidney (Vero) cells and subsequently titered by plaque assay (Brault et al. 2004). A genetically labeled virus (COAV997-MUT) was generated by site-directed mutagenesis of five nucleotides from an infectious clone derived virus of the original COAV997 strain to serve as the 2003 founding California virus for subsequent fitness competitions (Worwa et al. 2012). A previously described protocol (Worwa et al. 2012) was used to compete the field isolates against COAV997-MUT using an *in vivo* fitness competition in House Finches (*Carpodacus mexicanus*; HOFI) and *Culex (Cx.) tarsalis* mosquitoes. Briefly, mosquitoes and birds were infected with a mix of the COAV997-MUT and each of the sixteen field isolates at a starting ratio of 1:1 plaque forming units (p.f.u.). Groups of six feral HOFIs (previously screened for anti-WNV antibodies by plaque reduction neutralization assay) were inoculated subcutaneously with 1000 p.f.u./50μL of a 1:1 virus mixture. Serum was collected from all birds on days 1 to 7 post infection (dpi) and on the last day of the experiment at 14 dpi following euthanasia and necropsy. Starved *Cx. tarsalis* mosquitoes were fed for 2 hours on chicken blood spiked with a 1:1 mixture of each virus at a 7 $\log_{10}$ p.f.u./mL titer using a Hemotek membrane feeding system. Blood-fed females were transferred to separate containers and kept for 14 dpi at 28°C in a humidified atmosphere. Bodies, legs and expectorant were collected on 14 dpi. Mosquito bodies were homogenized with tissue lyser (Qiagen). RNA from bird sera and the mosquito body

homogenate was extracted using a MagMAX magnetic particle processor (Applied Biosystems). The quantity of template copies in the RNA samples was determined by a specific quantitative RT-PCR (qRT-PCR) and the ratios between the wildtype virus and COAV997-MUT were calculated from collected samples to determine the outcome of competitive replication.

RESULTS AND DISCUSSION

Results from the fitness competitions of 16 and 12 field isolates (wildtype virus) against the COAV997-MUT were summarized in Tables 1 and 2 for HOFI and *Cx. tarsalis*, respectively. Field isolates from SAYO, KERN and GRLA exhibited increased replicative fitness for HOFIs compared to COAV997-MUT representing the founding 2003 California strain which may indicate a phenotypic evolution towards increased avian fitness in those parts of California. In contrast, three out of four field isolates from COAV showed markedly reduced replicative fitness for HOFIs. Interestingly, these isolates came from sites close to the origin of the founding COAV997 isolate. There may have been little pressure for WNV to evolve phenotypically in COAV due to avian or vector-species differences compared to the other study sites. In addition, we noticed a generally reduced avian virulence of the 2007 and 2008 field isolates because infected HOFIs showed low mortality throughout the study with the exception of the early 2008 GRLA isolate which was collected during a WNV outbreak year. This might indicate a better adaptation of recent WNV strain to its bird host, but it is possible

Field isolates	RNA copy ratio (WT:MUT) per dpi							Mortality (n)	Winner
	1	2	3	4	5	6	7		
COAV late 2007	1:2	1:2	1:2	1:10	1:7	1:16	2:1	1	MUT
COAV early 2008	1:3	1:8	1:9	1:14	1:32	MUT	MUT	1	MUT
COAV mid 2008	MUT	1:4	1:5	1:12	1:57	1:48	1:44	0	MUT
COAV late 2008	3:1	23:1	46:1	53:1	133:1	128:1	WT	1	WT
GRLA late 2007	MUT	5:1	10:1	11:1	50:1	7:1	WT	1	WT
GRLA early 2008	3:1	22:1	60:1	68:1	58:1	58:1	WT	4	WT
GRLA mid 2008	5:1	6:1	11:1	21:1	4:1	2:1	3:1	0	WT
GRLA late 2008	7:1	13:1	36:1	146:1	39:1	69:1	7:1	1	WT
KERN late 2007	4:1	14:1	38:1	95:1	WT	WT	WT	1	WT
KERN early 2008	WT	23:1	82:1	224:1	252:1	WT	WT	2	WT
KERN mid 2008	MUT	8:1	26:1	42:1	22:1	WT	WT	1	WT
KERN late 2008	MUT	14:1	47:1	51:1	WT	WT	WT	0	WT
SAYO late 2007	3:1	5:1	13:1	12:1	9:1	7:1	WT	1	WT
SAYO early 2008	MUT	10:1	26:1	42:1	106:1	WT	WT	2	WT
SAYO mid 2008	0:0	12:1	16:1	39:1	25:1	34:1	WT	0	WT
SAYO late 2008	0:0	3:1	3:1	3:1	1:2	1:2	3:1	0	neutral

Table 1. Fitness competition results in HOFI. RNA copies are shown as ratios between wildtype virus (WT) and COAV997-MUT (MUT) as a mean of six infected birds per group and sampled between 1 and 7 dpi. If WT or MUT is shown, RNA was detected from that virus only. Mortality is presented as the number of birds (n) that died during the study. The virus with higher replicative fitness is indicated as the winner.

Field isolates	Overall infection rate (%)	RNA copy ratio on 14 dpi (WT:MUT)	Infection rate (%) for WT	Infection rate (%) for MUT
COAV late 2007	90	2:1	82	78
COAV early 2008	93	1:24	90	76
COAV mid 2008	90	1:10	80	80
COAV late 2008	50	1:4	46	38
GRLA late 2007	81	1:6	75	56
GRLA early 2008	65	1:4	53	43
GRLA mid 2008	80	1:4	78	48
GRLA late 2008	65	1:9	55	42
KERN late 2007	74	1:3	74	51
KERN early 2008	95	1:4	93	78
KERN mid 2008	81	1:10	74	74
KERN late 2008	70	1:3	66	36

Table 2. Fitness competition results in *Cx. tarsalis*. RNA copies are shown as ratios between wildtype virus (WT) and COAV997-MUT (MUT) detected in the mosquito bodies on 14 dpi. The overall infection rate indicates the percentage of bodies tested qRT-PCR-positive for both viruses. The infection rates for WT and MUT show the specific infection rate for either wildtype virus or COAV997-MUT, respectively.

that outbreak isolates retained high virulence that facilitated epidemics.

Field isolates from KERN and GRLA yielded low RNA copy numbers in the bodies of infected mosquitoes. However, these isolates also produced higher or equal infection rates compared to the COAV997-MUT, indicating that more mosquitoes were found positive by qRT-PCR. The early 2007 COAV isolate showed a "fitness trade-off" through compensation of its low avian fitness by elevated fitness for mosquitoes (higher RNA copy number and infection rate) compared to COAV997-MUT. However, this was not observed for the mid and late 2008 COAV isolates which replicated poorly in both *in vivo* models compared to COAV997-MUT. The exact fitness outcome in these mosquitoes will be determined after the amounts of RNA in the expectorant reveal which of the two viruses was transmitted.

On-going work will analyze the mosquito expectorants to determine transmission-specific fitness. In addition all sixteen isolates are currently being full-length sequenced to search for genetic differences. The fitness of additional strains from WNV epidemics in Los Angeles, Bakersfield and Sacramento during 2004, 2005, 2007, 2011 and 2012 will be assessed using the same *in vivo* system to elucidate outbreak-related changes in phenotype. Additional isolates made from *Cx. tarsalis* and *Cx. quinquefasciatus* mosquito pools collected in COAV will be screened for fitness in an avian primary cell culture system to determine vector species-specific co-evolution.

ACKNOWLEDGMENTS

Special thanks to Brian Carroll and Amy Jobe for trapping and husbandry of House Finches and the California mosquito control districts for providing mosquito pool samples. We thank Dr. Shirley Luckhart and her group for allocating insectary space. G. Worwa was funded by the Swiss National Science Foundation (SNSF; PBBEP3_128345) and the Swiss Foundation for Grants in Biology and Medicine (SFGBM; PASMP3_137034 / 1). We thank the National Institutes of Allergy and Infectious Diseases (NIH; RO1-AI55607) for partial funding support.

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Surveillance-Based Prediction of Human West Nile Virus Infection Risk over Space and Time

Jessica M. Healy[1], William K. Reisen[1], Vicki Kramer[2] and Christopher M. Barker[1]

[1]*Center for Vectorborne Diseases, University of California, Davis, CA, 95616, jhealy@ucdavis.edu,*
[2]*California Department of Public Health, 1616 Capital Ave. Sacramento, CA 95814*

ABSTRACT: West Nile virus (WNV) surveillance in California plays an integral role in guiding mosquito control operations to protect the public from serious illness resulting from WNV infection. Three components of the enzootic surveillance system were analyzed in space and time to determine the bounds within which each is most predictive of human West Nile neuroinvasive disease occurrence. The methods included are mosquito trapping and testing, collection and testing of dead birds and testing sera from sentinel chickens. The analysis included three mosquito control districts in northern, central and southern California, encompassing a range of climatic and anthropogenic habitats. Poisson mixed-effect modeling of the data showed that within four kilometers WNV-positive dead birds are most predictive at one to four weeks prior, whereas infection in mosquitoes collected in gravid or CO_2 traps was most predictive two and one to six weeks prior, respectively.

INTRODUCTION

West Nile Virus has become an endemic arbovirus in California since its introduction in 2003. The virus causes a wide range of illnesses with the most severe being West Nile neuroinvasive disease (WNND) which may present as meningitis, encephalitis or poliomyelitis (Murray et al. 2011); California has had 1,692 WNND cases reported since 2003 (California Department of Public Health 2012). Due to the lack of a human vaccine, preventing transmission of the virus to the human population depends on surveillance guided-control of the vector mosquito populations. In 2010 the U.S. Centers for Disease Control and Prevention, California Department of Public Health, the Center for Vectorborne Diseases and three Mosquito and Vector Control Association of California member agencies (Coachella Valley MVCD, Kern MVCD and Sacramento-Yolo MVCD) began a collaborative project to evaluate the methods of WNV enzootic surveillance employed in California. Here we describe a component of the study that aimed to determine whether enzootic surveillance provided early warning of WNND case occurrence and to define the spatio-temporal windows within which each method was most predictive.

MATERIALS AND METHODS

Data on surveillance of mosquitoes, chickens and dead birds were collected by the participating MVCDs for the years 2004 through 2011, and data on WNND cases within each district were provided by the CDPH. A WNND case was defined as a patient diagnosed with meningitis, encephalitis or acute flaccid paralysis who had tested positive for infection with WNV (Center of Disease Control and Prevention 2004). Census block groups were used as the spatial unit with any surveillance within a four kilometer buffer around each block group assumed to represent WNV exposure for the block group. Associations between surveillance indicators (exposure variables) and WNND cases (disease outcomes) were compared over a range of temporal lags to determine the period prior to human case occurrence when each surveillance method was most predictive.

For each surveillance method and spatio-temporal exposure window, a multi-level Poisson regression model was constructed with a random effect included to account for repeated observations over time for each block group (Baayen et al. 2008). The Akaike information criterion (AIC) was then used to select the model with the best fit to the data (Akaike 1974).

RESULTS AND DISCUSSION

Model results showed that having a WNV-positive dead bird within four kilometer of a particular census block group was most predictive one to four weeks prior to onset of symptoms in a WNND case. Presence of an

infected mosquito in a gravid trap was most predictive at two weeks prior and presence of an infected mosquito in a CO_2-baited trap was most predictive at one to six weeks prior within the four kilometer radius. As shown in Table 1, the change in the rate of case occurrence, or incidence rate, given a positive sample within the four kilometer radius of a block group, compared to the rate of case occurrence in the absence of a positive sample within the same four kilometer radius, was calculated for the most predictive temporal lag. The rate of WNND case occurrence given a WNV-positive dead bird, an infected mosquito in a gravid trap or an infected mosquito in a CO_2-baited trap is expected to increase by 433%, 405% and 325%, respectively. Although sentinel chickens did not show a statistically significant lead time within the four kilometer radius, preliminary results from analyses which include data from 2012 suggest that sentinel chickens are most informative within one kilometer of a census block group. This disparity could possibly be explained by the low spatial density of sentinel chicken flocks in comparison to mosquito trapping and the clustered spatial distribution of dead birds. However, these results would agree with the findings of Kwan et al. (2010) that sentinel chickens seroconversions occur concurrently with human cases of West Nile fever and WNND.

Surveillance Method	Temporal Lag	Rate Increase (95% CI)	P-value
Dead Birds	1 - 4 weeks	+ 433% (+256-697%)	<0.001
Mosquitoes, Gravid traps	2 weeks	+ 405% (+86-1,272%)	0.002
Mosquitoes, CO_2 traps	1 - 6 weeks	+ 325% (+48-1,116%)	0.007

Table 1. Temporal lags for each surveillance method that were most closely associated with human case occurrence within a 4-km spatial buffer around each census block group and the respective rate increase in case occurrence.

In our current analyses, we are performing the same procedure on each agency individually to determine if the predictive value of each method is affected by the differences in environment, population dynamics and relative densities of humans and avian hosts. Additionally, our analyses will include varying radii around census block groups to determine the distance and lead-time combination at which each method of surveillance is most predictive. Results of these analyses will be used to produce one or more predictive models to be used for predicting the risk of WNND case occurrence over space and time.

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Effort Analysis for Sentinel Chicken and Mosquito-based Surveillance

Jessica M. Healy[1], William K. Reisen[1], Vicki Kramer[2] and Christopher M. Barker[1]

[1]*Center for Vectorborne Diseases, University of California, Davis, CA, 95616,*
[2]*Vector-Borne Disease Section, California Department of Public Health,*
1616 Capitol Ave. Sacramento, CA 95814

INTRODUCTION

The value of the surveillance methods used to detect West Nile virus (WNV) depends upon their sensitivity for detecting the virus and whether they provide early warning of the risk for human infections. In this study, we used data from 2004 - 2011 from each of three collaborating mosquito control districts to compare the efficacy of surveillance based on mosquito infections and chicken serology. In particular, we were interested in determining which of the two methods provided earlier indication of the onset and peak of WNV activity and whether the results differed between urban and rural areas.

MATERIALS AND METHODS

The study areas included the Sacramento-Yolo, Kern and Coachella Valley MVCDs, and all mosquito and sentinel chicken samples tested for WNV RNA or antibody, respectively, were initially considered for inclusion. Our goal was to eliminate bias resulting from the typically higher spatial density of mosquito traps in comparison to chicken flocks, so each bleed date for each chicken flock was paired with the single nearest trap from which mosquitoes were tested for WNV, potentially selecting a different nearest trap for each week. This equalized and matched spatio-temporal sampling for the two methods and resulted in a time series for each flock-trap combination within each season. These time series were then compared based on whether they detected WNV within each year and the dates of initial and peak WNV detection. Initial detection was defined for each site and year as the first week in which a WNV positive was found, and the week of peak activity was defined as the first week at which the highest level of WNV activity was reached (minimum infection rate in mosquitoes or percent of chickens seroconverted).

RESULTS AND DISCUSSION

When WNV activity was detected by only one of the two methods, chickens detected WNV more frequently than mosquitoes (Figure 1), but this pattern differed between urban and rural areas. Chickens alone detected WNV twice as frequently as mosquitoes alone in rural areas (35 vs. 17 occurrences), but the reverse was true in urban areas (6 vs. 12 occurrences, respectively). These differences in annual detection may reflect the continuous exposure of chickens for long time periods (typically two weeks between bleeds) compared to CO_2-baited traps that were run for single nights.

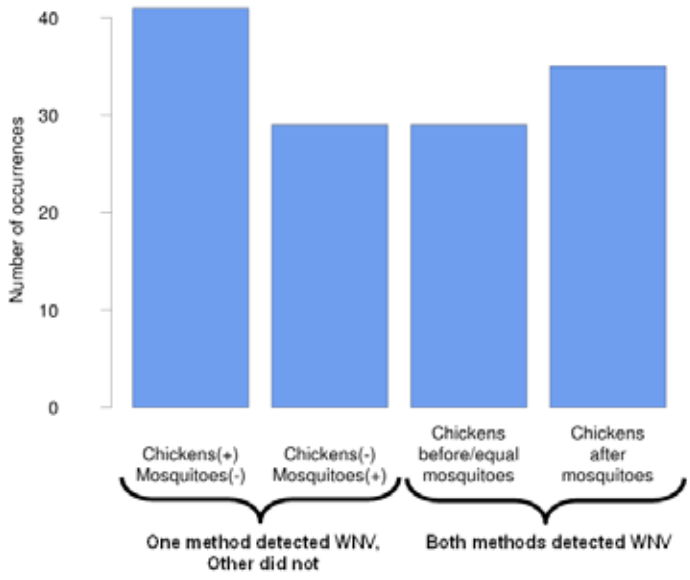

Figure 1. Frequency of WNV detection when only one method detected the virus (left pair of columns) and timing of WNV detection when both methods detected the virus (right pair of columns).

When WNV was detected by both methods within a particular season, mosquitoes most frequently detected WNV earlier than chickens (Figure 1), although there

were many individual cases where chickens were similar in timing to mosquitoes, and this trend was consistent for both urban and rural areas. The median week of first WNV detection by mosquitoes was 1.5 and 3 weeks earlier than chickens in rural vs. urban areas, respectively. Similarly, for median timing of peak activity, mosquitoes were 3.5 and 3.0 weeks earlier in rural vs. urban areas. Some of the difference in timing may be due to inherent differences in the time to detection of antibody (~ 10 days following infection) versus RNA (probably detected ~ 4-5 days after infection during the next host-seeking period).

Results of this study show that both mosquitoes and chickens continue to have relevance for sensitive and timely detection of West Nile Virus activity, although their utility depends upon context and sampling effort. In general, mosquitoes detected WNV earlier than chickens, which agrees with earlier findings in California (Reisen et al. 2009, Kwan et al. 2010b, Kwan et al. 2010a). The greater sensitivity of chickens in rural areas is interesting and shows that chickens are a valuable component of a complete surveillance program in these areas, especially if intensive mosquito trapping cannot be conducted. Ongoing analyses are focusing on estimating optimal sampling effort for chickens and mosquitoes. We are also working with the collaborating mosquito control agencies to estimate the costs for each surveillance method (similar to Scott et al. 2001), and further comparisons will consider the relative value of each method for a fixed cost.

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We especially thank our collaborating agencies: Coachella Valley MVCD, Kern MVCD and Sacramento-Yolo MVCD for their participation in this project. We also appreciate the help of our collaborators: Bborie Park at the Center for Vectorborne Diseases at UC Davis; Tina Feiszli, Carol Glaser, Maria Salas, Cynthia Yen and others at the California Department of Public Health; and Roger Nasci, Marc Fischer and Nicole Lindsey at the Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, who also provided financial support for this study through the cooperative agreement for Epidemiology and Laboratory Capacity for Infectious Diseases. CM Barker and WK Reisen also acknowledge support from the Research and Policy for Infectious Disease Dynamics (RAPIDD) program of the Science & Technology Directorate, Department of Homeland Security and Fogarty International Center, National Institutes of Health.

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Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2012

Tina Feiszli[1], Kerry Padgett[1], Bborie Park[2], Bruce Eldridge[2], Ying Fang[2], William K. Reisen[2], Cynthia Yen[1], Leslie Foss[1], and Vicki Kramer[1]

[1] *California Department Public Health, 1616 Capital Ave. Sacramento, CA 95899 tina.feiszli@cdph.ca.gov*
[2] *Center for Vectorborne Diseases, University of California, Davis, CA, 95616*

INTRODUCTION

The California Arbovirus Surveillance program is a cooperative effort of the California Department of Public Health (CDPH), the University of California at Davis Center for Vectorborne Diseases (CVEC), the Mosquito and Vector Control Association of California (MVCAC), local mosquito abatement and vector control agencies, county and local public health departments and physicians and veterinarians throughout California. Additional local, state and federal agencies collaborated on, and contributed to, the West Nile virus (WNV) component of the arbovirus surveillance program. In 2012 the surveillance program elements included the following:

(1) Diagnostic testing of specimens from human patients exhibiting symptoms of encephalitis, aseptic meningitis, acute flaccid paralysis or with unexplained febrile illness of more than seven days.
(2) Diagnostic testing of specimens from horses exhibiting clinical signs of viral neurologic disease compatible with western equine encephalomyelitis virus (WEEV), WNV and other arboviruses as appropriate.
(3) Monitoring mosquito abundance and testing mosquitoes for the presence of St. Louis encephalitis virus (SLEV), WEEV, WNV and other arboviruses as appropriate.
(4) Serological monitoring of sentinel chickens for SLEV, WEEV and WNV antibodies.
(5) Surveillance and WNV diagnostic testing of dead birds and tree squirrels.
(6) Weekly reporting of arbovirus testing results in California and arbovirus activity throughout the United States in the CDPH Arbovirus Surveillance Bulletin.
(7) Bi-weekly posting of WNV information, including test results, reports, maps, and public education materials on the California WNV website: www.westnile.ca.gov.
(8) Data management and reporting through the web-based California Surveillance Gateway.

Only West Nile Virus was detected in 2012, and a summary of WNV activity by county is shown in Table 1.

County	Humans[a]	Horses	Dead Birds[b]	Mosquito Pools	Sentinel Chickens	Dead Squirrels
Alameda	2	0	15	0	0	1
Alpine	0	0	0	0	0	0
Amador	0	0	0	0	0	0
Butte	10	1	53	27	45	2
Calaveras	0	1	0	0	0	0
Colusa	3	0	5	0	6	0
Contra Costa	4	0	66	19	7	0
Del Norte	0	0	0	0	0	0
El Dorado	0	0	5	0	0	0
Fresno	29	3	25	147	0	0
Glenn	9	1	16	4	8	0
Humboldt	1	0	0	0	0	0
Imperial	1	0	0	4	10	0
Inyo	0	0	0	0	0	0
Kern	28	2	14	571	74	0
Kings	3	0	2	102	0	0
Lake	1	0	10	23	4	0
Lassen	0	0	0	0	0	0
Los Angeles	179	0	271	339	135	15
Madera	3	1	2	14	0	0
Marin	0	0	1	0	0	0
Mariposa	0	0	0	0	0	0
Mendocino	0	0	6	0	0	0
Merced	13	1	42	4	14	0
Modoc	0	0	0	0	0	0
Mono	0	0	0	0	0	0
Monterey	1	0	0	0	0	0
Napa	0	0	1	1	0	0
Nevada	0	0	3	0	0	0
Orange	48	0	92	69	0	0
Placer	12	0	60	108	8	0
Plumas	0	0	0	0	0	0
Riverside	19	0	5	133	63	0
Sacramento	32	2	466	487	36	1
San Benito	0	0	0	0	0	0
San Bernardino	37	0	39	58	45	0
San Diego	2	0	1	0	0	0
San Francisco	1	0	1	0	0	0
San Joaquin	15	2	58	169	0	0
San Luis Obispo	0	0	1	0	0	0
San Mateo	0	0	5	0	0	0
Santa Barbara	0	0	0	0	0	0
Santa Clara	0	0	20	3	0	2
Santa Cruz	0	0	1	0	0	0
Shasta	1	5	36	17	6	1
Sierra	0	0	0	0	0	0
Siskiyou	0	0	0	0	0	0
Solano	2	0	28	3	2	0
Sonoma	0	0	8	3	1	0
Stanislaus	28	1	51	210	8	0
Sutter	8	0	29	19	38	0
Tehama	4	0	5	0	0	0
Trinity	0	0	0	0	0	0
Tulare	9	0	54	144	11	0
Tuolumne	0	0	0	0	0	0
Ventura	7	0	38	2	2	0
Yolo	11	1	98	154	4	1
Yuba	4	1	11	15	13	0
State Totals	**527**	**22**	**1,644**	**2,849**	**540**	**23**

[a]Includes asymptomatic infections
[b]Does not include chronic infections

Table 1. Infection with West Nile Virus in California, 2012.

RESULTS

Human Disease Surveillance. Serological diagnosis of human infection with WNV and other arboviruses was performed at the CDPH Viral and Rickettsial Disease Laboratory (VRDL), county public health laboratories and commercial diagnostic laboratories. Local laboratories tested for WNV using an IgM or IgG immunofluorescent assay (IFA) and/or an IgM enzyme immunoassay (EIA). Specimens with inconclusive results were forwarded to the VRDL for confirmation or further testing with a plaque reduction neutralization test (PRNT). Additional WNV infections were identified through testing performed at blood donation centers.

A total of 479 symptomatic and 48 asymptomatic infections with WNV were identified in 2012, the highest number of infections reported since 2005 (Table 2). Of the 479 clinical cases, 158 (33%) were classified as West Nile fever, 313 (65%) were West Nile neuroinvasive disease (WNND)(i.e., encephalitis, meningitis, or acute flaccid paralysis), and 8 were of unknown clinical presentation. Cases were residents of 31 counties and 280 (58%) were male. Incidence was highest (24.9 cases per 100,000 persons) in Glenn County (Figure 1). The median ages for West Nile fever and neuroinvasive cases were 52 years (range, 1 to 94 years) and 58 years (range, 2 to 93 years), respectively. The median age of the 20 WNV-associated fatalities was 82 years (range, 47 to 91 years). Dates of symptom onset ranged from May 23 – December 9, 2012.

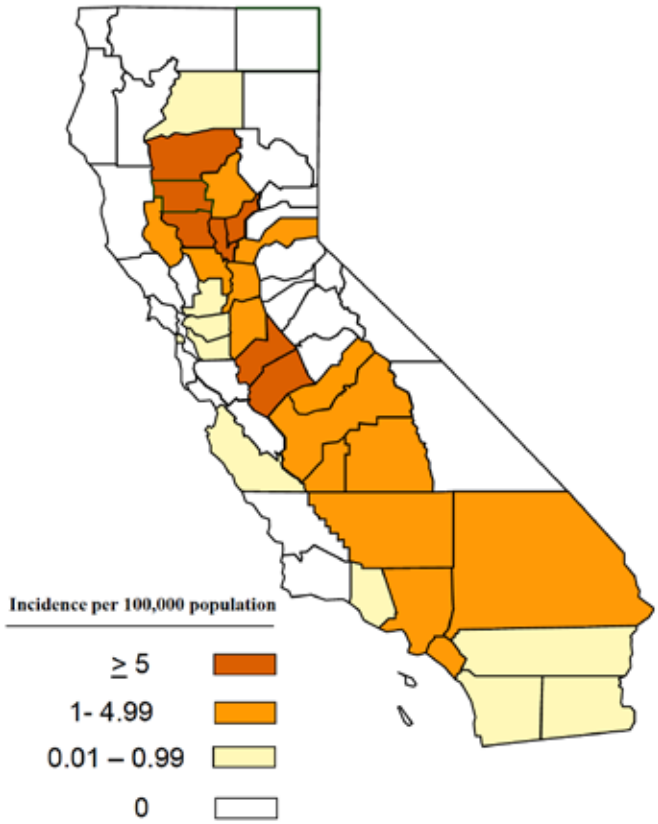

Figure 1. Incidence of human cases of West Nile virus, California, 2012.

County	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Incidence per 100,000 person years
Alameda	0	0	1	1	0	1	0	1	0	2	0.04
Alpine	0	0	0	0	0	0	0	0	0	0	0.00
Amador	0	0	3	0	0	0	0	0	1	0	1.06
Butte	0	7	24	51	16	6	2	1	3	10	4.52
Calaveras	0	0	2	0	0	1	0	0	0	0	0.66
Colusa	0	0	2	4	2	1	0	0	0	3	5.56
Contra Costa	0	0	11	8	3	4	5	4	3	4	0.40
Del Norte	0	0	0	0	0	0	0	0	0	0	0.00
El Dorado	0	0	1	2	0	1	1	0	1	0	0.33
Fresno	0	11	59	11	17	3	13	23	9	24	1.81
Glenn	0	3	13	12	7	1	0	2	1	7	16.27
Humboldt	0	0	1	0	0	0	0	0	0	0	0.07
Imperial	1	1	1	1	3	0	0	0	0	1	0.45
Inyo	0	0	0	0	0	0	0	0	0	0	0.00
Kern	0	59	67	49	140	2	18	15	18	25	4.64
Kings	0	0	32	1	7	2	3	1	1	3	3.26
Lake	0	1	0	2	0	0	0	0	0	1	0.62
Lassen	0	1	0	0	0	0	0	0	0	0	0.29
Los Angeles	1	305	40	13	36	156	20	4	58	163	0.81
Madera	0	0	18	0	2	0	1	7	2	3	2.17
Marin	0	0	0	1	0	0	0	0	0	0	0.04
Mariposa	0	0	0	0	0	0	0	0	0	0	0.00
Mendocino	0	0	0	0	2	0	0	0	0	0	0.23
Merced	0	1	25	4	4	1	4	1	1	13	2.09
Modoc	0	0	0	2	0	0	0	0	0	0	2.06
Mono	0	0	0	1	0	0	0	0	0	0	0.70
Monterey	0	0	0	0	0	0	1	0	0	1	0.05
Napa	0	0	0	1	1	0	0	0	0	0	0.15
Nevada	0	0	4	1	0	0	0	0	0	0	0.50
Orange	0	62	17	6	9	71	4	1	10	42	0.73
Placer	0	1	35	8	4	6	0	3	1	12	1.99
Plumas	0	0	1	0	0	0	0	0	0	0	0.50
Riverside	1	109	103	4	17	62	3	0	7	19	1.47
Sacramento	0	3	163	15	25	13	0	12	4	29	1.85
San Benito	0	0	0	0	0	0	0	0	0	0	0.00
San Bernardino	0	187	33	3	4	36	2	5	4	33	1.50
San Diego	0	2	1	1	15	35	4	0	0	1	0.19
San Francisco	0	0	2	0	0	0	0	1	0	1	0.05
San Joaquin	0	2	34	8	10	12	10	6	5	13	1.45
San Luis Obispo	0	1	0	1	0	0	0	0	0	0	0.07
San Mateo	0	0	1	0	0	0	0	0	0	0	0.01
Santa Barbara	0	0	2	0	0	1	0	0	1	0	0.09
Santa Clara	0	1	5	5	4	1	0	0	1	0	0.09
Santa Cruz	0	0	0	0	0	0	0	0	1	0	0.04
Shasta	0	5	1	4	9	1	0	0	0	1	1.18
Sierra	0	0	0	0	0	0	0	0	0	0	0.00
Siskiyou	0	0	0	0	0	0	0	0	0	0	0.00
Solano	0	0	5	8	1	1	0	0	0	2	0.41
Sonoma	0	0	1	0	1	0	0	0	0	0	0.04
Stanislaus	0	0	84	11	21	17	14	12	11	26	3.79
Sutter	0	0	9	12	3	0	0	0	0	8	3.34
Tehama	0	10	4	6	4	4	0	0	1	4	5.16
Trinity	0	0	0	0	0	0	0	0	0	0	0.00
Tulare	0	3	56	6	10	5	4	12	11	7	2.55
Tuolumne	0	0	1	0	0	0	0	0	0	0	0.18
Ventura	0	2	1	3	1	0	0	0	0	7	0.17
Yolo	0	1	11	27	2	1	2	0	0	10	2.68
Yuba	0	0	6	5	0	0	1	0	3	4	2.62
Total WNV disease	**3**	**779**	**880**	**278**	**380**	**445**	**112**	**111**	**158**	**479**	**0.97**
Asymptomatic Infections*	0	51	55	14	29	53	17	20	18	48	
Total WNV infections	**3**	**830**	**935**	**292**	**409**	**498**	**129**	**131**	**176**	**527**	**1.05**

*WNV infections detected through blood bank screening; no associated illness reported

Table 2. Reported West Nile Virus human cases by county of residence, California 2003 – 2012.

Table 3. Mosquitoes and sentinel chickens tested for St. Louis encephalitis[a], western equine encephalomyelitis[a] and/or West Nile Viruses, California, 2012.

County	Agency	No. mosquitoes tested[b]	No. mosquito pools tested	WNV + pools	No. flocks	No. chickens[c]	No. WNV positive flocks	WNV + sera
Alameda	Alameda Co. MAD	1,860	84	0	0			
Alpine		0			0			
Amador		0			0			
Butte	Butte Co. MVCD	8,643	176	27	7	77	6	45
Calaveras	Saddle Creek CSD	0			1	10	0	0
Colusa	Colusa MAD	0			1	10	1	6
Contra Costa	Contra Costa MVCD	8,801	286	4	5	50	2	7
Del Norte		0			0			
El Dorado		0			0			
Fresno	Consolidated MAD	21,751	637	81	0			
Fresno	Fresno MVCD	2,254	50	10	0			
Fresno	Fresno Westside MAD	16,336	398	52	0			
Glenn	Glenn Co. MVCD	1,635	33	4	1	11	1	8
Humboldt		0			0			
Imperial	Coachella Valley MVCD	3,026	63	4	1	10	1	10
Inyo	Owens Valley MAP	398	11	0	0			
Kern	Delano MAD	0			2	20	2	17
Kern	UC Davis, CVEC	110,562	2,917	565	8	80	7	57
Kern	Westside MVCD	6,742	164	6	0			
Kings	Consolidated MAD	205	8	2	0			
Kings	Kings MAD	27,549	746	100	0			
Lake	Lake Co. VCD	17,080	462	23	2	12	2	4
Lassen		0			0			
Los Angeles	Antelope Valley MVCD	552	28	1	10	60	7	40
Los Angeles	Greater LA Co. VCD	98,411	2,756	320	7	70	6	24
Los Angeles	Long Beach VCP	5,436	143		3	30	2	5
Los Angeles	Los Angeles Co. West VCD	12,388	359	18	18	108	12	53
Los Angeles	San Gabriel Valley MVCD	0			10	40	5	13
Madera	Madera Co. MVCD	4,594	155	14	0			
Marin	Marin-Sonoma MVCD	3,082	412	0	1	6	0	0
Mariposa		0			0			
Mendocino		0			0			
Merced	Merced Co. MAD	2,175	165	3	7	42	5	14
Merced	Turlock MAD	2,683	69	1	0			
Modoc		0			0			
Mono		0			0			
Monterey	North Salinas Valley MAD	690	15	0	2	20	0	0
Napa	Napa Co. MAD	3,257	139	1	0			
Nevada	Nevada Co. Agric. Dept.	0			2	20	0	0
Orange	Orange Co. VCD	35,375	1,530	69	0			
Placer	Placer Co. MVCD	36,536	1,742	108	8	48	3	8
Plumas		0			0			
Riverside	Coachella Valley MVCD	128,030	3,415	115	11	110	9	55
Riverside	Northwest MVCD	8,023	404	8	6	18	3	7
Riverside	Riverside Co. EH	30,932	707	8	5	60	1	1
Sacramento	Sacramento-Yolo MVCD	61,365	4,660	487	9	69	7	36
San Benito	San Benito Co. Agric Dept.	0			1	10	0	0
San Bernardino	San Bernardino Co. VCP	20,084	641	20	8	88	6	35
San Bernardino	West Valley MVCD	13,456	752	38	8	20	5	10
San Diego	San Diego Co. EH	2,480	96	0	2	20	0	0
San Francisco	Presidio Trust	247	6	0	0			
San Joaquin	San Joaquin Co. MVCD	30,369	1,502	169	0			
San Luis Obispo	Santa Barbara Co. MVMD	1,471	38	0	0			
San Mateo	San Mateo Co. MVCD	344	22	0	1	10	0	0
Santa Barbara	Santa Barbara Co. MVMD	7,921	209	0	5	50	0	0
Santa Clara	Santa Clara Co. VCD	3,288	237	3	7	49	0	0
Santa Cruz	Santa Cruz Co. MVCD	1,687	109	0	2	20	0	0
Shasta	Burney Basin MAD	0			2	12	0	0
Shasta	Shasta MVCD	21,766	680	17	5	50	3	6
Sierra		0			0			
Siskiyou		0			0			
Solano	Solano Co. MAD	2,939	100	3	3	36	1	2
Sonoma	Marin-Sonoma MVCD	25,149	1,294	3	3	18	1	1
Stanislaus	East Side MAD	1,727	82	3	2	16	2	8
Stanislaus	Turlock MAD	69,348	1,701	194	0			
Sutter	Sutter-Yuba MVCD	11,071	270	19	5	50	5	38
Tehama	Tehama Co. MVCD	0			3	30	0	0
Trinity		0			0			
Tulare	Delano MAD	0			1	10	1	6
Tulare	Delta VCD	16,339	621	125	1	10	1	5
Tulare	Kings MAD	1,215	33	3	0			
Tulare	Tulare MAD	3,400	104	16	0			
Tuolumne		0			0			
Ventura	City of Moorpark VC	0			1	8	1	2
Ventura	Ventura Co. EH	2,008	47	2	4	41	0	0
Yolo	Sacramento-Yolo MVCD	33,983	1,626	154	4	28	2	4
Yuba	Sutter-Yuba MVCD	3,317	88	15	2	20	2	13
Total		**933,980**	**32,992**	**2,815**	**197**	**1,577**	**112**	**540**

[a]No mosquito pools or sentinel chickens were positive for SLEV or WEEV in 2012.
[b]Tested by University of California at Davis Center for Vectorborne Diseases or local mosquito/vector control agency. Only includes pools tested by RT-PCR.
[c]Reflects planned standard number of chickens per flock. Actual number may vary due to mortality or replacement of seroconverted chickens.

Equine Surveillance. Serum or brain tissue specimens from approximately 180 horses that displayed neurological signs were tested for WNV at the California Animal Health & Food Safety Laboratory (CAHFS). West Nile virus infection was detected in 22 horses from 13 counties (Table 1). Eight of the horses died or were euthanized as a result of their infection.

Mosquito Surveillance. A total of 933,980 mosquitoes (32,992 pools) collected in 38 counties were tested at the University of California, Center for Vectorborne Diseases (CVEC) or at one of eight local agencies by a real-time (TaqMan) reverse transcriptase-polymerase chain reaction (qRT-PCR) for SLEV, WEEV, and/or WNV RNA (Table 3). Four local agencies also tested an additional 8,544 mosquitoes (386 pools) for WNV using a commercial rapid assay-RAMP® (Rapid Analyte Measurement Platform, Response Biomedical Corp).

West Nile Virus was detected in 2,849 mosquito pools from 28 counties; 2,815 were positive by RT-PCR, and 34 were positive by RAMP only (Table 1). Statewide, the minimum infection rate (MIR), defined as 1,000 times the number of infected mosquito pools divided by the number of mosquitoes tested, of WNV in all mosquitoes tested was 3.0; the MIR was highest (7.9) in Sacramento County (Figure 2, Table 3). Since 2003 the MIR of WNV in California has ranged from a low of 0.08 to a high of 3.0 (Figure 3); 2012 was therefore the year with the highest measured WNV infection rate in mosquitoes to date. West Nile Virus was detected in five *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma* and *Cx. tarsalis)* and *Aedes vexans* (Table 4). The first and last detections of WNV in mosquitoes in 2012 were from *Cx. tarsalis* pools collected in Riverside County on March 28 and December 4, respectively.

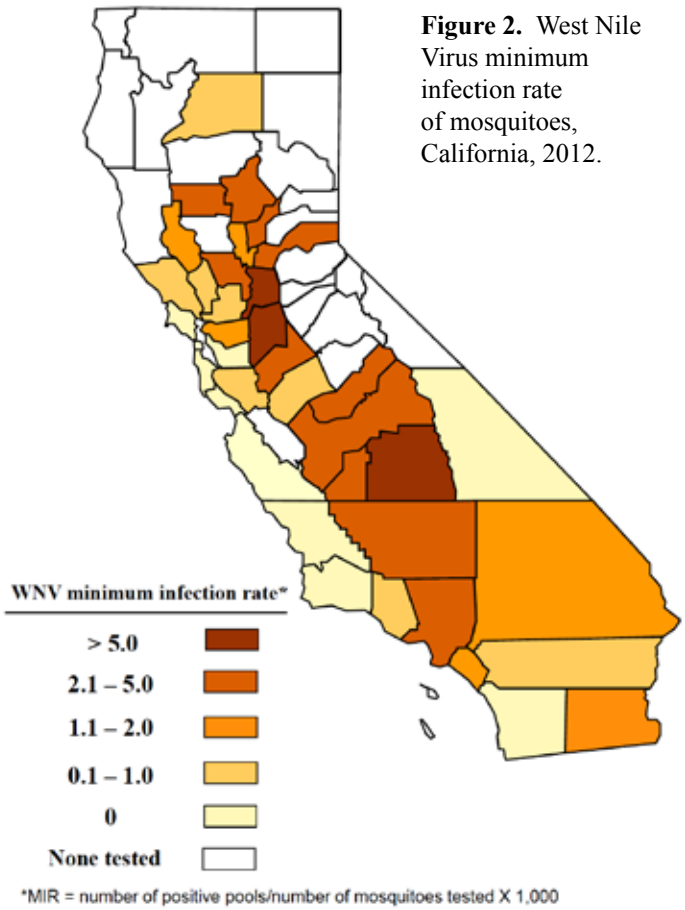

Figure 2. West Nile Virus minimum infection rate of mosquitoes, California, 2012.

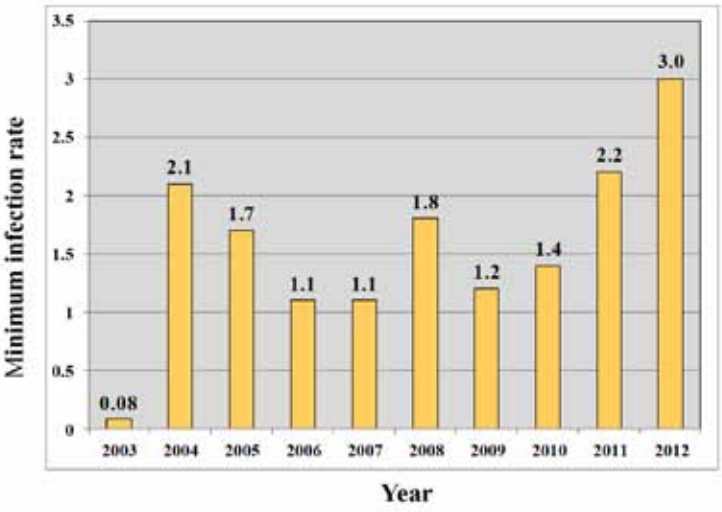

Figure 3. Annual minimum infection rate of West Nile virus in mosquito pools, California, 2003-2012.

Culex species	Pools	No. mosquitoes	WNV +	MIR*
Cx. apicalis	3	3	0	0.00
Cx. boharti	4	4	0	0.00
Cx. erythrothorax	2067	79,688	13	0.06
Cx. pipiens	7,488	143,234	598	4.17
Cx. quinquefasciatus	9,769	305,220	1,132	3.71
Cx. restuans	7	252	0	0.00
Cx. stigmatosoma	715	8,951	22	2.46
Cx. tarsalis	12,515	384,426	1,070	2.78
Cx. thriambus	68	148	0	0.00
unknown	9	116	0	0.00
All *Culex*	**32,645**	**922,042**	**2,835**	**3.07**

Anopheles species	Pools	No. mosquitoes	WNV +	MIR*
An. franciscanus	4	64	0	0.00
An. freeborni	80	2,086	0	0.00
An. hermsi	61	1,189	0	0.00
An. punctipennis	1	50	0	0.00
Unknown	1	25	0	0.00
All *Anopheles*	**147**	**3,414**	**0**	**0.00**

Aedes species	Pools	No. mosquitoes	WNV +	MIR*
Ae. dorsalis	6	203	0	0.00
Ae. melanimon	16	519	0	0.00
Ae. nigromaculis	1	12	0	0.00
Ae. sierrensis	6	91	0	0.00
Ae. squamiger	5	179	0	0.00
Ae. vexans	35	1,080	3	2.78
Ae. washinoi	12	351	0	0.00
All *Aedes*	**81**	**2,435**	**3**	**1.23**

Other species	Pools	No. mosquitoes	WNV +	MIR*
Culiseta incidens	300	6,103	0	0.00
Culiseta inornata	20	232	0	0.00
Culiseta particeps	34	748	0	0.00
Unknown	151	7,550	10	1.32
All other	**505**	**14,633**	**11**	**0.75**

* Minimum Infection Rate (MIR) = (No. pools positive/No. mosquitoes tested) X 1000

Table 4. Mosquitoes tested for West Nile Virus, California, 2012.

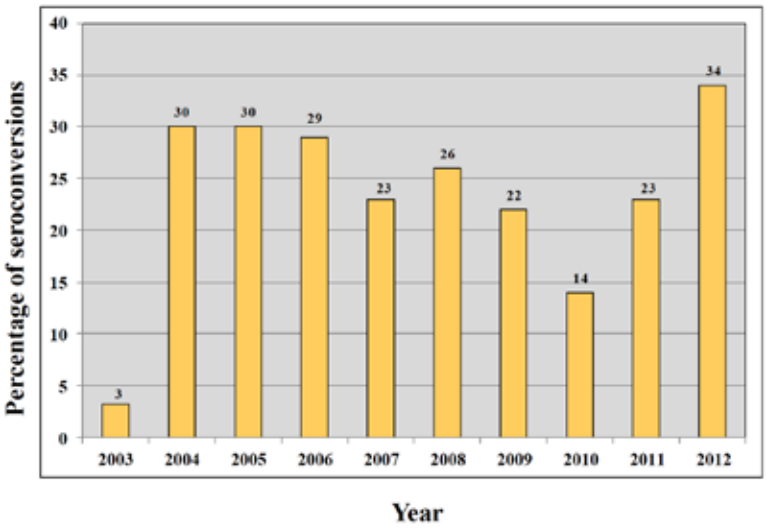

Figure 5. Annual percentage of sentinel chicken seroconversions to West Nile virus, California, 2003 - 2012.

Chicken Serosurveillance. In 2012, 39 local mosquito and vector control agencies in 33 counties maintained 197 sentinel chicken flocks (Table 3). Blood samples were collected from chickens every other week and tested for antibodies to SLEV, WNV and WEEV by an IgG EIA at the CDPH Vector-Borne Disease Section Laboratory (VBDS). Positive samples were confirmed at the VBDS laboratory by IFA and western blot. Samples with inconclusive results were forwarded to the VRDL for further testing by a PRNT.

Of 19,048 chicken blood samples that were tested, 540 seroconversions to WNV were detected among 112 flocks in 22 counties (Tables 1 and 3, Figure 4). Statewide, 34.2% of sentinel chickens seroconverted to WNV. Since 2003 the percentage of WNV seroconversions in chickens has ranged from a low of 3.2% to a high of 34.2% (Figure 5). In 2012 the first WNV seroconversion was detected in Riverside County on May 29, and the last seroconversion was detected in Los Angeles County on November 19.

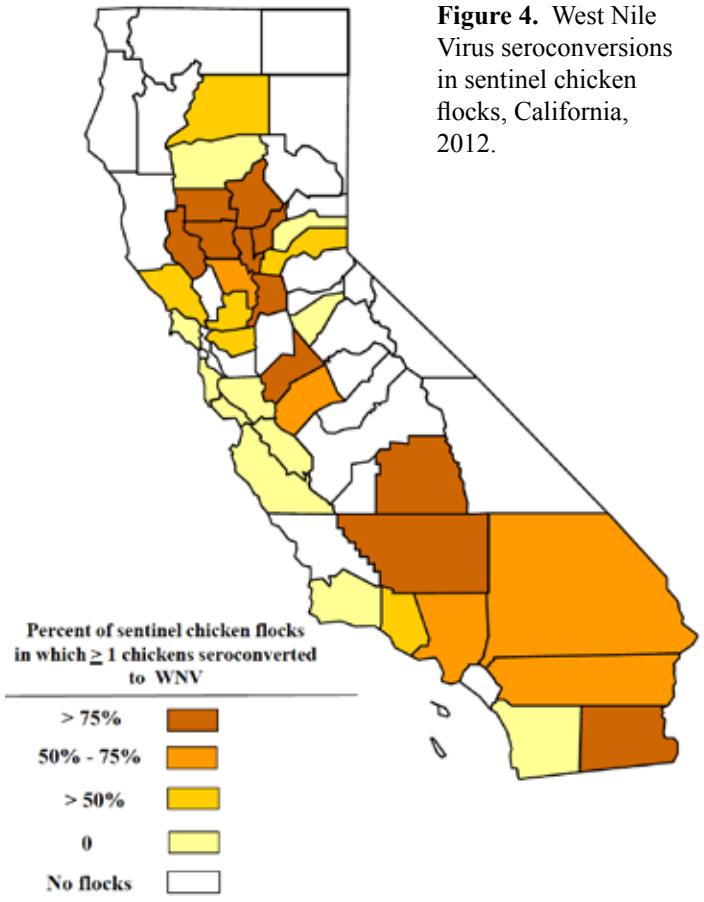

Figure 4. West Nile Virus seroconversions in sentinel chicken flocks, California, 2012.

County	Reported	Tested	Positive-acute	Positive-chronic
Alameda	591	118	15	23
Alpine	2	2	0	0
Amador	37	0	0	0
Butte	530	111	53	21
Calaveras	43	2	0	0
Colusa	52	8	5	0
Contra Costa	1816	106	66	8
Del Norte	0	0	0	0
El Dorado	377	99	5	21
Fresno	410	60	25	8
Glenn	57	28	16	10
Humboldt	12	4	0	1
Imperial	4	0	0	0
Inyo	7	0	0	0
Kern	368	58	14	9
Kings	67	13	2	4
Lake	97	22	10	0
Lassen	6	0	0	0
Los Angeles	3309	557	271	69
Madera	58	13	2	5
Marin	191	10	1	3
Mariposa	7	1	0	1
Mendocino	66	12	6	2
Merced	391	60	42	4
Modoc	7	4	0	1
Mono	2	0	0	0
Monterey	125	29	0	7
Napa	82	9	1	6
Nevada	79	16	3	8
Orange	306	353	92	17
Placer	506	320	60	33
Plumas	13	0	0	0
Riverside	351	32	5	4
Sacramento	3621	960	466	58
San Benito	20	4	0	2
San Bernardino	592	120	39	25
San Diego	192	111	1	0
San Francisco	118	19	1	7
San Joaquin	888	145	58	13
San Luis Obispo	131	16	1	3
San Mateo	383	54	5	17
Santa Barbara	71	13	0	5
Santa Clara	868	164	20	3
Santa Cruz	185	39	1	16
Shasta	163	69	36	5
Sierra	2	1	0	1
Siskiyou	10	2	0	1
Solano	564	49	28	2
Sonoma	398	55	8	16
Stanislaus	776	112	51	22
Sutter	167	43	29	2
Tehama	112	11	5	1
Trinity	4	0	0	0
Tulare	310	110	54	13
Tuolumne	19	1	0	0
Ventura	494	103	38	18
Yolo	591	201	98	10
Yuba	150	18	11	1
Totals	**20,798**	**4,467**	**1,644**	**506**

[a]Tested by University of California at Davis Center for Vectorborne Diseases or local mosquito/ control agency

Table 5. Dead birds reported, tested[a] and positive for West Nile Virus, California, 2012.

Dead Bird and Tree Squirrel Surveillance. In 2012 the WNV hotline and website received 20,798 dead bird reports from the public in 57 counties (Table 5). Dead bird carcasses were tested either at CVEC by RT-PCR or at one of 24 local agencies by RT-PCR, RAMP or VecTest (Medical Analysis Systems, Inc., Camarillo, CA). Of the 4,467 carcasses deemed suitable for testing, WNV was detected in 2,150 (48%) carcasses from 47 counties; 1,644 were reported as acute infections (i.e., recent within current surveillance season) from 39 counties, and 506 were reported as chronic infections (i.e., exposed at an undeterminable time in the past) from 44 counties (Tables 1 and 5, Figure 6). Since 2003 the prevalence of WNV positive dead birds has ranged from a low of 5% to a high of 56% (Figure 7). Of the acute infections, 1,453 were confirmed positive by RT-PCR, 142 by RAMP and 49 by VecTest. In 2012 the first WNV positive dead bird was a house finch reported from Sacramento County on January 18, and the last WNV positive dead bird was an American crow reported from Santa Clara County on December 27.

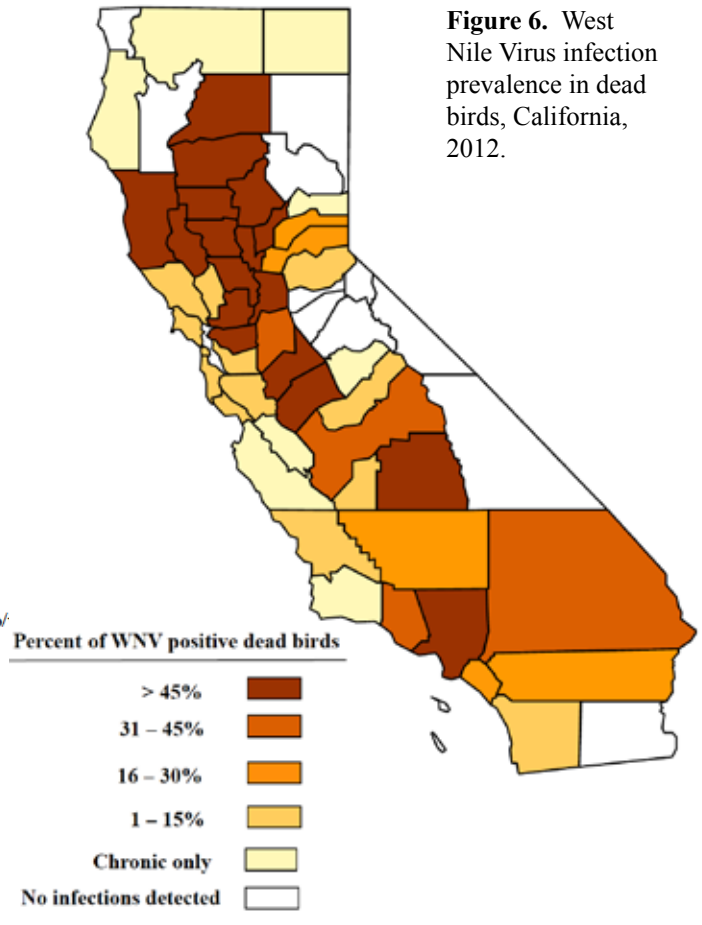

Figure 6. West Nile Virus infection prevalence in dead birds, California, 2012.

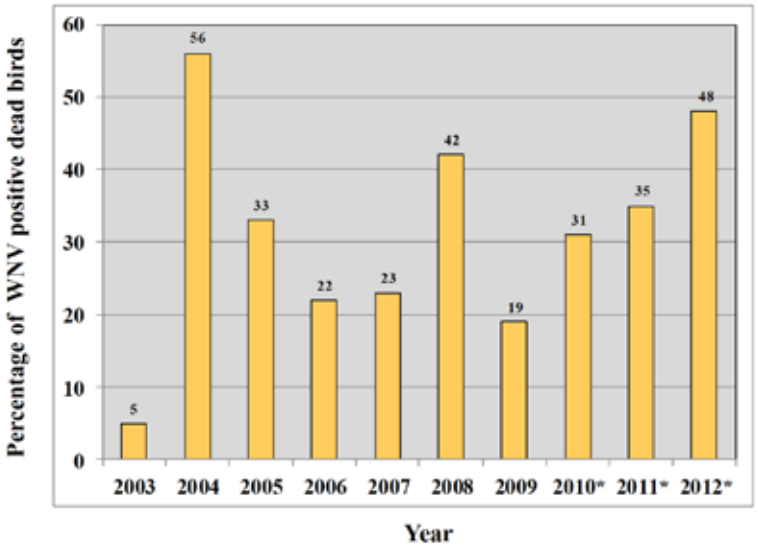

Figure 7. Annual percentage of WNV infection in dead birds, California, 2003-2012.

* Data include chronic and recent infections

In 2012, 686 dead squirrels were reported through the WNV Hotline; 184 carcasses were tested and WNV RNA was detected by RT-PCR in 23 (12.5%) carcasses from seven counties (Table 1). These included 15 fox squirrels (*Sciurus niger*), 2 eastern gray squirrels (*S. carolinensis*), 1 western gray squirrel (*S. griseus*), 2 California ground squirrels *(Otospermophilus beecheyi*) and 3 were of unknown species.

SUMMARY

In 2012, 527 human WNV infections, including 20 fatalities, were reported from 32 counties; this was the highest number of infections reported since 2005 (Table 2). The statewide case incidence per 100,000 persons was 1.27. The highest number of cases was reported from Los Angeles County, with 163 cases, although case incidence was higher in rural counties (Figure 1, Table 2). The proportion of WNND cases among all reported cases was high, with 65% reported in 2012.

Environmental surveillance for virus activity detected an increase in WNV enzootic activity throughout the state compared to the previous three years. Notably, the statewide minimum infection rate of WNV in mosquitoes and the proportion of sentinel chicken seroconversions to WNV were higher in 2012 than in any other year since surveillance began for WNV in 2000 (Figures 3 and 5). Additionally, the prevalence of WNV in dead birds was the third highest detected since 2003 (Figure 7). These data, along with the continued high proportion of WNND cases, suggest that there is significant under-diagnosis of WNV illness in humans.

Throughout California, environmental surveillance detected WNV activity during every season of the year, including the winter months. For the fifth consecutive year, only WNV was detected. WEEV was last detected in California in 2007, and SLEV has not been documented since 2003, perhaps indicating continued competitive displacement with WNV._

ACKNOWLEDGEMENTS

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West Nile Virus in Southwest San Bernardino County, California: A 10-Year Summary

Min-Lee Cheng and Tianyun Su

West Valley Mosquito and Vector Control District
1295 E. Locust St., Ontario, CA 91761, tsu@wvmvcd.org

ABSTRACT: Evidence that the exotic pathogen, West Nile virus (WNV), had invaded San Bernardino County, California in late 2003 was documented when a dead crow collected from the West Valley (the southwestern corner of the county) tested positive for WNV. In response to the invasion of WNV, the arbovirus surveillance program was enhanced in the West Valley Mosquito and Vector Control District (WVMVCD) by expanding mosquito trapping and testing; dead bird collection and testing, and sentinel chicken bleeding and testing. Additionally, a collaboration with San Bernardino County Department of Public Health and Department of Preventive Veterinary Health was established for monitoring human and equine infections. Enzootic, epizootic, endemic and sometimes epidemic levels of WNV transmission have occurred since then. In total between 2003 and 2012, WNV was detected by or reported to the WVMVCD from 576 positive mosquito pools, 338 positive dead birds, 36 sentinel chicken flockss, 98 individual sentinel chickens, 66 humans cases (1 fatality) and 12 equine cases.

We have learned that it is very difficult to predict reliably WNV activity when one considers the complex epidemiological dynamics of this virus. *Culex quiquefasciatus* Say, *Cx. stigmatosoma* Dyar, *Cx. tarsalis* Coquillett and *Cx. erythrothorax* Dyar are the primary WNV vectors in WVMVCD. The gravid trap is a highly valuable tool for monitoring mosquito populations and WNV transmission. WNV-positive mosquitoes and dead birds function as an early warning for enzootic WNV transmission, while sentinel chicken sero-conversion serves to confirm local WNV transmission. Occurrence of human and equine cases was sporadic. Human infections have been significantly underestimated because a majority of the infected individuals are either asymptomatic or show atypical symptoms. Underreporting is also problematic because of inadequate awareness of WNV infections among some clinicians. Equine cases declined over the years partially as a result of enforcing immunization by San Bernardino County Department of Preventive Veterinary Health. West Nile Virus is now well established in the WVMVCD and the rest of southern California. Enzootic and endemic transmissions persist, and epizootic and epidemic transmissions occur when the relevant ecological and socio-economic factors are favorable.

INTRODUCTION

The West Valley Mosquito and Vector Control District (WVMVCD) was established in 1983 and serves approximately 550,000 residents living in five cities and some unincorporated county areas in the southwestern corner of San Bernardino County, CA. The District's jurisdiction covers about 199 square miles of urban, suburban and rural (agricultural and riparian) landscape. West Nile virus (WNV) first appeared in North America in New York City in 1999, resulting in 62 human cases with 7 fatalities. This virus was first detected in WVMVCD in late 2003 in a dead American crow collected on November 6 in the City of Ontario. In response to the arrival of WNV, the WVMVCD implemented an enhanced arbovirus surveillance program by maintaining extensive mosquito trapping and testing, dead bird collection and testing, and sentinel chicken bleeding and testing on a year round basis. Information on WNV infections in humans and equines was acquired from the County Departments of Public Health and Preventive Veterinary Medicine. Collaborations with California Department of Public Health, University of California system and other mosquito and vector control agencies also were enhanced. Public outreach activities and field operations in the WVMVCD were also intensified in response to WNV epidemics. The mosquito and WNV surveillance data were summarized to reflect the enzootic to epizootic, as well as endemic and epidemic transmissions of WNV between 2003 and 2012.

MATERIALS AND METHODS

Mosquito Trapping and Testing. In total, 20 - 25 EVS traps and 20 - 25 gravid traps were deployed weekly between April 1 and November 30 and bi-weekly during rest of each year. Mosquitoes collected were identified to species and pooled for WNV testing by RAMP® test or real time RT-PCR. Minimum infection rates (MIR) were calculated by week, year, species and trap type.

Dead Bird Collection and Testing. Dead birds were collected in response to the requests from the general public or California Department of Public Health. Oral swab samples were collected upon arrival of dead bird carcasses in the laboratory. VecTest® or RAMP tests were performed to detect viral antigens, and carcasses were submitted to CAHFS (California Animal Health and Food Safety) laboratory for confirmation by real time RT-PCR.

Sentinel Chicken Bleeding and Testing. In total, 8 sentinel chicken flocks each consisting of 2 to 10 chickens (depending on different years) were maintained every year. Blood samples were collected every 10 to 14 days and tested by ELISA or in-house rapid test (Cheng and Su 2011). Blood samples were confirmed later by ELISA at the State Public Health Laboratory in Richmond, California.

Infections in humans and equines. Information of human and equine cases was acquired from Department of Public Health and Department of Preventive Veterinary Health in San Bernardino County, California. Additional inspections for mosquito breeding sources and larviciding, as well as trapping and testing of mosquitoes were carried out near the case locations usually for extended periods of time.

Temperature Monitoring. HOBO RH-Temp monitoring units (ONSET Computer Corp., Bourne, MA) were used to monitor outdoor temperatures on an hourly basis at the District headquarters. Weekly averages were calculated and related to WNV onset data.

RESULTS

Mosquito trapping and testing. High mosquito counts occurred during weeks 18-41 by EVS traps and during weeks 18-46 by gravid traps (Fig. 1a). The predominant species was *Culex quinquefasciatus* Say, followed by *Cx. stigmatosoma* Dyar and *Cx. tarsalis* Coquillett, *Cx. erythrothorax* Dyar, *Culiseta incidens* (Thompson) and other minor species. In total 270,622 mosquitoes making up 10,198 pools were submitted for testing, and 576 pools tested positive. Total MIR varied from year to year with peaks in 2004 (4.04), 2008 (2.93), 2011 (3.66) and 2012 (2.81). Over all, infected mosquitoes occurred during week 18 to 48 with a peak in week 33 (6.09). *Culex quinquefasciatus*, *Cx. tarsalis*, *Cx. stigmatosoma* had the overall comparable infection rates of 2.14, 2.13 and 2.36 respectively, while it was much lower for *Cx. erythrothorax*. No WNV infections were detected in other mosquito species, such as *Anopheles hemsi* Barr and *Culiseta incidens*. MIR in mosquitoes collected by gravid traps (2.77) was much higher than in those collected by EVS traps (1.72) (Figure 1b).

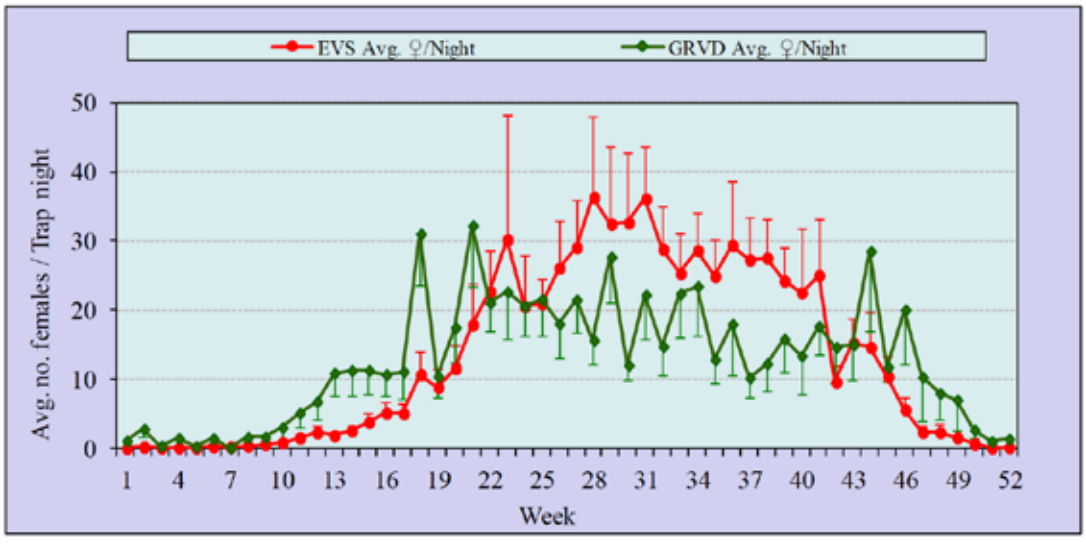

Figure 1a. Average mosquito population densities (with standard errors) (2004-2012, West Valley MVCD, Ontario, CA).

Figure 1b. Minimum infection rates (MIRs) of West Nile Virus in mosquitoes by year, week, species and trap types (2004-2012, West Valley MVCD, Ontario, CA).

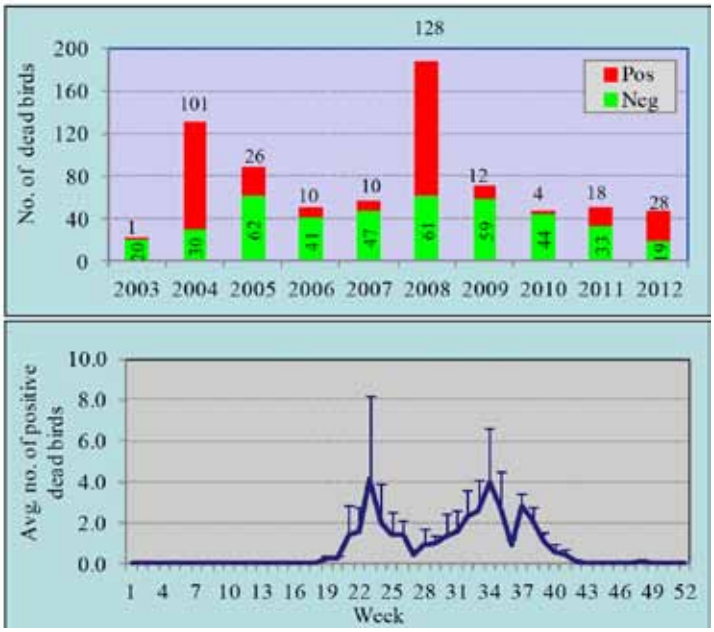

Figure 2. Dead birds tested and West Nile Virus positive dead birds by year and week (2004-2012, West Valley MVCD, Ontario, CA).

Dead Bird Collection and Testing. During 2003 - 2012, a total of 2,502 dead birds was reported to the District, 905 dead birds were collected, 762 tested and 338 tested positive for WNV. In 2008, the total number of dead birds tested and WNV positive dead birds were the highest, followed by 2004, 2005, 2012 and the remaining years. The onset of positive dead birds was during weeks 19 - 48, with peaks during weeks 23 and 34 (Figure 2). The weeks of WNV positive dead birds were about the same as those in positive mosquito samples.

Sentinel Chicken Bleeding and Testing. Numbers of sentinel chicken flocks that sero-converted against WNV were the highest in 2004 (n = 7) and 2005 (n = 7), followed by 2008 (n = 6), 2012 (n = 5), 2006 (n = 4) and the remaining years (n = 0 - 3). The onset for sero-conversion in sentinel chickens was during weeks 24 through 43 (Figure 3a). Numbers of individual sentinel chickens that sero-converted against WNV also showed the same trend with the highest in 2004 (n = 37), followed by 2005 (n = 19), 2008 (n = 15), 2012 (n = 10), 2006 (n = 7) and the remaining years (n = 0 - 5). The trend of onset weeks indicated by individual sentinel chicken sero-conversions was the same as that indicated by individual coop sero-conversions (Figure 3b).

Infections in Humans and Equines. There have been 66 human cases, including 1 fatality, reported within the service boundaries of WVMVCD since the arrival of WNV in 2003. Case reporting and confirmation were sporadic and variable, highest in 2004 (n = 25), followed by 2012 (n = 17), 2008 (n = 8) and 2005 (n = 6). There were no human cases in

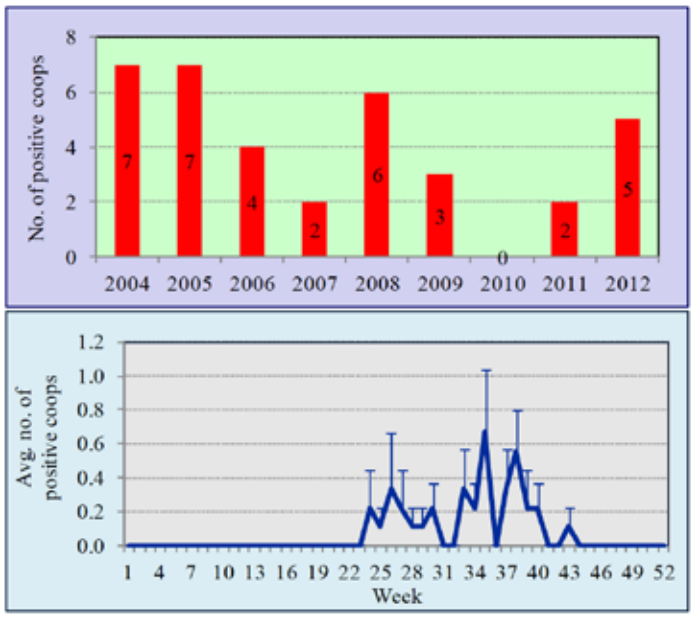

Figure 3a. Sentinel chicken coop sero-conversions against West Nile Virus by year and week (2004-2012, West Valley MVCD, Ontario, CA).

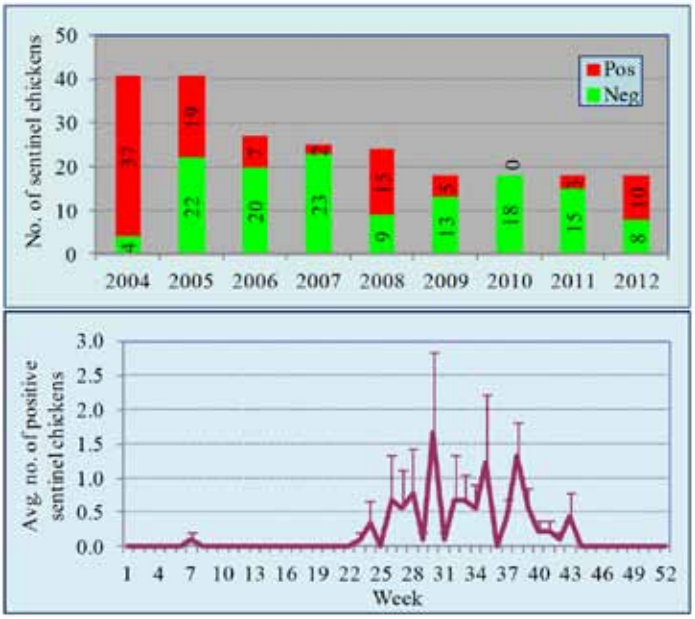

Figure 3b. Individual sentinel chicken sero-conversions against West Nile Virus by year and week (2004-2012, West Valley MVCD, Ontario, CA).

the other years. The onset for human cases was during weeks 27 - 45 with peaks during weeks 31 - 39 (Figure 4). Equine cases of WNV showed similar sporadic, temporal distribution pattern between 2004 and 2012. Equine cases were highest in 2004 (n = 9), followed by 2005 (n = 2) and 2008 (n = 1). There were no cases in the remaining years. The onset times for equine cases were weeks 30 - 44 with a peak in week 32 (Figure 5).

Comparison of Onset Data among Surveillance Tools. Mosquito infection onset started in week 18 and ended in week 48; this onset in times was almost

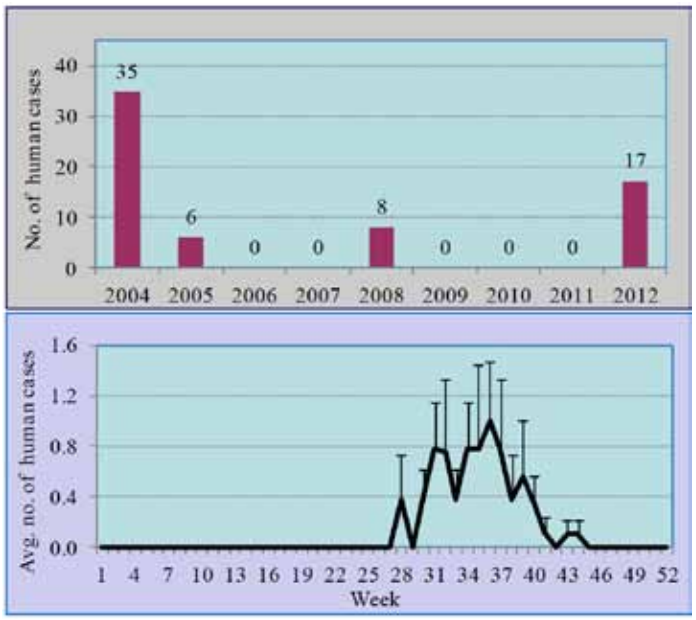

Figure 4. Human infections of West Nile Virus by year and week (2004-2012, West Valley MVCD, Ontario, CA).

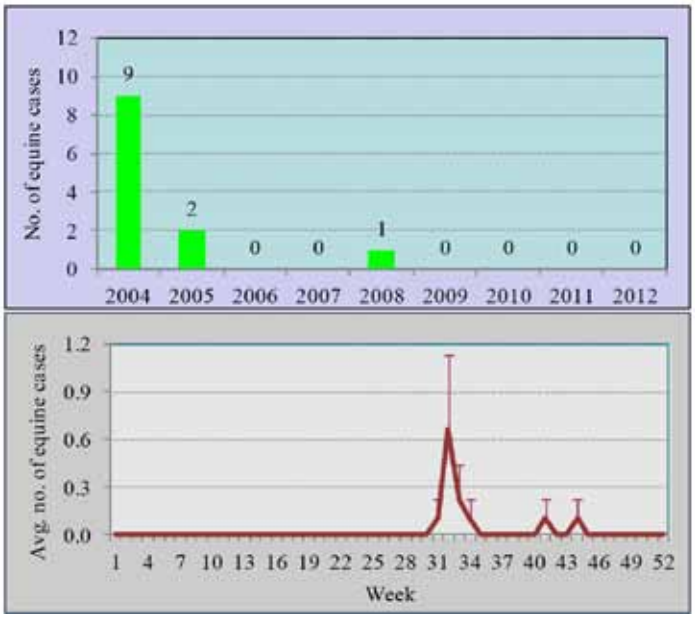

Figure 5. Equine infections of West Nile Virus by year and week (2004-2012, West Valley MVCD, Ontario, CA).

synchronous with positive dead bird data (onset weeks 19 - 48). Sentinel chicken sero-conversion onset was delayed to week 24 but ended earlier in week 43. Confirmed human (week 27) and equine (week 30) cases were reported even later and ended earlier in weeks 44 - 45. All WNV onsets were correlated to warmer average temperatures (Figure 6).

Culex quiquefasiatus and *Cx. stigmatosoma* are important vectors of WNV in urban and suburban areas, whereas *Cx. tarsalis* and *Cx. erythrothorax* play major roles in virus transmission in rural and riparian environment. The gravid trap is a highly valuable tool to detect WNV transmission as gravid females should have taken at least one blood meal and thereby have

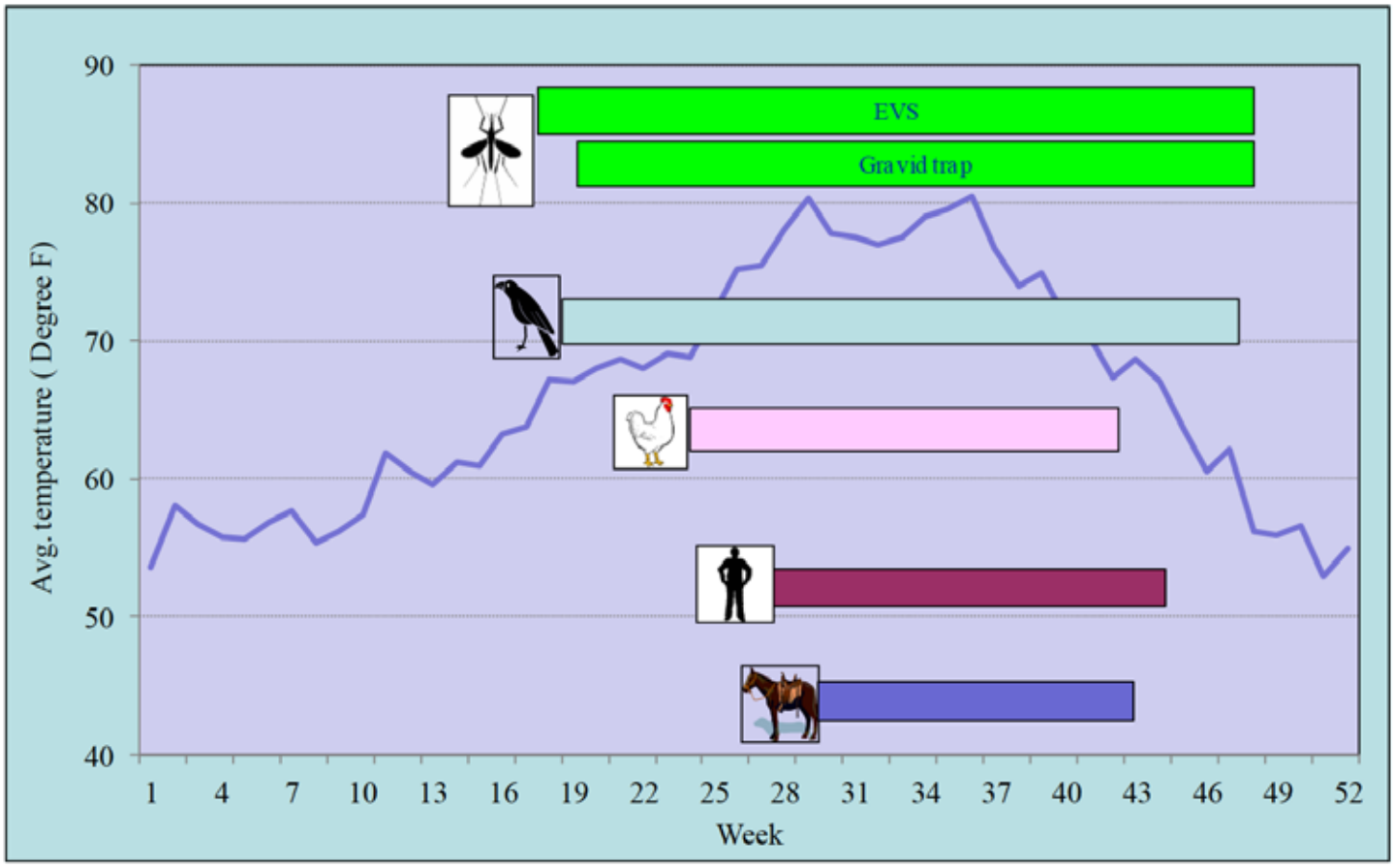

Figure 6. Summary of West Nile virus infection onset in mosquitoes, dead birds, sentinel chickens, humans and equines with comparison of average temperatures (2004-2012, West Valley MVCD, Ontario, CA).

DISCUSSION

During 2003 - 2012, 576 positive mosquito pools, 338 positive dead birds, 36 positive sentinel chicken coops, 98 individual positive sentinel chickens, 66 human cases (1 fatality) and 12 equine cases were detected or reported within the WVMVCD boundaries. The different surveillance tools showed a clear and consistent seasonal pattern of WNV activity among years.

a greater chance for WNV infection compared with females caught in EVS traps. Strategic locations, design and quality control in mosquito trapping and subsequent testing for WNV played critical roles in detection of WNV transmission in our District. Infected mosquitoes and dead birds served as early warning of enzootic transmission. Our studies on detection of WNV antigens and viral RNA in fly maggots retrieved from dead bird carcasses in advanced decomposition state significantly extended the post-mortem window time for WNV detection (Su and Cheng 2011).

Sentinel chickens primarily served to confirm WNVS activity, as sero-conversions mostly occurred after positive mosquito pools and dead birds had been detected. However the significance of sentinel chicken sero-conversions to WNV epidemiology is irreplaceable for specific localization of WNV transmission. Since 2008, a rapid dip-stick technique was developed, evaluated and applied in preliminary screening of sentinel chickens for sero-conversion to arboviruses (Cheng and Su 2011). This test requires minimum laboratory equipment and skills and provides preliminary qualitative results in about 45 minutes, which allows personal in vector control agencies to make immediate and appropriate decisions in operations.

It is most likely that human infections are significantly underreported (Roger Nasci, 44th SOVE Annual Meeting, St. Augustine, FL, 2012) because the majority of infected individuals are either asymptomatic or show atypical symptoms. In addition, inadequate awareness of WNV infections in clinicians has also probably led to under reporting. Equine cases in our District declined over the ten years analyzed here, primarily as a result of enforcing horse immunization by the San Bernardino County Department of Preventive Veterinary Health.

Based on the data collected between 2003 and 2012, it appears that the WNV has now well established into the ecological systems in the West Valley area of the San Bernardino County, California, as indicated by the stable enzootic activities every year (i.e., repeated detections of WNV infections in wild mosquito populations and dead bird carcasses). In most years except 2010, enzootic transmission was reflected later by sero-conversion in sentinel chickens. Human and equine cases, if any, ensued in late season following the detection of WNV activity in mosquitoes, dead birds and sentinel chickens.

ACKNOWLEDGMENTS

We appreciated multi-years' collaborations in management of West Nile virus with other local vector control agencies, California Department of Public Health, California Animal Health and Food Safety Laboratory, University of California, California State University at San Bernardino, California Polytechnic State University, and the industries involved in research, development and marketing of viral test kits and public health pesticides.

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Development and Validation of a Fine-scale, Spatio-temporal Model to Predict Abundance of Culex mosquitoes in the Coachella Valley

Jessica M. Healy[1], Bborie Park[1], Gregory S. White[2] and Christopher M. Barker[1]

[1]*Center for Vectorborne Diseases, University of California, 4206 Vet Med 3A, One Shields Avenue, Davis, CA, 95616, 530-752-0151, cmbarker@ucdavis.edu*
[2]*Coachella Valley Mosquito and Vector Control District, 43420 Trader Place, Indio, CA 92201*

ABSTRACT: In the Coachella Valley of Southern California *Culex quinquefasciatus* and *Culex tarsalis* are the primary vectors of West Nile Virus in urban and rural settings. The abundance of these vectors is controlled by the Coachella Valley MVCD to limit transmission of the virus. We have developed a model to predict the times and places that have the highest abundance of these vectors that is used by the MVCD in targeting control efforts. The most predictive models combine information on several factors shown to influence abundance in earlier studies, including temperature, precipitation and landscape to predict more accurately the abundance of the mosquito vector over time and space.

INTRODUCTION

Coachella Valley is a unique area in the deserts of Southern California that is composed of both urban and agricultural settings with minimal precipitation and temperatures that, on average for the time period of 2001 through 2012, surpass 38°C 127 days out of the year. In urban areas the main vector of West Nile Virus is *Culex quinquefasciatus,* while in rural areas the main vector is *Culex tarsali*s. Numerous ecological studies have been conducted on the spatial and seasonal patterns of *Cx. tarsalis* populations in the Coachella Valley. Results from these studies have shown that: (1) the abundance is greatest in the ecotones between elevated vegetation and another land-use type (Lothrop and Reisen 2001), (2) dispersal rates of the vector vary by season, but independently of temperature or humidity (Reisen and Lothrop 1995), and (3) temperature change may have selective pressure on the population of *Cx. tarsalis* mosquitoes (Reisen 1995). Similar studies in other parts of the United States also have shown the impacts of landscape and climate on *Culex* abundance (Barker et al. 2009, Barker et al. 2010, Pecoraro et al. 2007, Reiter and Lapointe 2007). The goal of the current study is to develop a statistical model based on landscape and climate that will predict abundance at unsampled locations and future time periods.

METHODS AND MATERIALS

Data. All available mosquito collection data from CO_2 and gravid mosquito traps were used for the years of 2001 through 2012. Mosquito trapping locations are dispersed throughout the valley encompassing both urban and rural settings. Because *Cx. quinquefasciatus* is the most significant vector in urban areas and *Cx. tarsalis* is most important in rural areas, we divided the valley into urban and rural designations. A 1 mile^2 grid was designated as urban if 50% or more of the area within the grid was occupied by developed land as classified through the National Land Cover Database; otherwise grids were designated as rural. The grid structure for the valley and land types within the grids are displayed in Figure 1. By dividing the valley we are able to construct a model to predict *Cx. tarsalis* in rural areas and *Cx. quinquefasciatus* in urban areas.

Predictive Model. The predictive models were constructed using negative binomial regression. Potential covariates considered in construction of the model included temperature and precipitation data (Nemani et al. 2007) taken at each site for each biweekly time-step at 2 - 8 week lags, the abundance in neighboring grids, the human population density within each grid cell derived from the 2010 Census data and a sinusoidal function to account for the typical seasonal variation in abundance. Additional variables include the types of soil within each grid cell and the

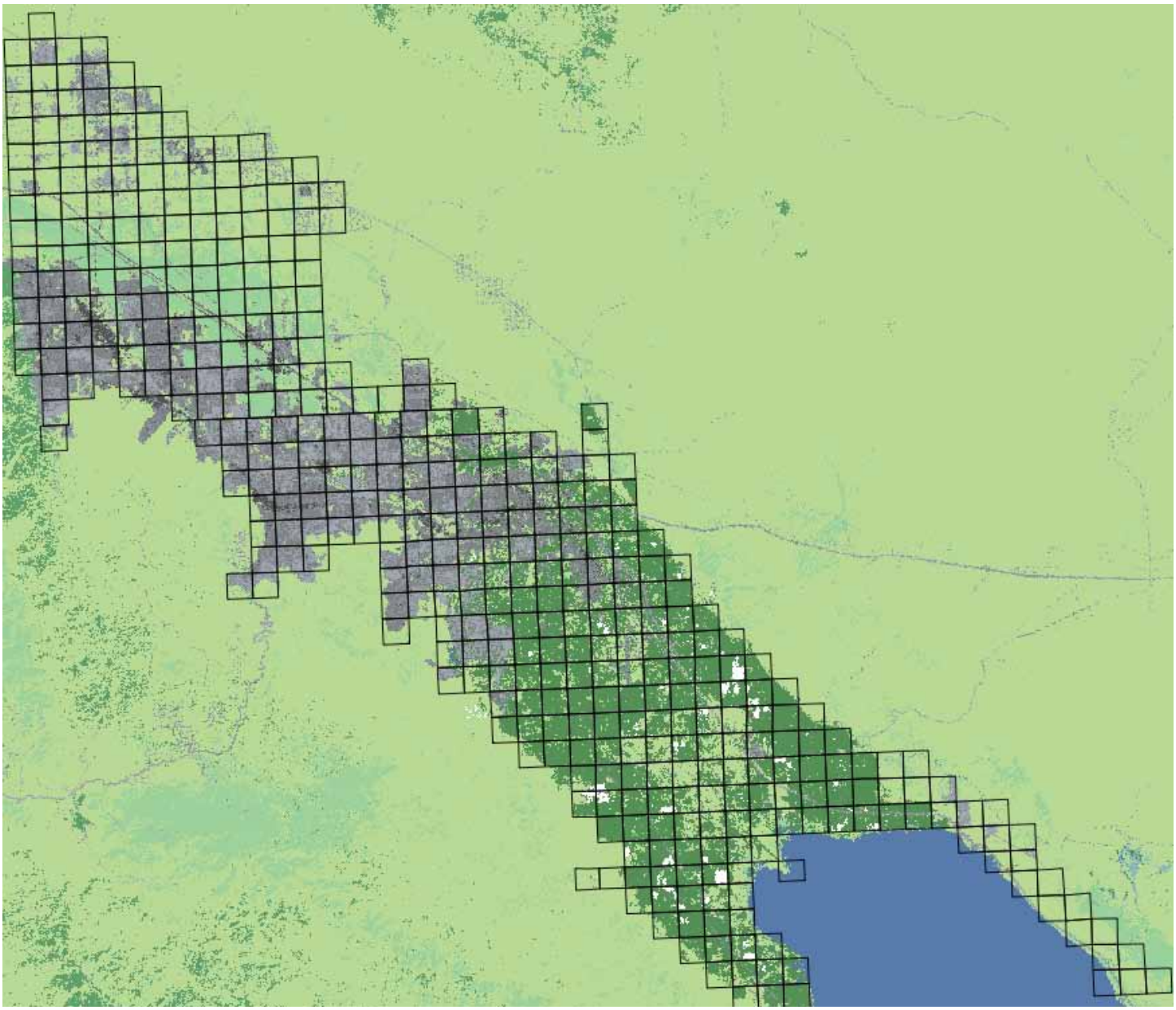

Figure 1. The one square mile cell grid structure for prediction with corresponding land types; developed land (greys) and agricultural and rural land types (greens).

interaction with precipitation and the percent change in abundance from the previous time period. The accuracy of candidate models was verified through internal and external validation, with accuracy being calculated as the percentage of predictions that fall within one standard deviation of the observed abundance for each biweek. Internal validation consisted of predicting the abundance for the next biweek at each time-step within the data, while external validation consisted of predicting the abundance for the next biweek at each time-step in the data using only the data leading up to that time point to inform the predictive model.

RESULTS AND DISCUSSION

Internal and external validation of the models was performed and used to select the best predictive models and their corresponding covariates. Table 1 displays the covariates and accuracy of prediction for the rural and urban models. For prediction of *Cx. tarsalis* abundance the most informative variables included the average abundance in neighboring grid cells within 10 kilometers, prior temperature and precipitation, human population density within the grid cell, the percent change in abundance from the previous time period

and a function for the seasonality of abundance. The model accurately predicted 49.95% and 53.42% of observations for the next time-step through internal and external validation, consecutively. For prediction of *Cx. quinquefasciatus* abundance, the most informative variables included the same variables as above, but with different time lags for both the precipitation and temperature variables. This model accurately predicted 29.2% and 32.88% of observations for the next time-step through internal and external validation, consecutively.

ACKNOWLEDGEMENTS

We thank Cary Roberts, Branka Lothrop, Jeremy Wittie, Ed Prendez, Jennifer Henke and Melissa Snelling at the Coachella Valley Mosquito and Vector Control District for their support in funding and providing data and advice for this project.

Setting and Species	Variables in Model	% Accuracy Internal	% Accuracy External
Rural, *Cx. tarsalis*	Average abundance at Neighbor sites Maximum Temperature (1-6 weeks prior) Precipitation (7-8 weeks prior) Human Population Density Seasonality (sin and cosine functions) % Change in abundance from previous biweek	49.95%	53.42%
Urban, *Cx. quinquefasciatus*	Average abundance at Neighbor sites Mean temperature (1-2 and 5-6 weeks prior) Precipitation (3-8 weeks prior) Human Population Density Seasonality (sin and cosine functions) % Change in abundance from previous biweek	29.2%	32.88%

Table 1. The variables included in the model for the rural and urban grids and their corresponding accuracies through internal and external validation.

The disparity seen between the accuracies of the urban and rural models can possibly be explained by the large amount of data that is available to inform the models for the rural grid cells in comparison to the urban grid cells. This is because not only are there more rural cells, but also because historically more mosquito trapping has taken place in rural than in urban areas in the valley. To improve the accuracy of the predictive models further, additional covariates will be considered for inclusion. These covariates may include the schedule of flooding of duck clubs around the Salton Sea shore, the levels of the Salton Sea and possibley spatial and temporal data on the use of stand pipes for agricultural irrigation. The predictive models produced from this work will be incorporated into a custom ArcGIS toolbox through a linkage with the R statistical software mediated by Python that can be used by the MVCD to determine the areas where control efforts may need to be focused in the coming weeks.

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Tick-Borne Spotted Fever Group Rickettsia Surveillance in Imperial County

Mary Joyce Pakingan[1], Kerry Padgett[1], Natasha Espinosa[2], Laura Apodaca[3], Paula Kriner[3] and Alex Espinosa[2]

[1] California Department of Public Health ,Vector-Borne Disease Section, 1616 Capitol Avenue, MS 7307, P.O. Box 997377, Sacramento, CA 95899
[2] California Department of Public Health,Viral and Rickettsial Disease Laboratory , 850 Marina Bay Parkway, Richmond, CA 94804
[3] Imperial County Public Health Department, 935 Broadway, El Centro, CA 92243

ABSTRACT: In response to a 2008-2009 outbreak of Rocky Mountain spotted fever (RMSF) in Mexicali, northern Baja Mexico, tick surveillance was conducted in 2009 and from 2011 to 2012 across the Mexican border in Imperial County (Eremeeva et al. 2011). In a preliminary study in 2009, 200 *Rhipicephalus sanguineus* (brown dog ticks or kennel ticks) were tested for spotted fever group *Rickettisa* from 35 shelter dogs; all were negative (Fritz et al. 2010). In 2011 - 2012, Imperial County Public Health Department (along with Valley Veterinary Clinic, Calexico Animal Control Shelter, Humane Society of El Centro and El Centro Animal Control Shelter) collected 523 ticks from 112 dogs. Vector-Borne Disease Section (VBDS), California Department of Public Health (CDPH), staff identified all ticks as *R. sanguineus*, including 201 females, 186 males, 133 nymphs and 3 larvae. Approximately half of the collected ticks, a total of 120 females, 115 males, 27 nymphs, and 2 larvae, were tested. DNA was obtained via a Qiagen extraction kit. Samples were then tested by the Viral and Rickettsial Disease Laboratory (VRDL), CDPH, using a SFGR real-time PCR designed to amplify a region of the rOmpA gene (Eremeeva et al., 2003). All 264 *R.sanguineus* ticks were individually tested for SFGR and were PCR negative. California shares six ports of entry with Mexico, two of which are in Imperial County. Travelers, stray dogs and pet owners looking for low-cost veterinarian care in Mexico increase the potential of *R. rickettsia* positive ticks and dogs crossing the US-Mexico border. Although SFGR is rare in California, Rocky Mountain spotted fever is endemic in the south central United States. *Rhipicephalus sanguineus* can bite people and have been shown to be a vector of Rocky Mountain spotted fever (Eremeeva et al. 2011). Continued tick surveillance and control of stray dog populations are vital to minimize the risk of rickettsial disease transmission to people.

ACKNOWLEDGEMENTS

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Surveillance of Rodent-borne Pathogens in Northwestern Riverside County During 2003-2012

Greg A. Williams[1], Bill Van Dyke[1], Nate McConnell[1], Lal S. Mian[2] and Major S. Dhillon[1]

[1]*Northwest Mosquito and Vector Control District, 1966 Compton Ave., Corona CA 92881-3318*
[2]*Department of Health Science & Human Ecology, California State University, San Bernardino, CA 92407-2393*

ABSTRACT: As part of the rodent-borne disease surveillance program at Northwest Mosquito and Vector Control District, rodent trapping was carried out at 19 sites in northwestern Riverside County between 2003 and 2012. Of the 1,188 rodents trapped and sampled for blood, 3 tested seropositive for arenaviruses and 56 for hantaviruses. None of the rodents (California ground squirrels and wood rats) collected at 9 different surveillance sites tested positive for the plague bacterium, *Yersinia pestis*.

INTRODUCTION

The Northwest Mosquito and Vector Control District (NWMVCD) provides vector control services to ~400,000 residents within an area of approximately 245 square miles in the cities of Norco, Corona, Lake Elsinore, parts of the city of Riverside, Canyon Lake, and several adjoining unincorporated communities (Figure 1). The disease and vector surveillance program is part of the District's coordinated effort to service the community effectively by detecting and controlling vector-borne diseases in the area. Surveillance for hantavirus and plague has been carried out at the NWMVCD for over one decade. Rodents at selected surveillance sites are collected and bled and their serum tested for antibodies to plague or hanta and arena viruses.

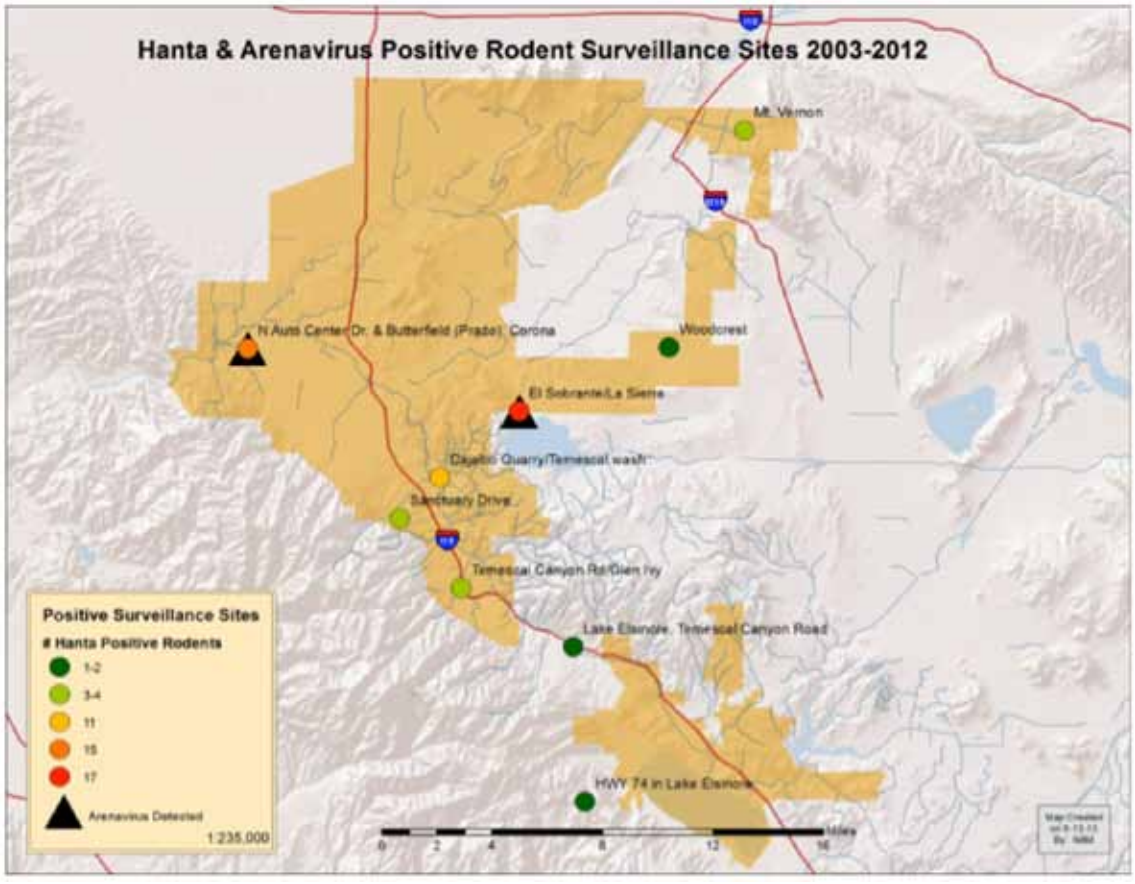

Figure 1. Map showing Northwest Mosquito and Vector Control District boundary along with sites found positive for arena and hanta viruses between 2003 and 2012.

Hantaviruses are responsible for two types of human diseases: the Hemorrhagic Fever with Renal Syndrome (HFRS) and the Hantavirus Pulmonary Syndrome (HPS). The latter type has been reported in the U.S. during the last two decades. The viruses are most commonly transmitted to humans through aerosolization of rodent excreta, but secondary aerosols, mucous membrane contact and skin breaches are also considerations. Nearly 200 cases of HPS have been reported in North America, whereas HFRS has proved to be very uncommon. A great number of New World rodent species, including deermice (*Peromyscus maniculatus*), wood rats (*Neotoma spp*.), voles (*Microtus spp.* and *Clethrionomys spp.*) and rats (*Rattus rattus* and *Rattus norvegicus*) have been found to have antibodies to hantaviruses. *Peromyscus maniculatus*, carrying the Sin Nombre virus, is responsible for the HPS cases in California and the western United States. Other hantaviruses may also be vectored by other rodent species; these include the El Morro Canyon virus carried by the western harvest mouse (*Reithrodontomys megalotis*) and the Isla Vista strain vectored by the California vole (*Microtus californicus*)(Bennet et al. 1999).

Arenaviruses are associated with rodent-transmitted diseases in humans worldwide. As with the hantavirus, arenavirus infections in humans result from inhalation of aerosols of rodent excreta or from direct contact of rodent excreta with open skin and mucous membranes. Human to human transmission may occur upon direct contact with infective fluids and fomites such as medical equipment. Ingestion of contaminated food with rodent excreta may also result in an infection. There are close to 20 known arenavirus strains belonging to two main serocomplexes,(Old World Lymocytic Choreomeningites [LCM]-Lassa and New World Tacaribe) (Buchmeier et al. 2001). Arenaviruses known to occur in North America include the LCM, White Water Arroyo (WWA) and Tamiami (TAM) viruses (Childs and Peters, 1993). The LCM virus, vectored principally by the house mouse (*Mus musculus*), causes mild flu-like symptoms leading to meningitis and/or encephalitis. The disease is usually not fatal but there are no specific treatments. Wood rats in the Southwestern United States are principal hosts of WWA virus, which is an agent of hemorrhagic fever in humans. The TAM virus transmitted by the hispid cotton rat (*Sigmodon hispidus*) in Florida causes Tamiami virus encephalitis.

In Southern California, antibodies to the Pichinde (PIC) and TAM viruses have been found in the dusky-footed wood rat (*Neotoma fuscipes*) and the desert wood rat (*Neotoma lepida*) (Kosoy et al. 1996). Antibodies to the Amapari (AMA) and/or WWA viruses have been reported in the desert wood rats, dusky-footed wood rats, brush mice, California mice, deer mice, cactus mice and harvest mice collected in the Los Angeles, Orange, Riverside, Northwestern San Diego and San Bernardino counties (Bennet et al. 2000a, 2000b; Mian et al. 2000). In 2002, Fulhorst et al. isolated a new arenavirus, named the Bear Canyon virus. This virus belongs to the Tacaribe serocomplex and was found in California mice collected in the Cleveland National Forest close to the Orange County and Riverside County line.

Plague was introduced into North America in 1900 when a ship from Hong Kong docked in San Francisco and released Norway rats carrying plague-infected fleas. Since then, 18 rodent species in California have been implicated in the epidemiological cycle of plague. The causative agent of plague, the bacterium *Yersinia pestis*, is maintained in wild rodents and other small mammals and transmitted within and among species by their fleas. The host species of plague include relatively resistant enzootic (maintenance) hosts and the susceptible epizootic (amplification) hosts. The enzootic hosts include *Peromyscus spp*. and voles; *Peromyscus maniculatus* and *M. californicus* are most significant in this respect (Davis et al. 2002). The epizootic host species include California ground squirrels, *Otospermophilus beecheyi,* wood rats and chipmunks (*Tamias spp*.). The ground squirrels and their fleas are most often associated with human plague cases in California (Nelson 1980).

In the present report, we summarize the data on rodent fauna and the pathogens they transmit in northwestern Riverside County during the past ten years, 2003 - 2012.

MATERIALS AND METHODS

In routine disease surveillance, small rodents including rats, mice and voles were trapped at 19

locations throughout northwestern Riverside County (Figure 1). Based on previous rodent surveillance studies at NWMVCD (unpublished data), locations with highest trap success for *Peromyscus spp.* are open fields containing some human refuse and scattered vegetation. For the present study, sites were selected based on these criteria.

In overnight surveys, 20 - 40 Sherman traps (3x3x10 in.) were used at different locations throughout the year (Figure 1). Each trap was baited with 3 g of rolled oats. Squirrels were trapped in Tomahawk live animal traps (7.6 x 8.9 x 22.9 inches) baited with peanut butter and rolled oats mixed together to form balls approximately 3.5 cm in diameter. Typically, 20 Tomahawk traps were set in the mid-morning and collected the same day in early afternoon.

All rodents were euthanized with carbon dioxide within hours after trap collection. The cardiac puncture technique was used to collect blood samples. For hantavirus antibody testing, whole blood samples collected from rats, mice and voles were shipped overnight to the Viral and Rickettsial Disease Laboratory, California Department of Public Health, Vector-Borne Disease Section (CDPH-VBDS). For arenavirus testing, blood sera were separated through centrifugation at 4500 rpm for 20 min and stored at -70°C until enough samples were accumulated for shipment. The serum samples were then shipped on dry ice for testing at the University of Texas Medical Branch, Department of Pathology, Galveston, Texas. For plague antibody detection, whole blood samples were adsorbed onto Nobuto blood filter strips and shipped to the Microbial Disease Laboratory, CDPH-VBDS for analysis during 2003 - 2007. Testing of samples for hantavirus and plague was performed by Orange County Vector Control District (OCVCD) beginning in 2006 and 2008, respectively, and have since been exclusively tested by OCVCD as of 2008.

RESULTS AND DISCUSSION

A total of 1188 rats, mice and voles were collected at 19 different surveillance sites between 2003 and 2012 (Figure 1). The most predominant species collected was *P. maniculatus* (405), followed by *N. lepida* (190), *Chaetodipus californiacus* (125), *O. beecheyi* (93), *N. fuscipes* (83), *P. californicus* (68), *M. californicus* (21), *R. megalotis* (16) and *M. musculus* (8) (Table 1). The abundance of *P. maniculatus* peaked (13/20 traps) in 2004, falling to 1 in 2007-2008, <1 in 2009-2010, and picking up to >2 in 2011 (Figure 2). Most other species showed a bimodal pattern of abundance, peaking in 2003-2005, dropping to near zero in 2006-2008, and then picking up from 2009 and onward. The dip in population abundance in 2006-2008 could partly be due to drought-like conditions during those years.

Among the 9 positive sites, 3 sites were responsible for more than three-quarters (75.4%) of the 57 positive specimens. Hantavirus activity started high (39) in 2003, 10 in 2004, 3 in 2006, and 2 and 2 in 2011 and 2012, respectively (Table 2). Hantavirus detected in 56/57 positive samples had two of these dually infected with arenavirus; one additional sample was positive

Scientific name	Common name	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
Chaetadipus californicus	California pocket mouse	47	36	0	1	0	0	7	9	13	12	125
Microtus californicus	California vole	4	6	8	0	1	0	0	0	2	0	21
Mus musculus	House mouse	2	1	2	0	0	1	0	0	1	1	8
Neotoma fuscipes	Dusky-footed woodrat	21	5	10	0	0	0	1	17	21	8	83
Neotoma lepida	Desert woodrat	58	32	17	0	0	11	11	23	16	22	190
Peromyscus californicus	California mouse	31	14	5	2	0	0	0	7	9	0	68
Peromyscus eremicus	Cactus mouse	22	14	0	13	9	22	12	39	21	27	179
Peromyscus maniculatus	Deer mouse	139	126	47	33	5	14	1	2	28	10	405
Reithrodontomys megalotis	Western harvest mouse	0	2	3	0	1	0	0	5	4	1	16
Otospermophilus beecheyi	California ground squirrel	57	16	12	1	0	3	1	3	0	0	93
	Total	**381**	**252**	**104**	**49**	**16**	**48**	**33**	**109**	**115**	**81**	**1188**
	Number of 20-trap events	23	10	5	6	4	9	3	10	11	7	
	Number per 20 traps	**16.6**	**25.2**	**20.8**	**8.2**	**4**	**5.3**	**11**	**10.9**	**10.5**	**11.6**	

Table 1. Numbers of rodent species trapped in rodent-borne virus surveillance in northwestern Riverside County, 2003-2012.

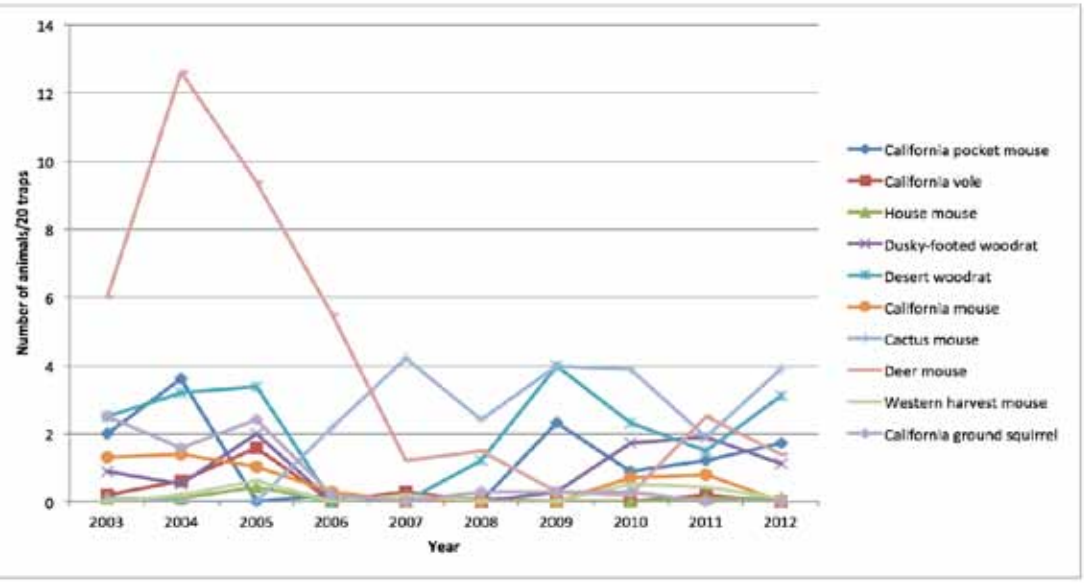

Figure 2. Abundance of rodent fauna collected in northwestern Riverside County, 2003-2012.

for only arenavirus. The dual infections occurred at two different sites (Prado and El Sobrante) both in *P. maniculatus* within a month of one another in the winter of 2003. The third arenavirus positive sample was also in a *P. maniculatus* from the same Prado site captured on the same night as the aforementioned specimen. Arenavirus was only tested in 291 samples yielding a detection rate of 1.03%.

Of the 21 *M. californicus,* 2 (9.52%) tested positive for hantavirus (Isla Vista). Sixteen *R. megalotis* were sampled over the 10 year period resulting in only one (6.25%) positive hantavirus sample (El Morro Canyon); the isolation was from the sole specimen collected in 2012. The following species tested positive for the Sin Nombre strain of hantavirus: *N lepida* (3.68%), *P. maniculatus* (8.89%), *P. californicus* (7.35%) and *P. eremicus* (2.79%). None of the 93 squirrels tested positive for plague, nor did any of the wood rats showed antibody to the plague bacterium.

Scientific name	Common name	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
Chaetadipus californicus	California pocket mouse	0	0	0	0	0	0	0	0	0	0	0
Microtus californicus	California vole	2	0	0	0	0	0	0	0	0	0	2
Mus musculus	House mouse	0	0	0	0	0	0	0	0	0	0	0
Neotoma fuscipes	Dusky-footed woodrat	0	0	0	0	0	0	0	0	0	0	0
Neotoma lepida	Desert woodrat	6	0	0	0	0	0	0	0	0	1	7
Peromyscus californicus	California mouse	3	0	0	1	0	0	0	0	1	0	5
Peromyscus eremicus	Cactus mouse	5	0	0	0	0	0	0	0	0	0	5
Peromyscus maniculatus	Deer mouse	23,3*	10	0	2	0	0	0	0	1	0	36, 3*
Reithrodontomys megalotis	Western harvest mouse	0	0	0	0	0	0	0	0	0	1	1
Otospermophilus beecheyi	California ground squirrel[+]	0	0	0	0	0	0	0	0	0	0	0
	Total	**39**	**10**	**0**	**3**	**0**	**0**	**0**	**0**	**2**	**2**	**56, 3***

*Arena virus positives

[+] all tested negative for the plague bacterium

Table 2. Rodent-borne virus surveillance in local fauna trapped in northwestern Riverside County, 2003-2012.

ACKNOWLEDGMENTS

We thank Chuck Fulhorst for testing the rodent blood samples for the arenavirus antibodies. We also greatly appreciate Steve Bennet, Bob Cummings and the late Jim Webb of the Orange County Vector Control District for professional cooperation and samples testing. Lastly, but not least, we thank CDPH-VBDS for testing our routine surveillance samples.

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Insecticide Resistance and Impacts on Successful Mosquito Control

Melissa Hardstone Yoshimizu

California Department of Public Health, Vector-Borne Disease Section,
850 Marina Bay Parkway, Richmond, CA 94804

SUMMARY

Insecticide resistance is a growing concern in the vector control community. Insecticide applications remain one of the most effective tools used to control mosquito populations, especially during disease outbreaks or in response to detection of pathogens in environmental samples or human cases. The rate of insecticide resistance evolution can be impacted by multiple factors such as those categorized as operational (mode of action of the compound, persistence of residues), biological (generation time, mating behavior) and/or genetic (stability of resistance gene, multiple resistance mechanisms). Understanding how insecticide resistance evolves and which factors are at play can help mitigate resistance to the limited number of compounds available specifically for vector control.

Insecticide resistance is a heritable trait that provides an individual the ability to survive doses of a toxicant that are usually lethal to a majority of individuals in a normal (susceptible) population (WHO 1957). This means that an individual mosquito having a random and rare mutation that allows it to survive an insecticide application will be able to propagate, carrying its genes into the next generation. As each generation undergoes exposure to the insecticide, natural selection will favor those individuals that carry the gene for resistance. Additionally, there are many types of resistance mechanisms (target site insensitivity, increased metabolism, and decreased penetration) that have evolved in response to exposure to various classes of insecticides (Brogdon and McAllister 1998).

Awareness of the presence of insecticide resistance in mosquito populations throughout California is an important step in determining which classes of insecticides may be the best to use under different scenarios (Figure 1).

Ideally, assessment of insecticide resistance status via routine resistance surveillance testing would allow for resistance management. Resistance management includes an ongoing cycle of prevention through education, detection through surveillance, action via best management practices and evaluation with bioassays. A resistance surveillance program as part of this management framework can provide: (1) Baseline data for planning and pesticide selection, (2) Detection of resistance at an early stage, and (3) Monitoring capabilities to determine the effectiveness of control strategies being implemented (Brogdon and McAllister 1998). A resistance management framework which includes surveillance can also allow control efforts to be increasingly proactive by knowing which insecticide tools are best to use in the area of concern; this allows control districts to direct their control efforts better, facilitating the best use of limited resources.

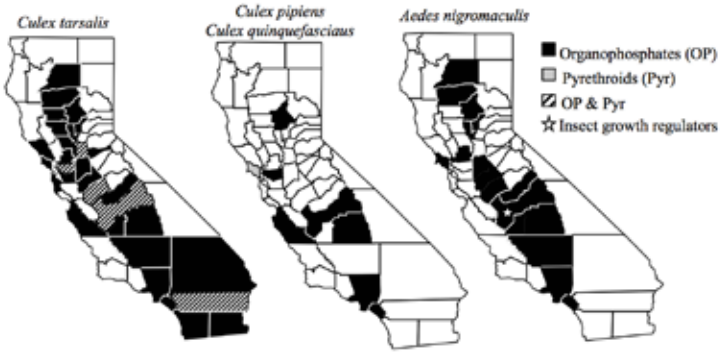

Figure 1. Insecticide resistance in mosquitoes from California.

ACKNOWLEDGEMENTS

Thank you to Tim Howard (CDPH, VBDS) for gathering historic data on insecticide resistance in California mosquitoes.

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Tough Mosquitoes – Why They Should Be Everyone's Problem

Marcia Reed, Paula A. Macedo, Gary Goodman and David Brown

Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA 95624, mreed@fightthebite.net

ABSTRACT: Periodic evaluation of insecticide susceptibility is part of the Sacramento-Yolo Mosquito and Vector Control District Integrated Vector Management Program and constitutes good pest management practice. In 2012 *Culex pipiens* populations from 13 locations were evaluated for susceptibility to two pyrethroids and one organophosphate insecticide. Mosquito populations with increased tolerance to permethrin and sumithrin were found in different areas in Sacramento and Yolo Counties.

INTRODUCTION

Insecticide resistance has been reported to every chemical class of insecticides (Brogdon and McAllister 1998a). Therefore, mosquito populations should be periodically evaluated for their susceptibility to the pesticides used in mosquito control programs. Current susceptibility data ensure timely decisions and should be considered when adjusting control strategies. Insecticide resistance may have severe implications in the mosquito control industry, such as limitations on products labeled or permitted for use in mosquito control, interference with the development of new products, and most importantly, the undesirable public health consequences of ineffective mosquito control in disease outbreak situations.

At the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD), the laboratory conducts periodic evaluation of insecticide susceptibility of *Culex tarsalis* and *Culex pipiens* populations in Sacramento and Yolo Counties. In 2012 the authors reported a population of *Cx. pipiens* from Orangevale, CA, which showed increased tolerance to permethrin (Reed et al. 2012). Further, it was shown that applications of pyrethroids or pyrethrins to that area for mosquito control had been infrequent and therefore not likely to have caused the tolerance levels observed. No increased tolerance has been observed in *Cx. tarsalis* populations in Sacramento or Yolo Counties. In 2013 the authors increased the number of *Cx. pipiens* populations tested throughout both counties to try to determine if the observed insecticide tolerance was an isolated occurrence, or if it was a widespread issue for the District.

MATERIALS AND METHODS

Study Areas. Mosquito populations from twelve urban and one rural site were evaluated (Figure 1). The urban sites were located in residential neighborhoods in either Sacramento County (ten sites) or Yolo County (two sites), and the rural site was in proximity to a dairy in Sacramento County.

Field Mosquito Populations. *Culex pipiens* populations were sampled by collecting gravid females using modified Reiter gravid traps (Cummings 1992). Gravid females were transferred to screened cardboard containers and allowed to lay eggs. The larvae were raised to the adult stage, and adults between three and five days old were subsequently evaluated using bottle bioassays.

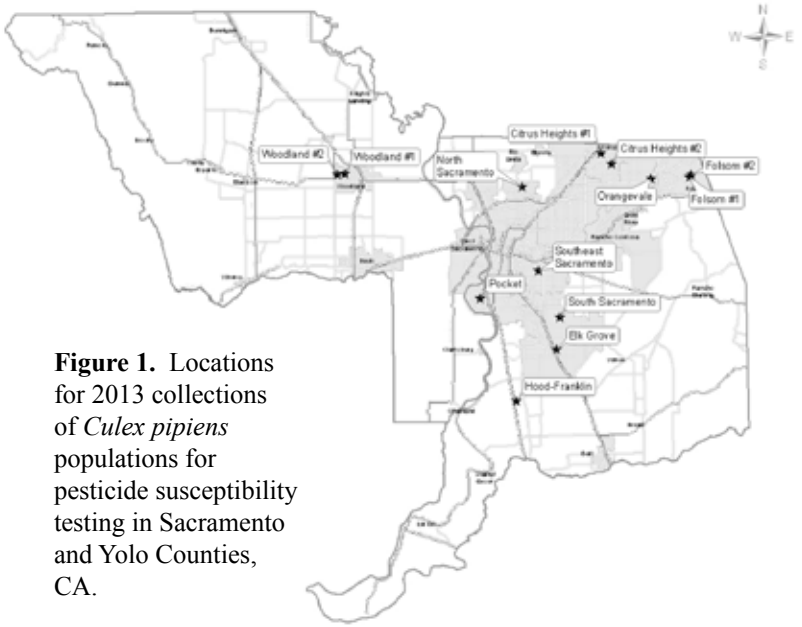

Figure 1. Locations for 2013 collections of *Culex pipiens* populations for pesticide susceptibility testing in Sacramento and Yolo Counties, CA.

Reference Colonies. The susceptible population used for comparison was the *Cx. pipiens quinquefasciatus* (CQ1) colony originally provided to our laboratory by Dr. Anthony Cornel at the University of California, Davis. This is the recommended reference colony for use in California according to the guidelines of the Mosquito Pesticide Resistance Monitoring Working Group. These guidelines were developed in 2008 to provide recommendations to the Mosquito and Vector Control Association of California (MVCAC) regarding the implementation of a pesticide resistance monitoring program. Susceptible mosquitoes used in the bioassays were approximately three to five days old.

Adult Bottle Bioassays. Mosquito populations were evaluated using the time-mortality method by Brogdon and McAllister (1998a, 1998b). In this procedure, glass bottles are dosed with a known amount of a pesticide, adult mosquitoes are introduced to the bottle and time to death is recorded. For each bioassay, four replicates of 25 adult females were used. Mortality was then recorded every 15 minutes for approximately three hours.

Resistance testing is part of SYMVCD's surveillance program and helps management make timely control decisions based on susceptibility data. Because of this, the products tested varied with the products that were used and needed in the field. In the beginning of the season, most of the bioassays were conducted using permethrin as a surrogate for any pyrethroid. As the season progressed, District personnel decided to evaluate the active ingredient that was actually being used in the field at that time, the pyrethroid sumithrin (d-phenothrin). Due to some low susceptibility issues observed in some field populations, some bioassays were then performed with the addition of piperonyl butoxide (PBO) to sumithrin to evaluate the effect that adding the synergist would have in those mosquito populations and to link the resistance to an oxidative mechanism to try to gain insight into the possible mechanism of resistance. For evaluation of the organophosphate class of pesticides, naled was used. Locations that were evaluated for each active ingredient are shown in Table 1. Doses for each active ingredient used in the bioassays are shown in Table 2.

Mosquito population	Naled	Permethrin	Sumithrin	Sumithrin and PBO
CQ1 susceptible	x	x	x	x
Woodland 1	x	x	x	x
Woodland 2	x		x	x
North Sacramento	x	x	x	x
Citrus Heights 1	x	x		
Citrus Heights 2	x	x		
Folsom 1	x	x		
Folsom 2	x	x		
Orangevale	x	x		
Southeast Sacramento	x	x		
Pocket	x	x		
South Sacramento	x	x		
Elk Grove	x	x		
Hood-Franklin		x		

Table 1. Locations of *Culex pipiens* populations and active ingredients tested.

Active ingredient	Dose (per bottle)
Permethrin	30 µg
Sumithrin (d-phenothrin)	22 µg
Sumithrin + PBO	22 µg + 22 µg
Naled	10 µg

Table 2. Doses (µg) of technical grade standards used per bottle for resistance testing in *Culex pipiens* populations.

Data Interpretation. Percent mortality at each time point was plotted, and response curves were generated. These response curves are not conducive to formal statistics, therefore testing and interpretation of results is based on comparison of field-collected populations with a susceptible reference colony over time.

RESULTS

Culex pipiens populations from 12 locations were evaluated for susceptibility to naled. As shown in Figure 2, 90 to 100% mortality was achieved by 60 minutes for 11 of the 12 populations; this was 15 minutes after 100% mortality was achieved in the susceptible CQ1 colony. *Culex pipiens* from Southeast Sacramento showed a slower response with only 74% dead at 60 minutes, but all mosquitoes were dead at 135 minutes. All populations tested had greater than 80% mortality within a reasonable time frame when exposed to naled.

Response curves varied greatly when mosquitoes from 12 locations were tested for permethrin susceptibility (Figure 3). The greatest response was from Hood-Franklin, with 94% of the mosquitoes dead at 60 minutes and 100% mortality at 105 minutes. This was the only rural population included in this study, and it remains susceptible to permethrin. We did not observe 100% mortality in any of the other 11 populations tested, even at 180 minutes. At 60 minutes the lowest mortality observed was from Woodland 1 (only 9%), and at 180 minutes only 79% mortality was observed. The lowest mortality observed at 180 minutes was from Folsom 1 (61%). Three *Cx pipiens* populations were used in bioassays with sumithrin and sumithrin + PBO (Figure 4). All three showed marked tolerance to these active ingredients at the doses evaluated. Percent mortality was affected by the addition of PBO (Table 3).

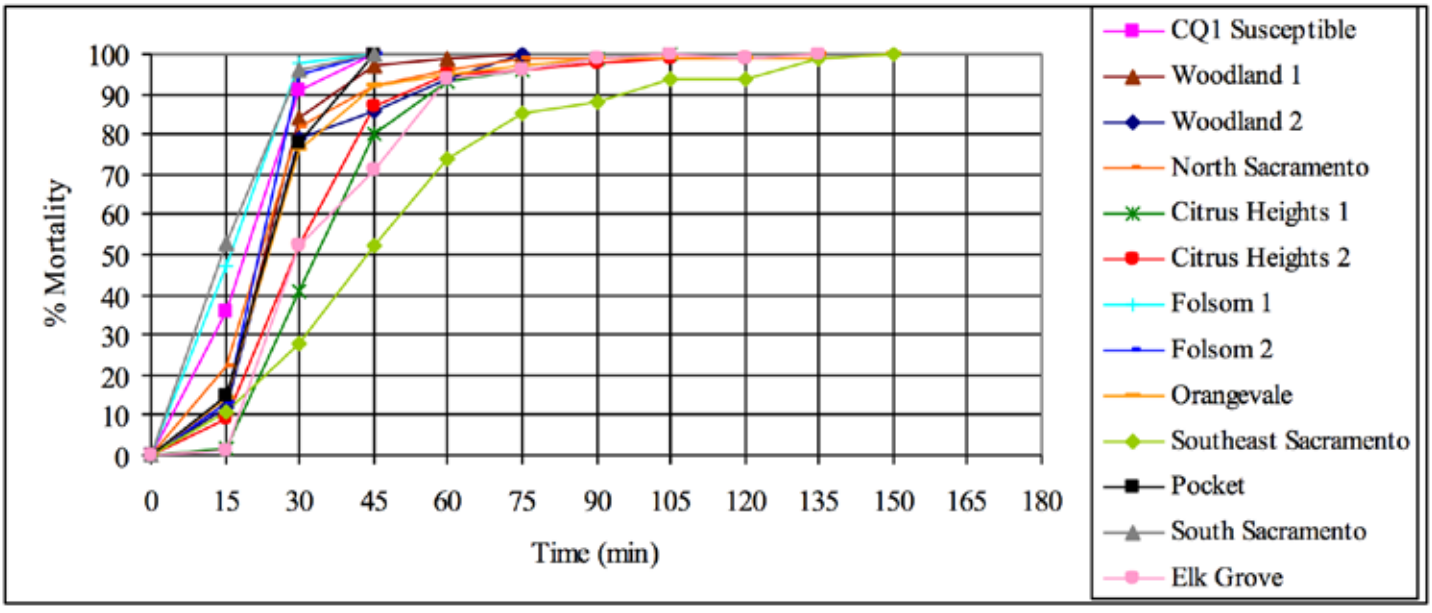

Figure 2. Percent mortality of *Culex pipiens* populations over time (minutes) in bottle bioassays using naled.

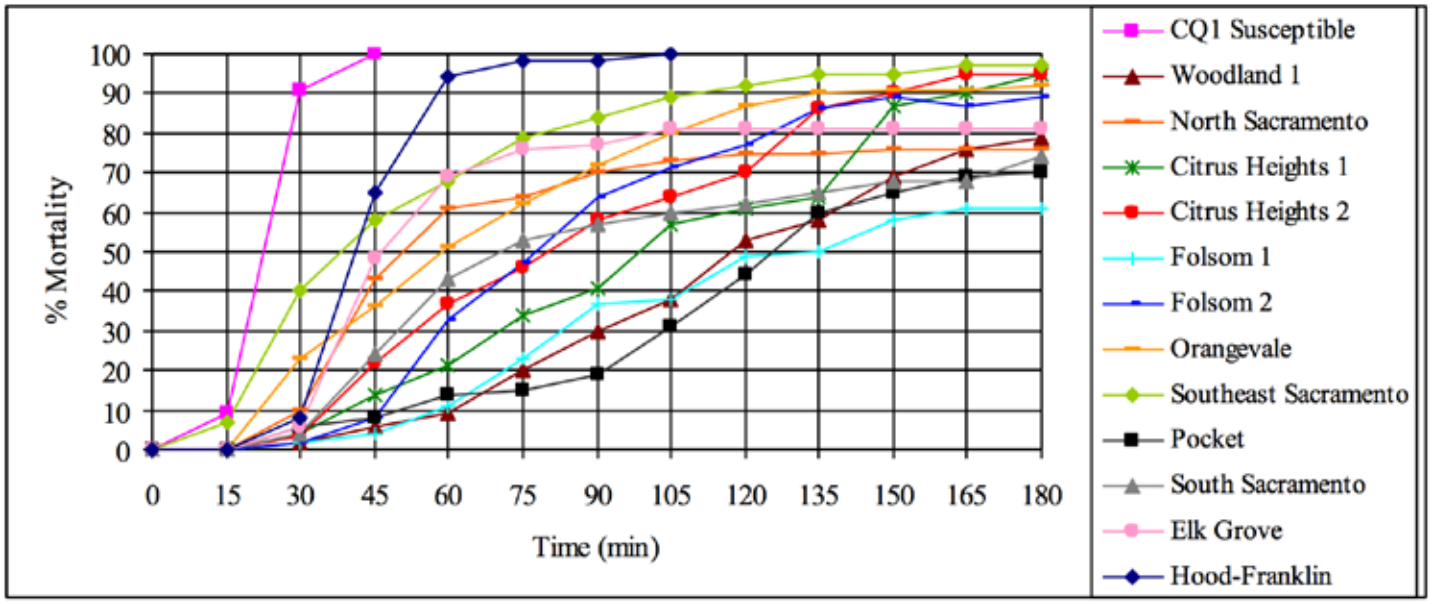

Figure 3. Percent mortality of *Culex pipiens* populations over time (minutes) in bottle bioassays using permethrin.

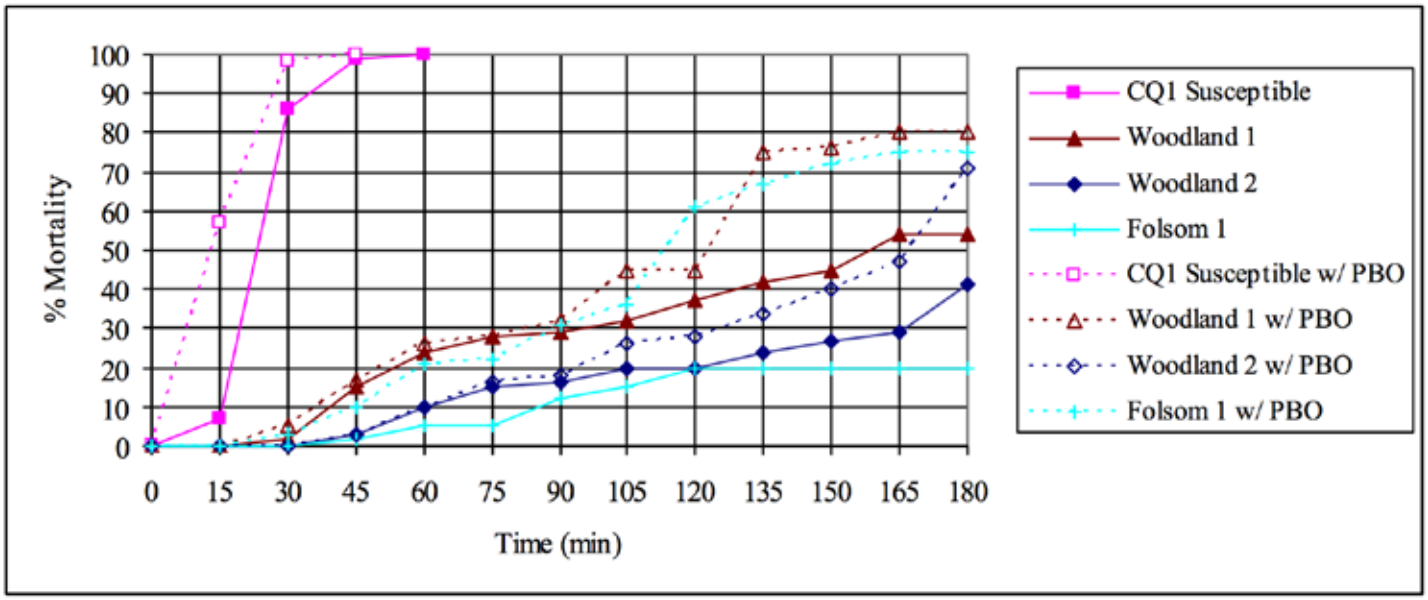

Figure 4. Percent mortality of *Culex pipiens* populations over time (minutes) in bottle bioassays using sumithrin with and without piperonyl butoxide (PBO).

Mosquito population	60 minutes		180 minutes	
	Sumithrin	Sumithrin + PBO	Sumithrin	Sumithrin + PBO
Woodland 1	24%	26%	54%	80%
Woodland 2	10%	10%	41%	71%
Folsom 1	5%	21%	20%	75%

Table 3. Percent mortality observed for three *Culex pipiens* populations tested in bottle bioassays for susceptibility to sumithrin with and without the addition of piperonyl butoxide (PBO).

DISCUSSION

In 2012 SYMVCD evaluated *Cx. pipiens* populations from 12 different locations in Sacramento and Yolo Counties, CA, against two pyrethroids and one organophosphate. Increased tolerance to pyrethroids was detected in all but one of the 12 populations. The levels of tolerance varied with location and active ingredient used. The addition of PBO to sumithrin resulted in increased mosquito mortality in the three populations tested, which may be an indication of an oxidative mechanism of resistance. As a next step the District will be evaluating these populations using microplate assays to confirm the mechanism of resistance, and it is currently investigating PCR-based diagnostic tests.

In addition to the data shown here, past mosquito control applications of pyrethroids in the same areas (data not shown) were also investigated to evaluate how much selection pressure our mosquito control applications were exerting on the mosquito populations evaluated. Applications were usually infrequent and should not have been a driving force for resistance in those mosquito populations. That fact is particularly significant for mosquito control programs because it would mean that, even if there were products available for mosquito control from a different chemical class that could be used to revert the resistance observed, the main driving force for resistance would still be unaffected because it is not from mosquito control. If true, this would render that class of chemicals ineffective in the fight to control mosquitoes and to interrupt transmission of the diseases they carry. Added to increasing regulations, potential loss of available chemicals and the general lack of availability of products for public health use can be devastating to the mosquito control industry and ultimately to public health.

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Results of Novel Surveillance/Education "Vector Inspectors" Program in Targeted Schools in *Aedes albopictus* Infestation Zone, San Gabriel Valley, CA

Kelly Middleton, Carol Anne Hagele, Tera Sorvillo and Kenn Fujioka

San Gabriel Valley Mosquito and Vector Control District, 1145 N. Azusa Canyon Road, West Covina, CA 91790

INTRODUCTION

Battling an *Aedes albopictus* infestation in a densely populated and ethnically diverse urban area presents many challenges. The San Gabriel Valley Mosquito & Vector Control District employed numerous surveillance and outreach tools to find infested properties and educate residents throughout the area. This paper describes a very successful and novel citizens' science project that trained elementary school-aged students to become 'vector inspectors' who, with family members, surveyed properties, collected larval samples and performed critical source reduction. While evaluating the efficacy of outreach programs is difficult and costly, through this program we were able to demonstrate effective learning that was transferred to family members and resulted in tangible behavior change.

The city of El Monte covers 9.56 mi^2 (6118 acres), contains more than 29,000 housing units and is home to 114,296 residents. The population of El Monte is ethnically diverse; only 16.2% speak English at home (US Census Bureau, 2012). *Aedes albopictus* was identified in El Monte in September of 2011 and shortly thereafter in South El Monte by the Greater Los Angeles County Vector Control District. Several isolated properties in the neighboring city of Arcadia and unincorporated Los Angeles County just north of El Monte were infested and treated successfully. Tremendous resources have been directed at eradication efforts since the initial discovery of *Ae. albopicatus* in the fall of 2011.

It soon became obvious that staff could not inspect enough properties each year to adequately address this infestation; we needed the public's help. Educating such a diverse populace was one challenge… motivating them to action was quite another. Since 52% of households in El Monte had school-aged children (US Census Bureau, 2012), the District developed an assembly program targeting elementary schools in and around the infestation zone. To be successful, the project had to:

1) *Motivate the students and through them, teach their families.*
2) *Bridge language barriers*
3) *Change behaviors*
4) *Help us identify additional infested properties*

The *Vector Inspectors Program* educated students about the problem and challenged them to become part of the solution. We created a science-based education and public health research project that appealed to students and educators alike. To convey detailed information effectively and ensure learning among students of various ages (kindergarten to 8th grade), learning styles and language abilities, we incorporated visual, auditory and kinesthetic elements into the program (Hawk 2007; Leite, Svinicki and Shi 2009). The following elements were important to the program's success.

The Vector Inspectors Machinima. The Vector Inspectors Machinima was developed to reach students of all ages and language abilities *on their level* and visually convey key elements of the project. A machinima is a video created using 3D virtual worlds or gaming platforms (such as Second Life) and is more flexible, less expensive and faster to produce than standard video or traditional animation. In the video, students follow Calvin and his family as they survey their yard for mosquitoes and use their Vector Inspector Kit to complete the activity. Younger students were mesmerized by the animation, music and buzzing mosquitoes while older students related to the 3D gaming-like visualizations and humor in the video. Machinima is a rapidly evolving technique that is finding an intriguing place in the traditional classroom setting.

A Hands-on Family Activity. This hands-on activity cemented the knowledge in the minds of the students, conveyed key public health information to their families (in their native/preferred language) and provided critical surveillance data to the District. Vector Inspector Kits were provided to each student and in a matter of weeks, the District's newly trained 'vector inspectors' and their families surveyed thousands of properties, collected mosquito samples and removed sources from targeted neighborhoods surrounding the infestation zone.

PROGRAM DESCRIPTION

The program was conducted in spring and fall of 2012. Schools were selected for participation based on proximity to the known or potential infestation zone. Some schools were asked to participate both times, while others only participated in spring or fall. School district superintendents and site principals were contacted, and 30-minute assemblies were scheduled for all students. Participation in the project was voluntary, however all students attended the assembly program and were given materials to take home. Each assembly began with a brief introduction into mosquito biology, a summary of the *Ae. Albopictus* infestation and its potential public health significance and a Vector Inspectors Program overview. Students then viewed a five minute machinima which visually illustrated how to complete the activity in a way that even the youngest students and English learners could follow. Each class was provided with a set of pre-labeled kits (name of the school, student, grade and teacher) and multi-lingual Invasive Asian Tiger Mosquito flyers.

Each kit contained a four ounce plastic soufflé sample cup and lid, a plastic transfer pipette, checklist, refrigerator magnet and a vector inspector sticker badge placed into a re-sealable plastic bag. During the assembly, students were taught what to look for and how to collect a water sample, provided with kits to take home and asked to survey their yards with an adult family member in attendance. Students recorded what type and how many different sources they found and wrote the information on the illustrated Vector Inspector checklist. They also placed samples of water (with or without visible larvae) from around their home into the collection cup. The checklist provided instructions and tips for eliminating any sources found.

Collection bins were left in common areas at each school site and the kits and checklists were collected approximately three to five days after the assembly. In the District laboratory, kits were sorted, samples identified and results entered into a database for analysis. Detailed summaries were compiled for each class describing what the students found, and a four color illustrated Key to Sample Results was provided to extend the learning experience. Each participating student was rewarded with a 5x magnifying viewer, and the teacher at each school with the highest level of student participation received a $50 gift card to enhance their science programs.

Program flyers, teacher prep materials, the Vector Inspectors machinima, and project summary can be viewed at: http://www.sgvmosquito.org/teach_index.html.

RESULTS

During the spring of 2012, nine schools participated in the Vector Inspectors Program. In the fall of 2012, seven schools participated; four were repeat schools from the spring effort. A total of 7,562 students attended assemblies and were provided program materials and 2,006 (27%) participated (Table 1). Participation was defined as collecting and turning in a water sample and/or a completed checklist. Participation rate among schools varied, ranging from 11% to 77%. Of participants, 1,524 (76%) submitted a water sample, and 355 (23%) of these samples contained mosquitoes. The distribution of participating students during the spring and fall programs in relation to known *Ae. albopictus* infested properties at their respective times is shown in Figure 1. None of the spring 2012 samples contained *Ae. albopictus* larvae. Thirteen samples submitted during the fall program were positive for *Ae. albopictus*.

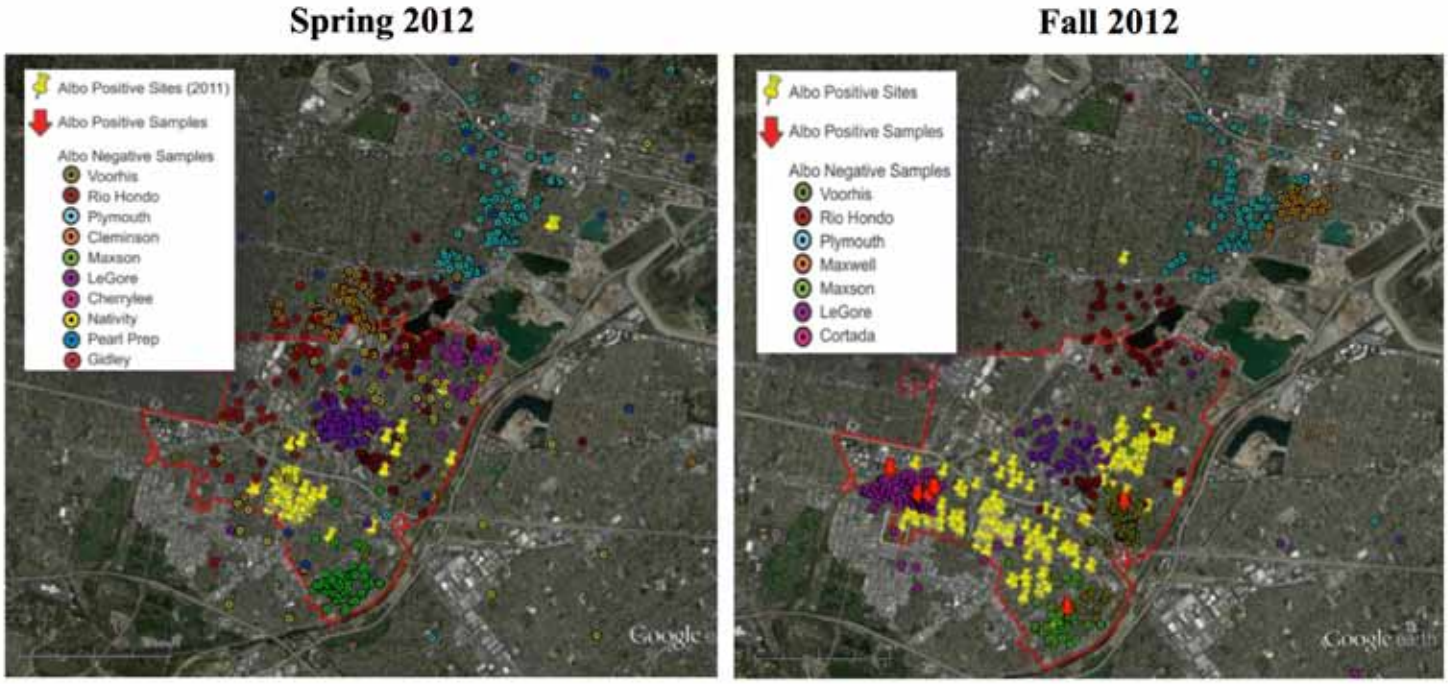

Figure 1. Distribution and results of samples submitted by program participants in relation to known infested properties in and adjacent to the City of El Monte (red border). Schools along the periphery of the known infestation were selected for participation.

Vector Inspector Program	# Schools	Enrollment	Student Participation	# Student Samples Submitted	% Student Samples with Mosquitoes	REPEAT SCHOOLS % Samples with Mosquitoes	REPEAT STUDENTS % Samples with Mosquitoes
Spring	9	3,755	4,052 (28%)	763	26%	33%	31%
Fall	7	3,807	954 (25%)	761	20%	17%	14%
Total	16	7,562	2,006 (27%)	1,524			
% Change					-23%	-48%	-54%

Table 1. Participation levels among students during spring and fall programs. Schools and students that participated both times submitted fewer mosquitoes in fall, verifying program efficacy.

To determine if the students successfully grasped the information we provided and were able to identify potential sources and collect immature mosquitoes, we compared the percent of student samples containing mosquitoes with the average percent of inspections positive for immature mosquitoes (expected level) reported during the same time period by our field technicians. Students successfully submitted larvae in about the same proportion as would be expected. When the same test was applied to schools individually, the results were more varied. Some schools submitted many more samples positive for mosquitoes than expected, while others submitted far fewer. Numerous studies indicate that economic variables can impact mosquito populations as well as disease risks (Reisen 2008, Harrigan 2010). Additionally, these programs were taught in English which might have impacted students' understanding of the instructions and affected results observed.

To determine if such factors may have contributed to the variability, schools were ranked using two indicators: 1) percent of students receiving free or reduced-cost lunches, and 2) percent English proficiency among students (Great Schools 2013). These two indicators were use to analyze the variability from expected larval submissions (Figure 2). While there did not appear to be a correlation between income and/or English proficiency and larval submissions, there was more variability in schools at the lower end of the two scales.

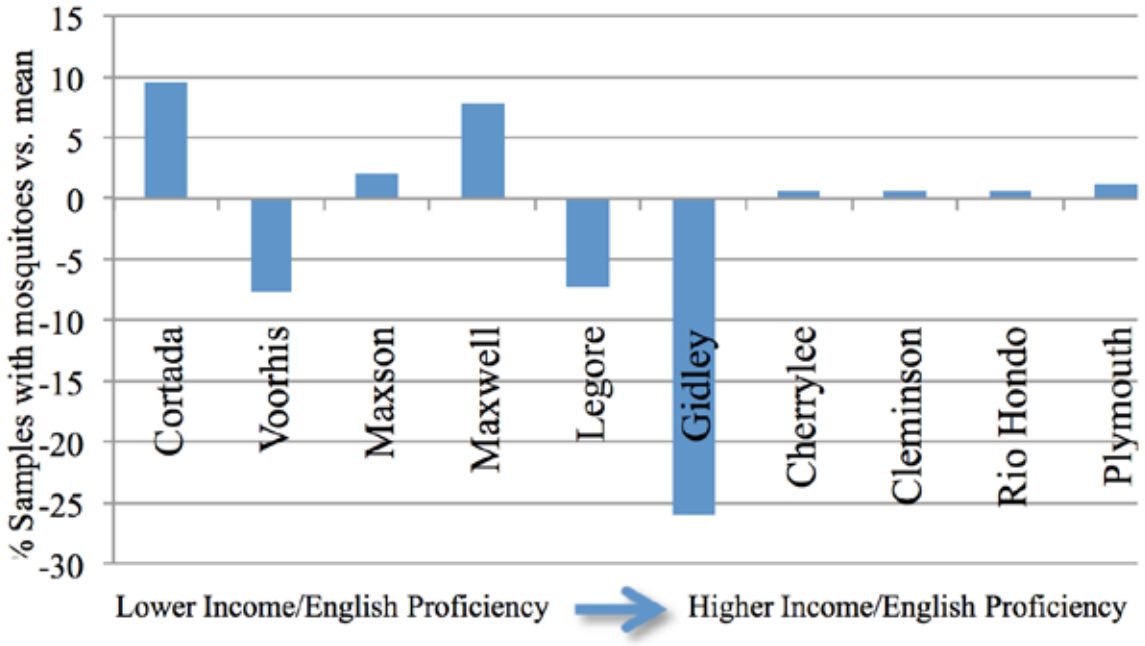

Figure 2. Do demographics matter? Schools were ranked by income and language indices and the percent of samples submitted containing mosquitoes compared to that seen throughout the District during the same time period.

Parent participation in this activity was recorded based on whether parents signed the checklist. An impressive 78% of student's checklists contained parent signatures, and approximately 80% also provided their contact information. Many of the forms, especially in the lower grades, were filled out by the parents. Between 15% and 43% provided email addresses. Parent participation was also evaluated using the above criteria and only a slight correlation between parent participation indicators and income levels and/or English proficiency was seen with the exception of email addresses which increased steadily as income and English proficiency levels rose. Attempts to evaluate the number and type of sources found on each property (via the checklist) were unsuccessful as the forms were not interpreted and filled out consistently.

To evaluate how effective the program was at changing behavior, we looked specifically at those schools that participated in both the spring and fall programs (4 schools, ~ 2,400 students). The overall percent participation decreased 31% from spring to fall; however, a higher percentage of participating students submitted samples the second time. Of those, proportionally fewer samples contained mosquitoes compared to that seen in the District during the same time periods. Among students at the four schools that participated both times (paired samples), the percent of samples containing mosquitoes dropped an impressive 54% from spring to fall.

Looking specifically at each of these four repeat schools, income and English proficiency levels may have contributed to the variation from expected levels of mosquito sample submissions. The two schools that scored highest on income and English proficiency scores had fewer samples containing mosquitoes than would be expected, whereas the lower income/lower English proficiency schools recorded breeding similar to expected showing no improvement between collection periods.

DISCUSSION

A unique program designed specifically to educate residents in and around a known infestation zone, motivate behavior change *and* increase the District's knowledge about the current infestation was highly successful on many fronts.

Timing and Seasonality. The spring program conducted from May 14 through June 5, 2012 was at the end of the school year and incidentally coincided with the seasonal re-emergence of *Ae. albopictus* in the

infestation zone. The ecology of this mosquito in El Monte was unknown at the time, but populations were found by District staff to be extremely low when the students were collecting. Additionally, schools invited to participate in spring were along the periphery of the known infestation zone, likely accounting for the lack of *Ae. albopictus* mosquito collections. Schools in the San Gabriel Valley finish the school year in early to mid-June. Conducting this program in spring put considerable time constraints on staff to get results and participation rewards back to students before the end of the school year and yielded little information with regard to the *Ae. albopictus* population.

The fall program (October 12 through November 15) was successful in identifying new properties infested with *Ae. albopictus* in an area adjacent to the primary infestation zone, but the timing made it difficult to act on this knowledge before the population declined due to seasonal factors. Scheduling programs earlier in the fall term will eliminate this difficulty and make the program more valuable as a surveillance tool.

Workload. The cost of materials to conduct this program was minimal; however, significant staff hours were required to assemble the kits, identify and record sample contents, analyze the results and report these findings to individual classrooms. Future analysis of this program will evaluate its fiscal efficacy. Students appeared able to inspect effectively thousands of properties in a very short time period and provide the District with important information about the extent and potential spread of the *Ae. albopictus* infestation. Targeting schools outside the known infestation zone will generate hundreds to thousands of samples and accomplish education and source reduction goals, supplementing efforts of control and surveillance staff and allowing them to reprioritize their schedules to address infested properties more effectively.

Evaluating Success. Determining the efficacy of outreach programs is inherently difficult and often requires costly surveys. We were able to reach thousands of students successfully and through them their families, thereby bridging the language barrier. This allowed the students and their families to inspect thousands of properties in a very short time period, eliminate standing water sources, change behavior and document a reduction in mosquito populations in yards of participating students. The results are summarized below:

1. This program educated more than 7,500 students and motivated 2,006 (27%) of them to volunteer to go out into their yards with family members to look for mosquitoes and remove standing water sources.
2. Students were successfully able to identify and collect mosquito larvae in proportion to their estimated abundance in the environment.
3. Results from schools that participated twice (spring and fall) showed the direct benefit of the program. The reduction in mosquito samples submitted was 13% greater than expected.
4. Individual students that participated in the program both times showed a 54% reduction in mosquitoes collected from their yards, 19% more than expected.
5. Students identified 13 additional properties infested with *Ae. albopictus* in an area District staff had not yet had time to revisit.

Our analysis shows that students understood the materials, could provide valuable surveillance data, and successfully motivated behavior change in their families. Further evaluation of this program will look closer at the data to determine if these results are statistically significant and consider alternatives to program implementation allowing participation by schools throughout the San Gabriel Valley and other interested groups such as scouts or individual residents.

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Predicting the Spread of *Aedes albopictus* in Los Angeles

Christopher M. Barker[1], Mary E. Danforth[1], Susanne Kluh[2], Kenn Fujioka[3], Tera Sorvillo[3], Harold Morales[2] and Tanya Posey[2]

[1]*Center for Vectorborne Diseases, University of California, Davis, CA, 95616, cmbarker@ucdavis.edu, Greater Los Angeles County Vector Control District[2], 12545 Florence Avenue, Santa Fe Springs, CA 906703, San Gabriel Valley Mosquito and Vector Control District, 1145 N. Azusa Canyon Road, West Covina, CA 91790*

INTRODUCTION

Aedes albopictus is a vector of global health concern because it bites humans and is a competent vector of many arboviruses, including Dengue, West Nile Virus, and Chikungunya (Gratz 2004). This species invaded California repeatedly during the last four decades (Kluh et al. 2002, Linthicum et al. 2002, Linthicum et al. 2003, Tietze 2003), including a recent infestation detected during late 2011 in the cities of El Monte and South El Monte in Los Angeles County. *Aedes albopictus* has already established populations in similar climates in southern Europe (Mitchell 1995, Aranda et al. 2006, Carrieri et al. 2011, Munnoz et al. 2011, Roiz et al. 2011). The aim of this study was to develop spatial models that simulate adult mosquito reproduction and movement between locations. These aspects of invasion ecology are particularly important for targeting control of *Ae. albopictus* as well as other invasive species because of the potential for spread beyond the containment area or repopulation from neighboring areas following successful local control (Richards et al. 2008). Here we describe the first year of the study in which we developed a model for receptivity to *Ae. albopictus* based on climatic and edaphic covariates. We then used the model to compare the potential for establishment following introduction via eggs or adult mosquitoes.

MATERIALS AND METHODS

The study area included the incorporated cities of El Monte (San Gabriel Valley MVCD) and South El Monte (Greater L.A. County VCD), which have been the foci of surveillance and detection of *Ae. albopictus* since its discovery in 2011. Census blocks (n = 1,148) were chosen as an appropriate scale for modeling receptivity and movement because they provided fine resolution of habitats, but were coarse enough to make the computations for mosquito movement feasible. Data used to generate the receptivity model consisted of all mosquito collection efforts by the respective vector control agencies since late 2011, with presence defined as a detection of *Ae. albopictus* at any time since surveillance began. A logistic regression model was constructed to model the probability of *Ae. albopictus* presence using the mosquito collection data, climatic variables, housing density and land use. The resulting probability surface was used to weight the probabilities of *Ae. albopictus* movement among census blocks, with greater probability of moving to parcels with higher probabilities of presence.

For each day in the movement model, mosquitoes randomly stay within a block or move to one of the eight nearest blocks, with probabilities depending on model-based receptivity as described above and distance, with movement to nearer blocks more likely than distant ones. Females alternate between blood-feeding and egg-laying, and eggs hatch and mature according to literature-based biological parameters governing biting frequency, immature development, fecundity and survival of eggs and adults (Hawley 1988 and references therein). Movement patterns generated by the model were validated by confirming that model-based dispersal distances matched those of mark-recapture studies.

RESULTS AND DISCUSSION

The best-fit model for receptivity predicted *Ae. albopictus* presence based on the existence of medium-intensity single-family housing within a census block, with higher housing densities having a higher probability of presence. This model was used to define probabilities of movement for all census blocks within the study area, and initial results showed that introduction of a single adult female mosquito would have a much lower probability of establishment compared to introduction via a batch of 20 eggs (e.g., in a container) due to a lower probability that any single female will survive to lay eggs (Figure 1).

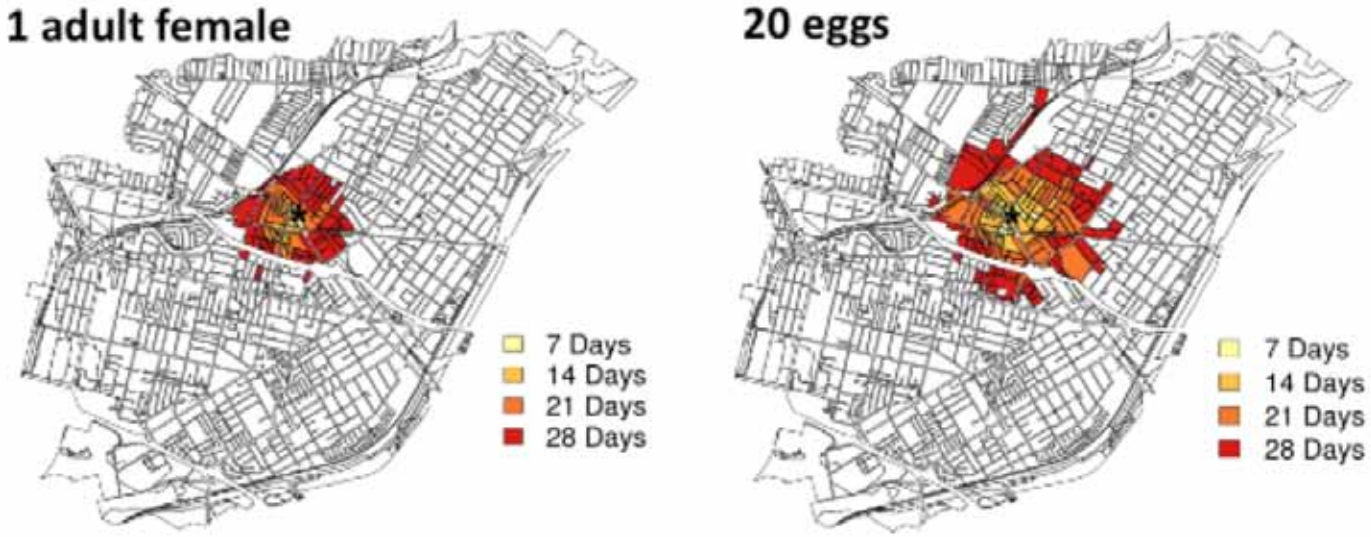

Figure 1. Single simulations from the stochastic movement model that show the spatial extent of spread following a single introduction of a single adult female (left panel) or a batch of 20 eggs (right panel) on day 0. Due to the random nature of mosquito survival, introductions of single females frequently fail to become established.

We are in the process of updating the receptivity model using additional surveillance data collected during late 2012 and early 2013, and this model will be used to inform the final movement simulation model. Ongoing research will use this model to estimate *Ae. albopictus* spread rates to define an appropriate surveillance buffer radius, and we will use the model to compare the efficacy of control methods for slowing spread and eradicating *Ae. albopictus*. Results will also provide useful information in the event of future invasions of *Ae. albopictus* or similar container-breeding species.

ACKNOWLEDGMENTS

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Survey Results from State Health Departments on Endemic Flea-borne Typhus

Matthew Reighter[1], Laura Krueger[2], Sokanary Sun[2], Michele Cheung[3] and Robert Cummings[2]

[1]*Orange County Environmental Health*
1241 E. Dyer Road, Ste. 120, Santa Ana, CA 92705
(714) 433-6091 email: mreighter@ochca.com

[2]*Orange County Vector Control District,*
13001 Garden Grove Blvd, Garden Grove, CA 92843
(714) 971-2421 e-mail lkrueger@ocvcd.org

[3]*Orange County Health Care Agency*
1719 W. 17th Street, Santa Ana, CA, 92706
(714) 834-7729

ABSTRACT: In recent years the Orange County Vector Control District (OCVCD) has been faced with the reemergence of flea-borne typhus. In 2012 alone, 32 possible human cases of flea-borne typhus (including suspected, probable or confirmed cases) were investigated by OCVCD's flea-borne typhus program, with a total of 85 investigated since 2006. The program's response to human cases of disease has included case investigations and providing public education through door-to-door campaigns or flyer distribution near disease cases. Specifically for case investigations, the steps taken include patient interview, environmental assessment of the exposure site, opossum trapping near the exposure site, flea collection and testing for the presence of *Rickettsia* spp. bacteria in flea and opossum specimens. In an attempt to evaluate and improve OCVCD's surveillance, response and prevention efforts, a survey was developed to understand the prevalence of flea-borne typhus nationally while aiming to identify other response programs implemented in endemic areas. The results of this survey have provided invaluable information for assessing the current roles of state health departments in case investigations of flea-borne typhus, while painting an overall picture of the extent of flea-borne typhus.

INTRODUCTION

Murine typhus, or "endemic" flea-borne typhus, was reported throughout the United States from 1919 to 1950. Subsequently, with the adoption of rodent control practices, the number of human cases began to decline significantly. As of 1993 the Centers for Disease Control (CDC) no longer listed murine typhus as a national reportable disease. After a thorough review of state health department websites, it appears that several state health departments (SHD) have followed suit; many states no longer list the disease on their notifiable disease list. Recently, however, flea-borne typhus has reemerged as a public health threat in several areas of the United States. It has resurfaced through an alternative transmission cycle that involves a newly-recognized infectious agent, *Rickettsia felis*, in conjunction with the cat flea, *Ctenocephalides felis*, as the vector and the opossum (*Didelphis virginiana*) and domestic cat (*Felis catus*) as vertebrate hosts (Sorvillo et al. 1993, Azad et al. 1997, Eremeeva et al. 2012). The transmission cycle of cat fleas – opossums/cats – humans (cat flea typhus) exists in many areas of the United States including Southern California and Texas (Boostrom et al. 2002, Reif and Macaluso 2009) and is different from the classic murine typhus transmission cycle of rat fleas (*Xenopsylla cheopis*) – rats (*Rattus* spp.) – humans with *R. typhi* as the etiologic agent (Dyer 1944, Civen and Ngo 2009). Human cases of flea-borne typhus in the U.S. are primarily reported from Texas, Southern California and Hawaii, and since 2000 each of these states has had increases in the number of cases.

The Orange County Vector Control District (OCVCD) is tasked with preventing cases of vector-

borne disease. In 2006, after a 15-year absence of human cases, flea-borne typhus returned to Orange County, California. Over the next six years, OCVCD investigated 85 suspected, probable or confirmed cases. During the investigation process, OCVCD employed both reactive and preventative measures for the purpose of inhibiting further transmission within adjacent communities. In order to evaluate the effectiveness of the OCVCD's Typhus Program and the role of local vector control districts in addressing flea-borne typhus cases, SHDs were contacted to understand better the prevalence of flea-borne typhus in the United States. The objectives of the survey were:

1) To identify those states that list flea-borne typhus as reportable.
2) To assess the national distribution of flea-borne typhus cases.
3) To compare case definitions for identifying flea-borne typhus cases, including infectious agent, transmission cycle and antibody titer thresholds.
4) To determine the role of state agencies in the response, surveillance and control of flea-borne typhus.
5) To identify local agencies that have reported cases within the last year.

METHODS

An electronic web-based survey was developed to determine which states required reporting of flea-borne typhus cases, whether the SHD had received cases within the last 5 years, the number of cases reported to the SHD in 2010 and 2011, the case definition used for reporting cases and the role SHDs take in the response to cases. Prior to distribution, the survey was reviewed by the California Department of Public Health and Orange County Health Care Agency to ensure clarity of the questions and content applicability. SHDs across the United States were contacted via email, and a link to the survey was provided within the email. SurveyMonkey® was used for the collection and distribution of the survey. Contact information for either state vector ecologists or state epidemiologists for each of the SHDs was collected through an extensive internet search of each department's website. If adequate contact information was not provided on the website, calls were made to available phone numbers listed on the state website. The link remained open and active for a month to allow for data collection. After a month, follow-up calls were made to SHDs that had not responded to the survey, and an additional email was provided with the link, if requested. Ninety-four percent (47/50) of SHDs completed the survey in its entirety.

RESULTS AND DISCUSSION

As previously mentioned, the CDC de-listed typhus from the national list of reportable diseases in 1993; subsequently, several SHDs followed suit as indicated by a thorough search of SHD websites which showed that only 42% (21/50) of states listed typhus as reportable. However, of the 47 states that responded to our survey, only 15 (32%) indicated that flea-borne typhus was reportable in their state. Potentially, the three states that failed to respond to the survey accounted for a portion of the observed difference, but there were at least another three states that listed typhus as reportable on their website but did not require flea-borne typhus reporting per their response to the survey. For those respondents indicating that flea-borne typhus is not reportable in their states, this signaled the end of the survey, and no further information was collected for those states.

An attempt was made to understand better the extent of flea-borne typhus nationally by asking respondents if cases had been reported from 2007-2011 in their states. More specifically, respondents were asked how many cases were reported in the calendar years of 2010 and 2011. Sixteen (two of which indicated in the survey that flea-borne typhus is not reportable to the SHD) of the 47 (34%) respondents indicated that they had received reports of cases from 2007 to 2011. Of those 16 states, Texas reported the highest number of cases with 135 and 286 for 2010 and 2011, respectively; the increase between years was 112%. Texas represents, on average, nearly 75% (421/565) of the reported cases annually in the U.S. After Texas, the number of cases reported annually dips significantly as California, Hawaii and Ohio account for 18% (101/565), 4% (24/565) and 2% (13/565) of the reported cases, respectively, with the remaining states representing only 1% (6/565) of all cases. Table 1 provides a complete listed of SHDs that reported cases in 2010 and 2011.

		Calendar Year		
		2010	2011	Totals
Reporting State	Texas	135	286	421
	California	50	51	101
	Hawaii	10	14	24
	Ohio	7	6	13
	Illinois	1	2	3
	Michigan	1	0	1
	Wisconsin	1	0	1
	Indiana	0	1	1
	Totals	205	360	565

Table 1. Reported Human Cases of Flea-borne Typhus by State, 2010 and 2011.

In an attempt to control for varying case definitions from state to state, we asked the 15 states with flea-borne typhus to report on various aspects of their case definition in order to elucidate the variation that may occur without a national standard for case definition. Respondents were asked to provide information on how flea-borne typhus is classified on their reportable disease list, the causative agent typically implicated and the antibody titer thresholds used to establish cases. Six of the responding SHDs (40%) indicated that flea-borne (murine) typhus is listed exclusively on their reportable disease, while the remaining nine (60%) indicated that endemic (or murine) typhus is grouped with other rickettsial diseases. All of the respondents with the exception of two, who opted to skip the question, specified that *Rickettsia typhi* is a causative agent for flea-borne typhus according to their case definition. In addition to *R. typhi*, *Rickettsia felis* was identified specifically as a causative agent in the case definition by two (13.3%) of the respondents, while *Rickettsia prowazekii* was specifically identified by two (13.3%) of the respondents. Moreover, two of the respondents (13.3%) indicated that all *Rickettsia* spp. are potential causative agents for their case definition. Titer thresholds with regards to IgG or IgM reactivity for establishing a case varied among respondents as six (40%) and three (20%) identified a titer threshold of 1:64 and1:128, respectively, while six (40%) responded "unsure/not established/failed to answer the question."

Lastly, we assessed the role that SHDs take in the response and investigation of flea-borne cases nationally by asking respondents to identify the different activities implemented by the SHD and available literature they may provide to either assist local agencies or the public. All of the respondents indicated that they performed some form of data collection and consolidation of cases in preparation for year-end totals and/or reviews. Additionally, several SHDs indicated that they perform some form of active response through case investigations (73.3%), public outreach or notification (56.7%) or surveillance or follow-up activities (20%). Conversely, only four respondents (26.7%) stated that they have developed educational materials to promote public awareness of flea-borne typhus. Of the four SHDs that have developed materials, all of them provide the material electronically, while only one provides hard copies of the material on a regular basis.

CONCLUSION

Although 37% of SHDs (16/47) indicated receiving reports of flea-borne typhus cases within the last 5 years, only 9% (4/47) of SHDs have developed educational materials for public outreach and/or to aid local health departments in response efforts. In addition, the lack of comparable case definitions from state-to-state, whether it's the difference in titer thresholds or implicated causative agent, makes comparisons of case counts difficult. Therefore, given the recent increase in flea-borne typhus cases in certain areas of the U.S. and the changing ecology of the vectors involved in the transmission cycle, additional surveillance may help

better elucidate the etiologic agent(s), specific vectors and hosts involved, while providing evidence for a comprehensive case definition.

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Changing Epidemiology of Flea-borne Typhus in California

Charsey Cole Porse

California Department of Public Health, Center for Infectious Disease, Vector-Borne Disease Section, Sacramento, CA 95814, charsey.porse@cdph.ca.gov

INTRODUCTION

Murine typhus, also known as endemic or flea-borne typhus, historically occurred across the United States with large outbreaks between 1931 and 1946. Transmission of the typhus-causing bacteria, *Rickettsia typhi*, existed in an urban cycle between rats (*Rattus rattus*) and the oriental rat flea (*Xenopsylla cheopis*) (Azad 1990). Today few cases of typhus are reported annually, and they are predominantly clustered in areas of Hawaii, Texas and California (Eremeeva et al 2008, Boostrom et al. 2002). The epidemiologic triad for disease causation has likely expanded to include *Rickettsia felis* as an additional disease causing agent, opossums as reservoirs or mechanical vectors and the cat flea (*Ctenocephalides felis*) as both a possible reservoir and vector of *R. felis (*Beck and Van Allen, Adams 1970, Azad et al. 1997, Sorvillo et al. 1993, Civen and Ngo 2008, Eremeeva et al. 2012). Public health surveillance for human typhus cases is also changing as case definitions are established and diagnostic test are developed and improved.

DISCUSSION

Between 1916 and 1948, 504 cases of typhus were reported to the California Department of Public Health (CDPH). The majority of these cases (319) were from Los Angeles, San Diego (82), and Orange (26) Counties (Beck and Van Allen 1947, 1950). The use of pesticides and rat control measures post World War II drastically reduced the number of typhus cases reported in California and limited the distribution of cases to small suburban pockets in Los Angeles and Orange counties (Figure 1) (Adams et al. 1970, Civen and Ngo 2008). Recent increases in reported cases since 2009 prompted an epidemiological comparison of historic cases (1916-1948) and cases over the last decade. Between 2001 and 2012, 335 cases of typhus were reported to CDPH, with over a threefold increase in cases since 2010. In addition to the recent increase in cases reported, cases are more limited geographically, and seasonality trends have changed when compared to historic data.

Figure 1. Distribution of typhus cases in California, 2001-2012.

Historically (1916 – 1948), the reported typhus cases peaked in the fall (October), decreased in winter to a nadir in spring (May-April) (Beck and Van Allen 1947, 1950). Current reported onset dates of typhus do not demonstrate this seasonality; there is no single monthly peak; rather a prolonged elevated incidence from May through January, with the fewest cases reported February – April (Figure 2). These changes in incidence, distribution, and seasonality support the idea that the ecology and epidemiology of typhus may be changing.

Potential exposure for the 335 case patients reported to CDPH between 2001 and 2012 included exposure to

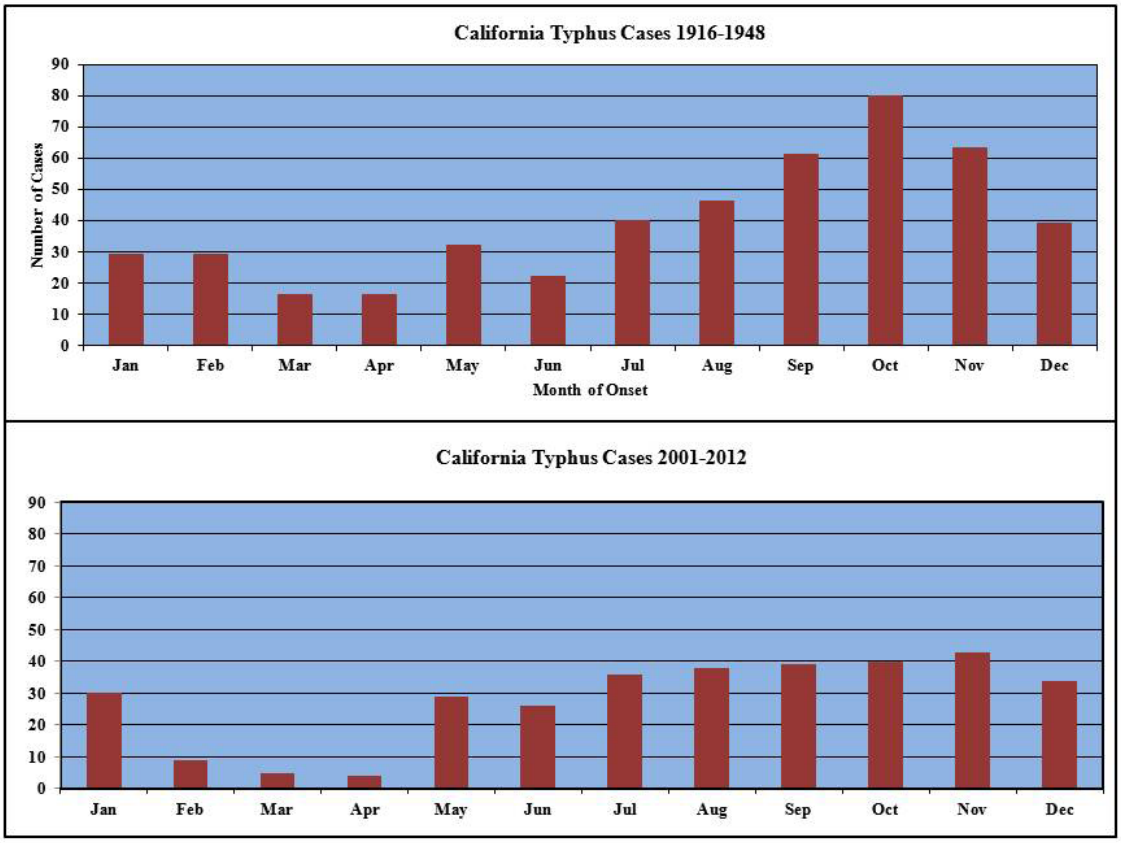

Figure 2. Seasonality of typhus cases in California 1916-1948 and 2001-2012.

cats (56%) or opossums (55%), one third had exposure to both, and nearly 35% of case patients reported exposure to fleas. Similar exposure information is not available for historic cases. Continued surveillance and determination of possible exposure routes is necessary to describe more accurately the agent, reservoir and vector for typhus in Southern California.

The non-specific nature of human typhus cases, in combination with diagnostic tests that potentially detect cross-reactive antibodies (Garcia 2010), make confirmation of cases difficult. Limited resources for case follow-up require accurate reporting and case evaluation to target resources to identifying human risk for typhus and accurately informing public health policy and action. The changing ecology and epidemiologic trends in typhus cases and the agent(s) responsible for disease are important public health concerns given the increase in reported cases in southern California. This has prompted enhanced surveillance by CDPH, including on-going projects to determine risk based on geographic location and agent prevalence. Human typhus cases are reportable to CDPH under Title 17 of the California Code of Regulations and are closely monitored to document possible routes and location of exposure. CDPH works closely with local health agencies to promote prevention of typhus by limiting exposure to urban wildlife and keeping pets on effective flea control.

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Development of an ELISA for Determining the Presence of Rickettsial Antibodies in the Virginia Opossum, *Didelphis virginiana*

Carrie Fogarty, Laura Krueger, Kiet Nguyen, Robert Velten, Stephen Bennett, Art Tilzer, Sokanary Sun and Robert F. Cummings

Orange County Vector Control District, 13001 Garden Grove Blvd, Garden Grove, CA 92843; (714) 971-242; e-mail contact: cfogarty@ocvcd.org

ABSTRACT: The purpose of this study was to develop and validate an indirect enzyme linked immuno-assay (ELISA) for detection of antibodies in the Virginia opossum, *Didelphis virginiana*, to *Rickettsia typhi* and *R. felis,* the etiologic agents of flea-borne typhus. While ELISAs have proven to be highly sensitive and reliable for detecting infections from a variety of pathogens, no ELISAs are commercially available for testing many of the vertebrate species involved in flea-borne typhus transmission and enzootic maintenance. Under guidance from the Bacterial and Rickettsial Diseases Laboratory at the Walter Reed Army Institute of Research (WRAIR), the Orange County Vector Control District (District) received training and materials (protocols, antigens and controls) from WRAIR for developing the ELISA. District staff trapped opossums during ecologic investigations of human typhus cases, received opossums from public submissions and processed flea and blood samples from each animal for serologic and quantitative real-time polymerase chain reaction (qPCR) testing. Serologic data from the ELISA and qPCR results on fleas from their respective opossum hosts were compared and evaluated to identify the potential disease agent. Of 202 opossum sera evaluated by ELISA, 84 (41.6%) reacted to *R. typhi* antigen; qPCR testing of cat fleas (*Ctenocephalides felis*), the most abundant flea species on the opossums, yielded *R. typhi* and *R. felis* infection rates of < 1% and > 45%, respectively. Based on these data, it is probable that *R. felis* was the etiologic agent responsible for the seropositive reactions in the cross-reactive ELISA test. This hypothesis, however, could not be confirmed serologically due to the lack of *R. felis*-positive opossum control sera.

INTRODUCTION

Surveillance of hosts and vectors for the etiologic agents of vector-borne diseases is essential in understanding disease transmission cycles and assessing prevention strategies. Serologic methods, when used for disease surveillance, may be highly insightful, since antibodies can persist after infection and provide evidence of prior exposure to a pathogen. Complement fixation (CF) tests were used in early epidemiologic investigations of murine typhus to assess infections rates of the putative agent, *Rickettsia typhi*, in host animals and humans (Dyer 1944, Bodily et al. 1950, Adams et al. 1970, Sorvillo et al. 1993). In subsequent studies, CF was replaced by immunofluorescent assays (IFA) because of their ease of use and shorter processing time. Currently, the direct IFA is used most often by diagnostic laboratories to show evidence of murine typhus infections in humans and may include multi-antigen screening for more than one rickettsial pathogen. However, no enzyme linked immunosorbent assays (ELISA) are available commercially to test many vertebrate species for infections caused by *R. typhi* or *R. felis*, a closely-related rickettsiae also implicated in flea-borne typhus infections in animals and humans (Williams et al. 1992, Higgins et al. 1996, Perez-Osorio et al. 2008). (The term "flea-borne typhus" can be used interchangeably for disease caused by either human pathogen). Although classified in different rickettsial groups (*R. typhi*, typhus-group; *R. felis*, transitional group, Gillespie et al. 2007), both organisms have been known to induce antibody responses in vertebrate hosts indistinguishable by most serologic methods (Williams et al. 1992, Azad et al. 1997, Raoult et al. 2001).

From December 2006 to 2012, 85 suspected, probable, and confirmed human cases of flea-borne typhus were reported to the Orange County Health Care Agency, with 32 cases in 2012 alone (Figure 1). Prior to 2006, no flea-borne typhus infections had been reported in Orange County since 1991. The Orange

County Vector Control District (OCVCD) collected only small numbers (< 5/yr.) of opossums, *Didelphis virginiana* Kerr, before the outbreak years, but expanded trapping efforts in recognition of this species' role as a significant host of the cat flea, *Ctenocephalides felis* Bouché, the primary flea-borne typhus vector in southern California and Texas (Adams et al. 1970, Sorvillo et al. 1993; Boostrom et al. 2002, Eremeeva et al. 2012). The purpose of this study was to develop an in-house ELISA with sera from opossums. ELISA results were then compared with quantitative real-time polymerase chain reaction (qPCR) testing of fleas. Together, these data from vertebrate hosts and insect vectors could prove useful in identifying epidemiologic patterns of flea-borne typhus transmission and enzootic maintenance in hosts.

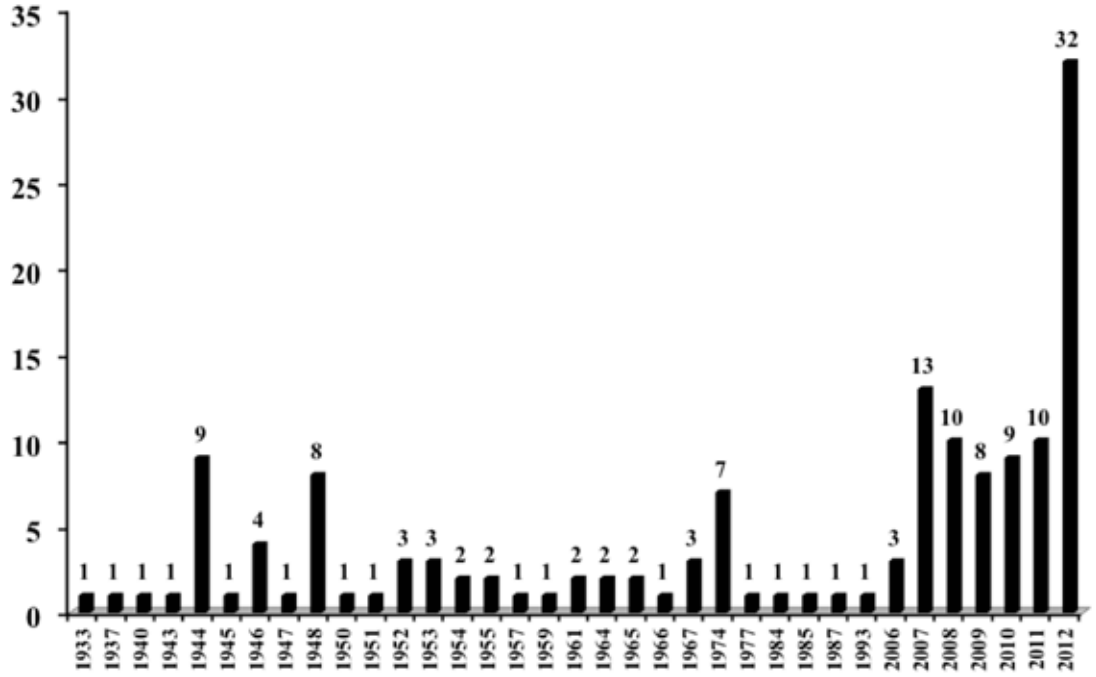

Figure 1. Historical and current suspected, probable, and confirmed human cases of flea-borne typhus in Orange County, CA, 1933 – 2012.

METHODS

Opossums were caught in small mammal traps (Tomahawk Live Trap®, Tomahawk, WI) either around case homes during ecological investigations by the District (2006 – 2012), or by the public and submitted as nuisance animals (1998 – 2006). Animals were euthanized in a CO_2 gas chamber according to American Veterinary Medical Association protocols. Blood and fleas from opossums were collected, processed and stored from 1998 – 2012 for testing. After 2006 most samples were taken by District staff, following the rise in flea-borne typhus cases in Orange County.

An ELISA was designed using *R. typhi* and spotted fever group (SFG) antigens provided by the Bacterial and Rickettsial Diseases Laboratory at the Walter Reed Army Institute of Research (WRAIR, Silver Spring, Maryland). Anti-opossum conjugate was made using unlabeled anti-opossum antibody from Bethyl Labs, Inc. (Montgomery, TX) and KPL Sure-Link HRP conjugation kit (Gaithersburg, MD). A basic checkerboard titration was used for the conjugate to find the optimum dilution using sera from opossums with fleas positive for *R. felis* by qPCR. Antigen titration was confirmed using positive human serum samples provided by WRAIR, one each for *R. felis* and Rocky Mountain Spotted Fever (RMSF), *R. rickettsii*. Testing was performed according to the ELISA protocol from WRAIR using *R. typhi* and SPG antigens (Richards et al. 2007). All positives were titrated for confirmation and determination of end-point titers.

Since no positive RMSF or *R. felis* opossum control sera were available for this study, we tried to validate the ELISA by testing 15 serum specimens (10 *R. typhi* –reactive ELISA positives and 5 *R. typhi* ELISA negatives) for *R. felis* with an IFA normally used on human serum at Fuller Laboratories (Fullerton, CA).

The District tested samples of opossum sera and fleas for *R. typhi* and *R. felis* DNA using an ABI 7300 PCR thermocycler (Life Technologies, Grand Island, NY) with a qPCR assay for the outer membrane protein (*omp*B) gene using species-specific probes (Henry et al. 2007). For *R. felis* testing by the qPCR assay, we used a newly redesigned, specific primer/probe set developed in response to the discovery of a previously unknown rickettsial species in flea samples that WRAIR characterized from Kenya (Jiang et al. 2013). The previous version of the *R. felis* qPCR test was assumed to be specific until sequence data demonstrated the presence of this novel organism. Additional flea samples were also tested at WRAIR by qPCR according to their protocols (Jiang et al. 2013).

RESULTS

In total 202 blood samples from an equal number of opossums were collected from 1998 – 2012. Prior to the first outbreak year (2006), samples had been taken from 34 opossums, while 124 were collected from December 2006 – 2011. As a result of a record number of flea-borne typhus cases in Orange County during 2012 (Figure 1), the District collected 44 opossums for the year: 36 during ensuing ecological case investigations and 8 from submissions by the public as nuisance animals (none of these was associated with a human case).

All 202 serum samples were tested by ELISA, and 84 (41.6%) were positive for *R. typhi* antibodies; none of the sera reacted against the SFG antigen (Table 1). In the IFA subsample validation test by Fuller Laboratories, all (10/10) *R. typhi* ELISA-positives demonstrated cross-reactivity between *R. typhi* and *R. felis* antigens on the IFA slides; none (0/5) of the ELISA-negatives tested positive in the IFA (Table 1).

One interesting result is that serum samples from all animals (n = 34) collected before the outbreak year of 2006 were negative for *R. typhi* antibodies by ELISA. Of the 44 opossums collected in 2012, 30 (68.2%) were positive by ELISA for *R. typhi* antibodies. Also, 29 of 36 (80.6%) opossums collected in 2012 near human flea-borne typhus cases tested positive for *R. typhi* antibodies, while only 1 of 8 (12.5%) opossums submitted as nuisance animals by the public for the year was found antibody-positive for *R. typhi* by ELISA. No proximity analysis to human cases was performed on the animals captured from 2006 – 2011.

No rickettsial DNA was detected by qPCR in any sera samples (n = 202). However, 67.1% (145/216) and 47.4% (72/152) of cat fleas tested by WRAIR and the District, respectively, were positive for *R. felis* DNA; only 0.5% (1/216) and none (0/152) of the flea specimens tested by WRAIR and the District, respectively, tested positive for *R. typhi* DNA.

SUMMARY

Some of the data showed interesting patterns: all seropositive opossums were collected after the outbreak year and although small in numbers, opossums from non-case sites had a lower antibody prevalence rate, 12.5% vs. 80.6%. These findings are similar to those reported for Corpus Christi, Texas (Boostrom et al. 2002). Based on the high abundance of *R. felis* relative to *R. typhi* infecting cat fleas in this study (67.1% vs. 0.5% [WRAIR] and 47.4% vs. 0% [District], respectively), it is conceivable that most of the positive serum

	Serologic Assays for Opossum Sera Samples Positive/Total Tested		
Serologic Test	***R. typhi***	***R. felis***	**SFG**
ELISA[a]	84/202	N.D.	0/202
IFA[b]	10/10	10/10	N.D.

Table 1. Antibody seroprevalence to *Rickettsia* spp. in 202 Virginia opossums, *Didelphis virginiana* Kerr, collected in Orange County, CA, 1998 – 2012.

[a] ELISA, enzyme-linked immunosorbent assay with *R. typhi* and SFG antigens (WRAIR, Silver Spring, MD).
N.D., not done; IFA, indirect immunofluorescence assay.
[b] IFA slides for *R. typhi* and *R. felis* using FITC (fluorescein isothiocyanate) anti-opossum conjugate (Fuller Labs, Fullerton, CA).

samples were the result of cross-reactivity of the *R. typhi* antigen in the ELISA to *R. felis* antibodies in the opossum sera. However, the possibility of dual infections cannot be ruled out entirely.

Although the ELISA worked well in detecting *R. typhi*-reactive antibodies, none of the results could be validated for *R. felis* antibodies because of the lack of *R. felis*-positive opossum control sera, supplies of which are currently unavailable to the District. For a comprehensive understanding of flea-borne typhus epidemiology, serologic reagents that can reliably identify infections caused by different *Rickettsiae* in vertebrate hosts are essential for disease surveillance. Most individual local agencies do not have the resources to develop the "proof of validity" for diagnostic tests and need the support of national organizations like WRAIR. The District will continue with its efforts to obtain *R. felis* antigen from several potential sources with the hope of incorporating species-specific materials in future flea-borne typhus seroprevalence studies in Orange County.

ACKNOWLEDGEMENTS

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The Orange County Vector Control District's Involvement in Flea-Borne Typhus

Laura Krueger, Kiet Nguyen, Robert Velten, Stephen Bennett, Carrie Fogarty,
Sokanary Sun and Robert Cummings

Orange County Vector Control District, 13001 Garden Grove Blvd,
Garden Grove, CA, 92843, (714) 971-2421, lkruger@ocvcd.org

ABSTRACT: The Orange County Vector Control District flea-borne typhus prevention and response program consists of two main components. The first component is an ecological investigation to determine neighborhood risk and the second component is a public education program. Results from the ecological investigation guide the geographic extent of the public education campaign.

INTRODUCTION

Orange County, California, has experienced a resurgence in human cases of flea-borne typhus. From 2006 to 2012, 85 suspected, probable and confirmed human cases of flea-borne typhus were reported to the Orange County Health Care Agency. Previous to 2006 no flea-borne typhus infections had been reported in Orange County since 1991. From 2006 to 2008, the Orange County Vector Control District (OCVCD) collaborated with the Centers for Disease Control, Division of Vector-borne Diseases, to investigate pathogen, vector and host relationships in Orange County for flea-borne rickettsiosis. Results from this collaborative study identified the suburban cycle of flea-borne typhus transmission (backyard wildlife - fleas - humans) in Orange County (Eremeeva et al. 2012), similar to earlier findings in neighboring Los Angeles County (Civen and Ngo 2008). Three species of fleas commonly found on opossums, feral cats, raccoons, skunks, domestic cats and domestic dogs were found to harbor *Rickettsia felis* and/or *R. typhi*, the causative agents of flea-borne rickettsiosis (Table 1)

The most prevalent flea species in southern California, *Ctenocephalides felis* Bouche´, regularly infests backyard wildlife and companion cats and dogs and is an avid human biter. It is believed that backyard wildlife such as opossums, feral cats, raccoons and skunks maintain populations of *C. felis* in the environment (Adams 1970). *Ctenocephalide felis* then infests companion cats and dogs not currently on

Location, flea species	Number of Flea Pools Tested (Total Fleas)	Number of Positive Pools (%)			Minimum Infection Rate (%)	
		R. typhi	*R. felis*	Dual Positive	*R. typhi*	*R. felis*
Orange County						
Ctenocephalides felis	727 (1405)	7 (0.9)	428 (58.9)	22 (3.0)	2.1	32
Pulex irritans	55 (109)	4 (7.3)	9 (16.4)	0	3.7	8.3
Echidnophaga gallinacea	2 (2)	0	0	0	0	0
Diamanus montanus	3 (4)	0	2 (66.7)	0	0	50
Leptosylla segnis	1 (1)	0	0	0	0	0

Table 1. Detection of *R. typhi* and *R. felis* DNA in Flea DNA Samples (Excerpt from Eremeeva et al. 2012)

flea control. The companion cats and dogs bring the fleas into the home environment where they bite and infect their owners with the causative agents of flea-borne rickettsiosis, *Rickettsia felis* and *R. typhi* (Table 1 - CDC, 2009, Eremeeva et al. 2012, Karpathy et al. 2009, Reif 2009). Humans become infected with *R. typhi* through accidental contact with infected fleas and their feces by inhalation or scarification into damaged skin (Azad 1990). *R. felis* and *R. typhi* are not known to cause illness in wildlife and/or pet cats and dogs, although *R. felis* has been identified from hepatic and renal tissue of opossums (Eremeeva et al. 2012).

OVERVIEW OF FLEA-BORNE TYPHUS AND RESPONSE PROGRAM

In response to this increasing disease burden, OCVCD established a flea-borne typhus prevention and response program consisting of two main components: an ecological investigation to determine neighborhood risk through surveillance of backyard wildlife and fleas and a public education program. Results from the ecologic investigation guide the geographic extent of the public education campaign. The ecological investigation consists of the following elements:

1) Inspection for backyard wildlife – opossums, feral cats, raccoons, skunks, rats, mice.
2) Inspection for conditions conducive to infestations such as harborage, food and water sources.
3) Inspection of companion cats and/or dogs for evidence of flea infestations;.
4) Inspection for evidence of flea infestations on property.
5) Trapping of opossums for determination of the flea index (# fleas/animal) as an indication of neighborhood risk.

From 2006 - 2012, OCVCD trapped 169 opossums associated with flea-borne typhus exposure sites. The average flea index was 53.51 fleas per opossum with a range of 0 - 501 fleas per animal. In neighborhoods where the flea index is significantly higher per opossum trapped, the geographic extent of the neighborhood notification is increased.

OCVCD's public education campaign occurs in/around known flea-borne typhus exposure sites. OCVCD staff goes door-to-door in the area directly adjacent to the flea-borne typhus exposure site providing information on the disease. If conditions are conducive to backyard wildlife, OCVCD educates residents on ways to make their properties less attractive to these animals. Residents are advised to put their pet animals on flea control and/or contact a structural pest control company for environmental flea control. When appropriate, properties with significant issues, such as hoarding, are referred to other governmental agencies for assistance.

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Overview of the Stormwater Symposium

Marco E. Metzger

Vector-Borne Disease Section, California Department of Public Health, 2151 Convention Center Way, Suite 218B, Ontario, CA 91764, USA

Over the past decade, the subject of "stormwater" has gone from a relatively unknown issue to one of the top priorities for many mosquito and vector control agencies in California. The series of studies spearheaded by the California Department of Public Health, Vector-Borne Disease Section between 1999 and 2003 that focused on mosquito presence, abundance and control in modern stormwater treatment structures (Caltrans 2004) was largely aimed at raising awareness of this emerging issue in California (Metzger 2004) and at the national level (United States 2002, CDC 2005). Today, stormwater management and treatment structures are included in the routine surveillance and control operations of most mosquito and vector control agencies in the state.

The function of engineered stormwater treatment structures is to protect public health and improve environmental quality by capturing a portion of suspended and dissolved pollutants carried by runoff and by controlling runoff volume to reduce downstream erosion. Increasingly stringent water quality regulations have forced continuous improvements to these types of structures and to the overall approach to managing stormwater runoff. As a result, familiar stormwater "Best Management Practices" such as extended detention basins are rapidly being retrofit or replaced with more sophisticated devices. At the same time, the paradigm for managing stormwater runoff has steadily progressed from a regional approach to a parcel approach. The future of stormwater runoff management therefore will be focused primarily on Low Impact Development (LID), a philosophy of maintaining pre-contruction area hydrology (i.e., no net increase in post-development runoff) by integrating stormwater management devices into individual properties. The LID concept opens up a whole new world of potential water-holding structures from rain barrels and cisterns to water-holding "rain gardens". Mosquito and vector control agencies are beginning to include these new devices into their growing list of potential mosquito sources.

This symposium was organized for the purpose of providing a much-needed update on issues related to stormwater that have and will continue to impact mosquito surveillance and control into the future. The speakers were intentionally selected from a wide range of backgrounds including regulatory, engineering and operations in order to offer the audience broad perspectives on the subject and provide a unique opportunity to interact with a group of subject matter experts. The following were the presenters, their affiliations, and the titles of their presentations:

- Marco E. Metzger, Ph.D., California Department of Public Health, Vector-Borne Disease Section
  Introduction to Stormwater
- William Hereth, P.E., State Water Resources Control Board, Municipal Stormwater Section
  An Insider's View on Stormwater NPDES Permits
- Scott Taylor, P.E., D.WRE., RBF Consulting
  Design Considerations in Stormwater Management and Treatment Structures: When can a Mosquito Habitat be Minimized?
- David Tamayo, County of Sacramento Stormwater Quality Program
  Regulatory and Organizational Challenges for Public Works Departments for Meeting Municipal Stormwater (MS4) NPDES Permit requirements in Relation to Mosquito Control in Urban Areas
- Mark Daniel and Susanne Kluh, Greater Los Angeles County Vector Control District
  The Potential Impact of the New Low Impact development (LID) Ordinance in Los Angeles on Mosquito Control Operations

- Eric Schulz, San Mateo County Mosquito and Vector Control District
  The Impact of the Municipal Regional Stormwater NPDES Permit on Mosquito Control Operations in the San Francisco Bay Area

- Marty Scholl, B.S., Sacramento-Yolo Mosquito and Vector Control District
  Adapting Mosquito Control Strategies to Changing Stormwater requirements in Sacramento and Yolo Counties

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A Story of *Culex stigmatosoma* Investigations into the Biology of a Lake County Mosquito

Bonnie M. Ryan, Michelle L. Koschik, Brittany M. Nelms, Tara C.Thiemann and Jamesina J. Scott

Lake County Vector Control District, 410 Esplanade St, Lakeport CA, 95453, (707) 263-4770, bryan@lcvcd.org

ABSTRACT: *Culex stigmatosoma* is a competent vector of West Nile virus (WNV) in California. While much attention has been devoted to *Culex tarsalis* and the *Culex pipiens* complex and their roles in WNV transmission, the role of *Cx. stigmatosoma* in the amplification and overwintering of WNV is not well understood. This paper describes the combined efforts of the Lake County Vector Control District and the Center for Vectorborne Diseases at UC Davis to describe the seasonality, efficacy of various trapping methods, minimum infection rate, larval habitats and onset and termination of diapause of *Cx. stigmatosoma* in Lake County, CA.

INTRODUCTION

Lake County is located in Northern California between the Central Valley and the Pacific Coast and includes Clear Lake, a eutrophic, warm polymictic lake (Suchanek et al. 2008). At 17,670 hectares, Clear Lake is the largest freshwater lake fully contained within California (Eagles-Smith et al. 2008). Lake County is comprised of mountainous terrain and agricultural land interspersed with rural communities.

West Nile virus (WNV) has been a recognized health problem in California since 2003 (Reisen et al. 2004); it was first detected in Lake County in 2004 (Ryan et al. 2006). Two mosquitoes are known to have an instrumental role in WNV transmission in California: *Culex tarsalis* Coquillett and mosquitoes in the *Culex pipiens* complex. Many resources have been devoted to elucidating the details of their vector ecology. In contrast, comparatively little is known about *Culex stigmatosoma* Dyar (Reisen 2012). This species is seldom collected by the commonly employed methods of CO_2-baited traps (CO_2), New Jersey Light traps (NJLT) or Large Red Boxes (LRB) (Reisen and Pfuntner 1987). Gravid traps have been incidentally successful, but the specific efficacy of this method has not been investigated (Du and Millar 1999). With no robust means of collection described, our ability to study *Cx. stigmatosoma* and its role in WNV transmission is limited.

Throughout Lake County, collections of *Cx. tarsalis* are nearly ubiquitous, *Cx. stigmatosoma* are localized and *Cx. pipiens* complex mosquitoes have been rare (Breuner et al. 2013). If a species' significance in WNV transmission is relative to its trap abundance *Cx. stigmatosoma* might play a major role, along with *Cx. tarsalis*, in Lake County. In this article we summarize the efforts of the Lake County Vector Control District (LCVCD), in collaboration with the Center for Vectorborne Diseases (CVEC) at UC Davis, to describe the local vector ecology of this mosquito. We review what we have learned about the minimum infection rate (MIR), seasonality, larval habitats, efficacy of various trapping methods, identified bloodmeal hosts and diapause dynamics of *Cx. stigmatosoma* in Lake County, CA. In an effort to put these data in context, these characteristics are contrasted with those of local *Cx. tarsalis*, *Culex erythrothorax* Dyar and *Cx. pipiens* complex mosquitoes whenever the data are available.

LARVAL HABITATS

In Lake County we have confirmed two larval habitats for *Cx. stigmatosoma*. We repeatedly collect larvae from winery effluent ponds and blooms of cyanobacteria in Clear Lake. We collect larvae from un-aerated winery effluent ponds in large numbers; these collections are homogeneous. Periodically, Clear Lake will have tremendous blooms of cyanobacteria in the genus *Lyngbya*. Floating mats clog man-made channels and tributaries, obstructing or killing mosquito predators. In 2012 this created larval habitat for tremendous populations of both *Cx. tarsalis* and *Cx. stigmatosoma* in Clearlake Oaks, CA.

Adult collections suggest the presence of additional larval habitats that are difficult to sample and from which we have not collected any *Cx. stigmatosoma* larvae. Each year emergent vegetation (especially tules, cattails and creeping water primrose) along the shores of Clear Lake creates a suspect habitat. Both the location and the timing of adult collections suggest wild rice fields are larval *Cx. stigmatosoma* habitat. Before the rice is harvested, adult collections are relatively low and increase post-harvest as the water quality deteriorates.

TRAPPING METHODS AND SEASONALITY

The LCVCD uses CDC suction traps (Sudia and Chamberlain 1962) that have been modified by removing the light and adding CO_2 bait (3.2kg of dry ice) to attract host-seeking mosquitoes. Traps are set overnight either weekly or bi-weekly. Large Red Boxes are modified after Meyer 1985. These consist of plywood boxes measuring 1.8m x 1.2m x 1.2m enclosed on four sides with the front and bottom open and all exposed surfaces painted red. Resting mosquitoes are collected with a hand-held vacuum before noon. Both CO_2 traps and LRBs are operated seasonally. The number and location of these collection methods are adjusted seasonally to meet surveillance and research objectives. Two NJLTs are operated within the county. One is at the wild rice fields north of Clear Lake and the other is east of the lake in Clearlake, CA. New Jersey Light traps are collected weekly and are the only trapping method operated year round.

Inclusive of all trapping efforts, *Cx. stigmatosoma* were collected between the first weeks of March and November (disease weeks 10 - 45) during an 11 year period (2002-2012). This mosquito's seasonality is very similar to *Cx. tarsalis* which were collected from the third week in March through the first week in November (disease weeks 12 - 45).

To determine the best trap collection method for *Cx. stigmatosoma* with comparison to *Cx. tarsalis*, eleven years of CO_2, NJLT and LRB data were averaged for the entire county and graphed by disease week (Figure 1). *Culex tarsalis* is collected in all three trap types; CO_2 traps were the most effective. The same data show that *Cx. stigmatosoma* do not readily come to CO_2 or NJLTs; they were most abundant in LRBs.

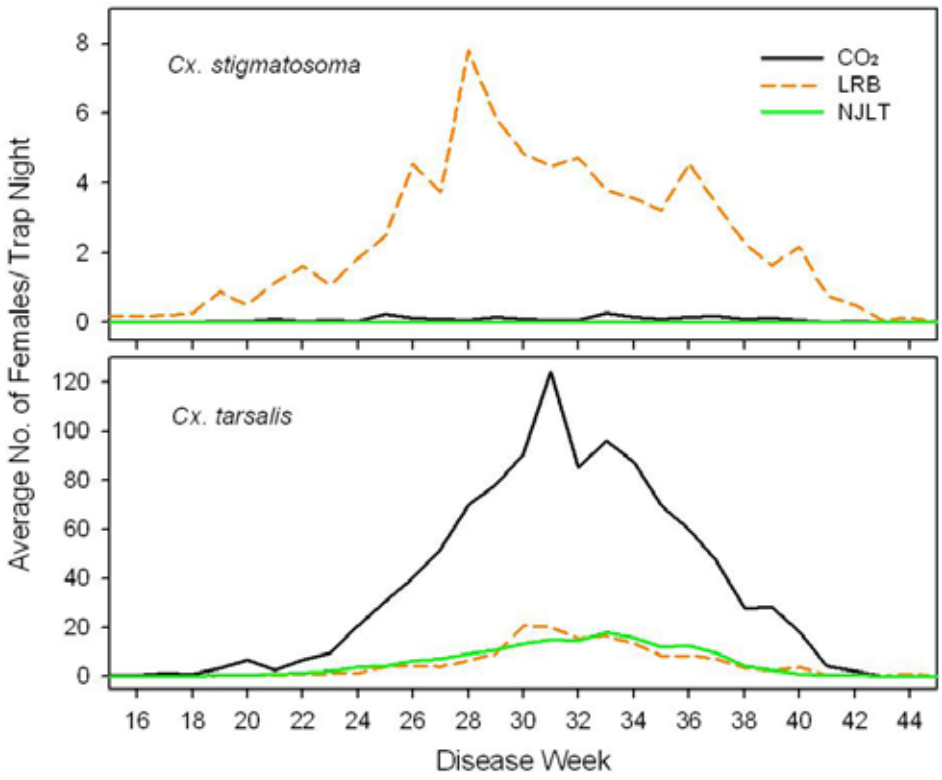

Figure 1.
Weekly abundance of mosquitoes collected 2002–2012 by three methods: Large Red Box (LRB), CO_2-baited trap (CO_2) and New Jersey Light trap (NJLT).

A portion of the mosquitoes collected from CO_2 traps and LRBs are submitted for WNV testing. We have observed that the majority of host-seeking females collected by CO_2 are the same gonotrophic stage (empty) and appear to be newly emerged. In contrast, females collected from LRBs often vary in gonotrophic stage (empty, bloodfed or gravid). Any bloodfed or gravid mosquito may have been exposed to WNV while taking the bloodmeal. Method of collection may, therefore, have an effect on overall Minimum Infection Rate (MIR) of each species.

MINIMUM INFECTION RATE

From 2003 to the present, mosquitoes being examined for WNV were pooled and submitted to CVEC. In that time, more than 125,000 *Culex* spp. mosquitoes were tested for WNV. The majority were *Cx. tarsalis* (86%); *Cx. stigmatosoma* and *Cx. erythrothorax* were approximately seven percent each. No mosquitoes of the *Cx. pipiens* complex were tested from Lake County because they were either collected in numbers too low for pooling or the specimens were used for research. West Nile virus has been detected in *Cx. tarsalis* annually from 2004 through 2012. Over the same period, WNV has also been detected in *Cx. stigmatosoma* in all but three years: 2007, 2009 and 2010. *Culex erythrothorax* pools have tested positive in three years; the first two years that WNV was detected in Lake County (2004 and 2005) and again in 2012. For each year that WNV was detected in *Cx. stigmatosoma*, this species had the highest MIR of the three (Figure 2).

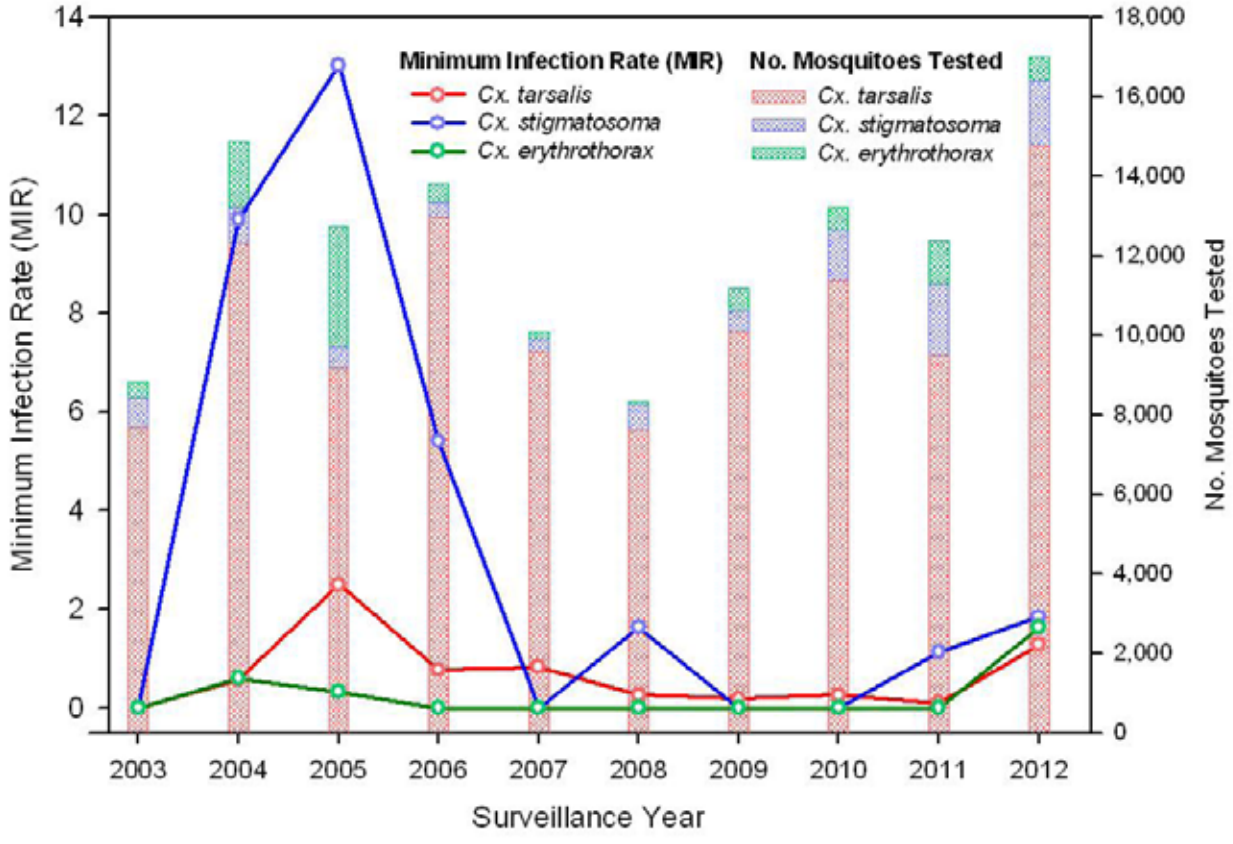

Figure 2. Annual number and minimum infection rate of mosquitoes tested for WNV collected throughout Lake County from Large Red Boxes and CO_2-baited traps 2003–2012.

West Nile Virus was detected in *Culex* spp. mosquitoes over a fourteen-week period between the fourth week in July and the fourth week in October (disease weeks 29 - 42). When data were combined from 2003 through 2012, a weekly minimum of one pool each of *Cx. tarsalis* and *Cx. stigmatosoma* was tested. West Nile Virus was detected each week in pools of *Cx. tarsalis* mosquitoes and in 10 of 14 weeks from *Cx. stigmatosoma*. For each week that WNV was detected in the *Cx. stigmatosoma* population, the MIR was higher than it was in the *Cx. tarsalis* for the same week. *Culex tarsalis* MIR exceeded *Cx. stigmatosoma* only in weeks when no WNV was detected in the later species. In the three weeks that WNV was detected in *Cx. erythrothorax*, this species' MIR was similar to *Cx. tarsalis* for the same week (Figure 3).

While no *Cx. erythrothorax* that were pooled for WNV testing were collected from LRBs, similar numbers of *Cx. tarsalis* and *Cx. stigmatosoma* were collected by this method; approximately 7,400 and 8,600 mosquitoes, respectively. Large Red Box-collected *Cx. stigmatosoma* consistently had higher MIR than *Cx. tarsalis*, except during the third week of August (disease week 32) when the no positive *Cx. stigmatosoma* positive pools were detected (Figure 4).

BLOODMEAL IDENTIFICATION

There are three avenues by which a mosquito may become infected with WNV: [1] From an infected female passing it to her offspring (vertical transmission), [2] From a host while taking a bloodmeal (horizontal transmission), and [3] From an infected male to a female during mating (venereal transmission). Because venereal transmission has rarely been documented, it will not be considered here. Horizontal transmission has been demonstrated under laboratory conditions for both *Cx. tarsalis* and *Cx. stigmatosoma* (Goddard et al. 2002). Vertical transmission has been demonstrated in *Cx. tarsalis* but has not yet been evaluated in *Cx. stigmatosoma* (Goddard et al. 2003).

Mosquito bloodmeal identification provides excellent detail into the horizontal transmission dynamics of WNV; these hosts are potentially infecting susceptible mosquitoes within a particular habitat. Maintenance and amplification WNV bloodmeals from avian hosts in the Order Passeriformes, especially those in the Family Corvidae, are particularly interesting. During 2008 and 2009, we collected bloodfed *Cx. tarsalis* (n = 58) and *Cx. stigmatosoma* (n = 124) from LRBs in an oak woodland in Lakeport, CA. Bloodmeals were identified by T. Thiemann at CVEC. Dr. Thiemann sequenced the mitochondrial gene, cytochrome c oxidase (*COI*) as described previously in Kent et al. 2009 and Thiemann et al. 2012. In our study, *Cx. tarsalis* fed on birds (86%), mammals (12%) and reptiles (2%). In contrast, 100% of the bloodmeals identified from *Cx. stigmatosoma* were from birds. Avian bloodmeals taken by *Cx. tarsalis* and *Cx. stigmatosoma* were from 47% and 77% passerine and 14% and 29% corvid hosts, respectively (Table 1).

	Culex stigmatosoma	*Culex tarsalis*
Birds	**100.00**	**86.21**
Order: Passeriformes	76.61	46.55
Family: Corvidae	29.03	13.79
American Crow	0.81	0.00
Common Raven	0.81	3.45
Western Scrub-Jay	27.42	10.34
Other Bird Orders	23.39	39.66
Mammals	**0.00**	**12.07**
Reptiles	**0.00**	**1.72**

Table 1. Percentages (%) of host bloodmeals identified from *Culex stigmatosoma* (n = 124) and *Culex tarsalis* (n = 58).

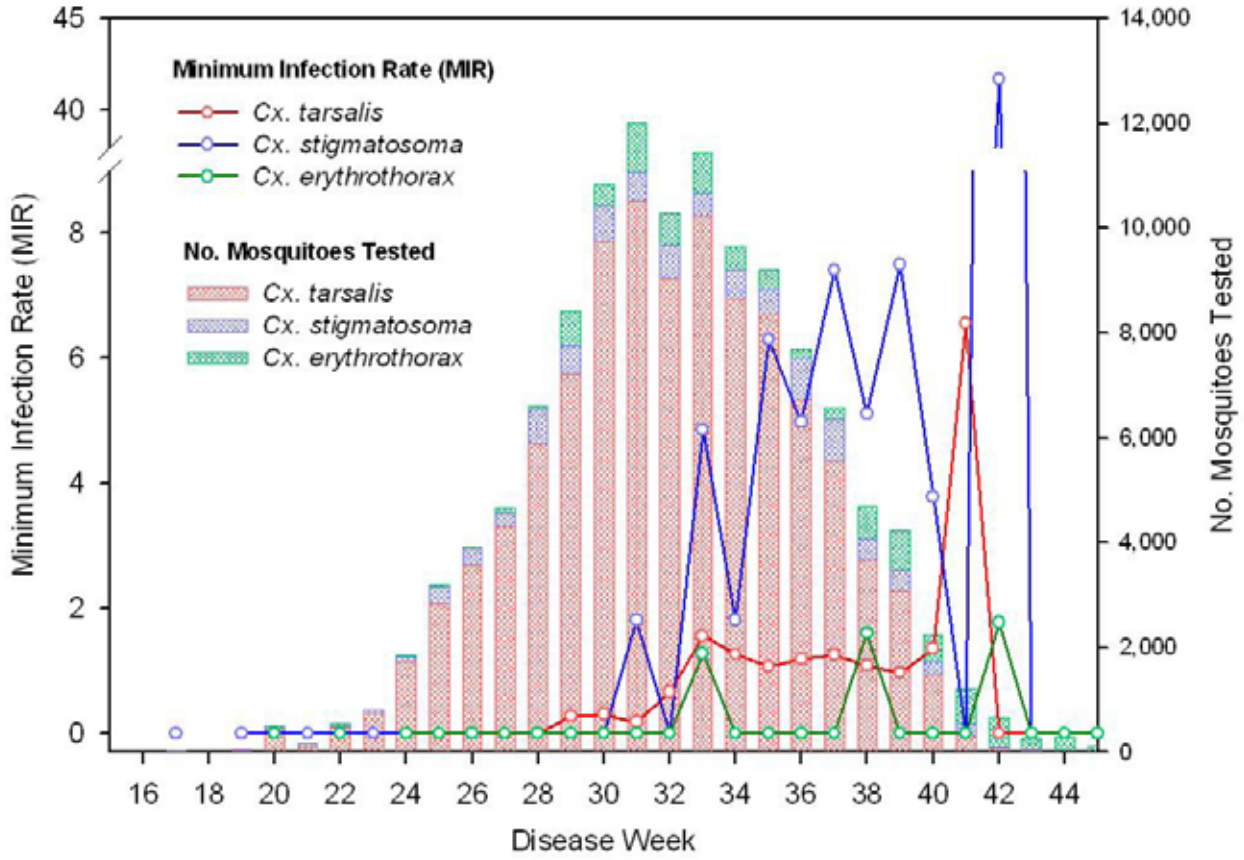

Figure 3. Minimum infection rate and total numbers of mosquitoes tested for WNV from Lake County Large Red Box and CO_2-baited trap collections graphed by disease week from 2003–2012.

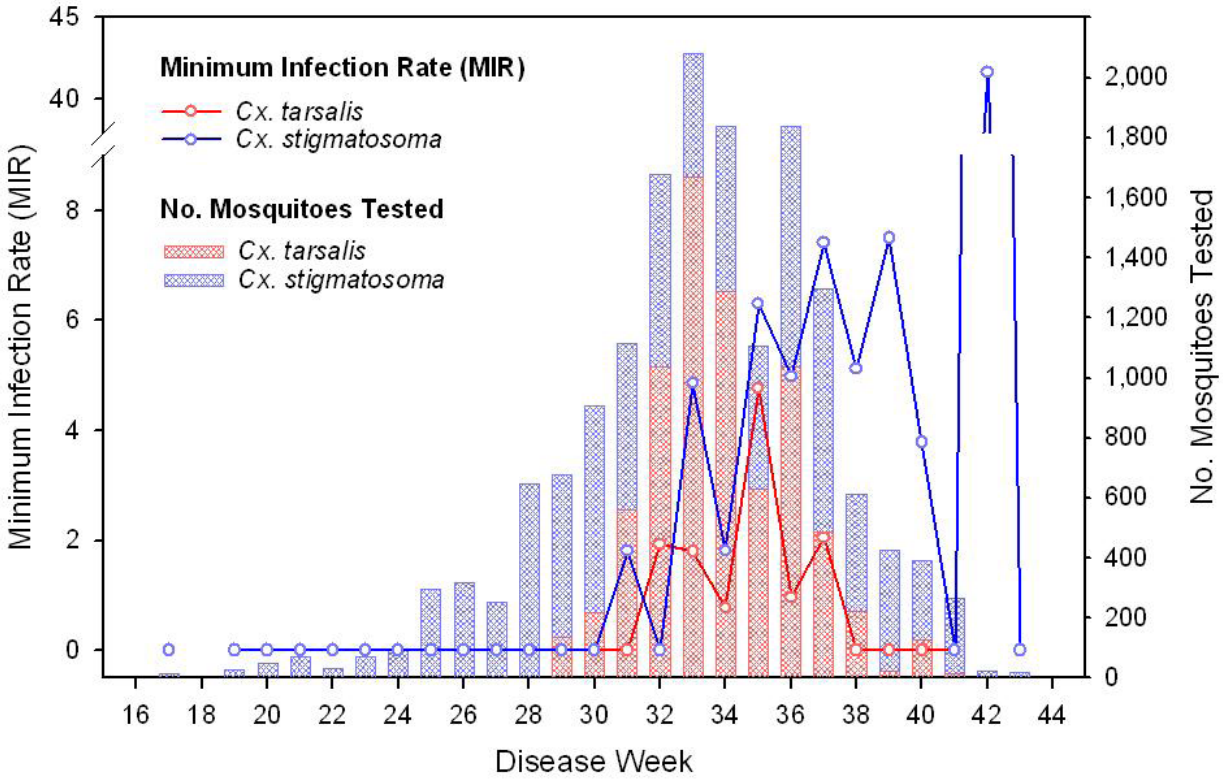

Figure 4. Minimum infection rate and total numbers of mosquitoes tested for WNV from Lake County Large Red Box collections graphed by disease week from 2003–2012.

From the last week in May through the third week in September (disease weeks 22 - 38), bloodfed *Cx. stigmatosoma* were collected each week. Specimens collected early in the season fed predominantly on passerines other than corvids. On the third week of June (disease week 25), the first bloodmeal from a corvid was identified. Bloodfed *Cx. tarsalis* collections were intermittent prior to the second week in July (disease week 27). The earliest passerine bloodmeal was detected in the second-to-last week of May (disease week 21). Bloodmeals from corvids and mammals were first detected during the last week of July (disease week 29) (Figure 5).

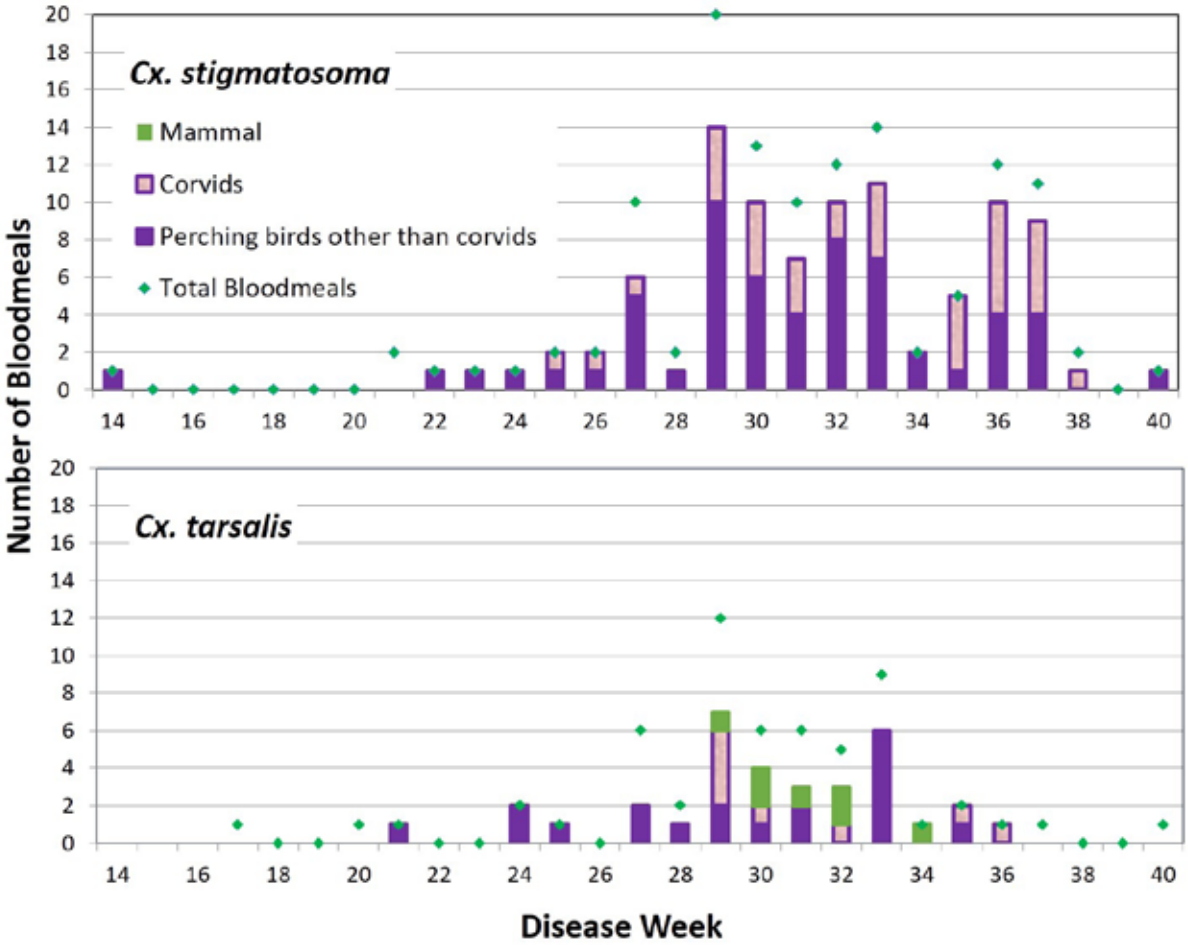

Figure 5. Bloodmeal hosts identified from *Culex stigmatosoma* and *Culex tarsalis* bloodmeals. The mosquitoes were collected from LRBs in an oak woodland in Lakeport, CA during 2008 and 2009 (Thiemann et al., in prep.).

Within this habitat, *Cx. tarsalis* and *Cx. stigmatosoma* bloodfeeding overlapped temporally and shared some avian hosts. No mammalian bloodmeals were identified from *Cx. stigmatosoma* despite the availability of mammalian hosts (e.g., 12% of *Cx. tarsalis* bloodmeals were mammalian). *Culex stigmatosoma* may have little significance in transmitting WNV to mammals. These data provide additional evidence for *Cx. tarsalis* and *Cx. stigmatosoma* as having different, but complementary, roles in WNV transmission; as bridge and maintenance vectors respectively.

OVERWINTERING DYNAMICS

West Nile virus has persisted in competent host or vector species or has been serially introduced to Lake County each year from 2004 through 2012. West Nile virus infects *Culex* spp. mosquitoes that survive the winter and may initiate WNV transmission the following spring. When and whether WNV-competent *Culex* spp. mosquitoes initiate and terminate reproductive diapause temporally defines the local transmission

season. To investigate the overwintering dynamics of *Culex* spp. in Lake County, we reared adults from eggs laid after the Autumnal Equinox. These specimens were collected at intervals throughout the winter and early spring to determine the onset and termination of diapause.

Two wading pools were set up on September 14th, 2011 at the District's property on Todd Road in Lakeport. Egg rafts were collected from wading pools filled with grass emulsion (Table 2). We collected at least 225 egg rafts from these pools ending October 3rd, 2011. The larvae and adults were held in a "mosquito house" that protected them from rain and predators but exposed the mosquitoes to seasonally variable day length and temperature (Nelms et al. 2013). Larvae were reared in 18" x 14" x 4.5" black plastic trays filled with well water. The trays were covered with window screen to prevent emerged adults from escaping. Larvae were fed a 1:1 mixture of rabbit chow and Tetra® fish food ground to a powder with a mortar and pestle. Temperature and light intensity were recorded hourly from inside the "mosquito house" and from the larval rearing trays with Onset HOBO® Pendant Temperature/Light Data Loggers. Emerged adults were collected by hand aspirator and transferred to one-gallon ice cream containers with an opening fitted with a cotton sleeve. Cohorts of adults were separated by emergence week and provided a 10% sucrose-soaked cotton ball placed on the screened lid. A minimum of five mosquitoes were retrieved and examined from each population at semi-regular intervals before and after the winter solstice.

1.) Loosely fill a 5-gallon bucket with partially composted grass clippings; cover clippings with water
2.) Ferment grass-clipping slurry overnight
3.) Add 1 gallon of slurry-water to 75 gallons of clean water in a wading pool
4.) Collect egg rafts using a small spatula daily or every-other-day

Table 2. Medium for attracting gravid *Culex stigmatosoma* and methodology for egg collection.

We reared adults of three species from the collected egg rafts: *Cx. stigmatosoma*, *Cx. pipiens* complex mosquitoes and *Culiseta incidens* (Thomson). *Culiseta incidens* adults were discarded. A total of 83 *Cx. stigmatosoma* females and 67 *Cx. pipiens* complex females were reared and examined for diapause status. Regrettably, *Cx. tarsalis* adults were not included in this investigation because late-season gravid females were rarely collected from LRBs, and the number of F1 adults reared were insufficient for examination.

Ovarial dissection and examination were conducted at CVEC as described by Mills et al. 2009. Diapause status was determined by B. Nelms using a combination of three methods: [1] Morphological examination of primary follicles (Kawai 1969), [2] Relative size of primary to secondary follicles (Spielman and Wong 1973, Reisen 1986) and [3] Degree of vitellogenesis (Clements and Boocock 1984). None of the females of the *Cx. pipiens* complex that were examined after the winter solstice were in reproductive diapause; thus, this population did not enter diapause. A portion of the *Cx. stigmatosoma* samples terminated diapause in the interval between the winter solstice and disease week 10, the first week in March (Figure 6). We were ultimately unable to determine the week that diapause terminated because a majority of specimens remained in diapause when the sample population was exhausted.

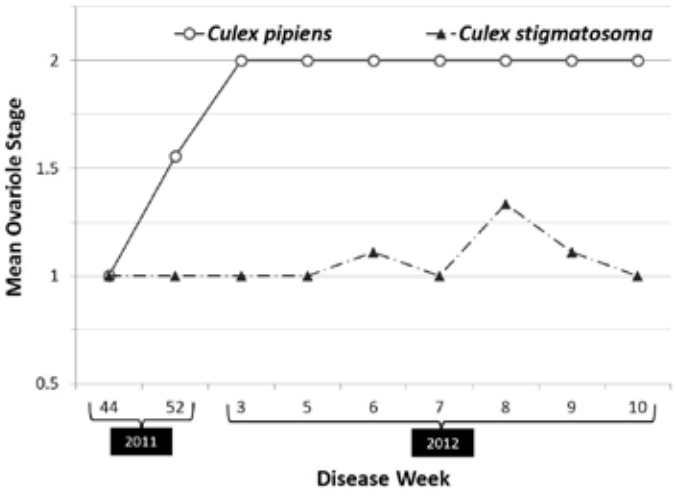

Figure 6. Average ovarial stage of a minimum of five *Culex stigmatosoma* and *Culex pipiens* examined before and after the winter solstice (week 51), 2012 (B. Nelms, unpub. data). Individuals in ovarial stage "one" are maintaining diapause; at ovarial stage "two" diapause has terminated.

DISCUSSION AND CONCLUSIONS

LCVCD data from nine years show that regardless of collection method, *Cx. stigmatosoma* had a higher MIR than *Cx. tarsalis*. Small, but similar, numbers of *Cx. stigmatosoma* and *Cx. tarsalis* were collected and tested from LRBs. On average, fewer than 1,000 LRB-collected-mosquitoes were tested each year, so the difference in MIRs may not be statistically significant. In contrast, CO_2-collected *Cx. tarsalis* were very numerous and may give an improved estimation of MIR overall. However, we are unable to collect similar numbers of *Cx. stigmatosoma* for comparison.

Our data show that the local population of *Cx. stigmatosoma* is feeding readily on hosts that amplify WNV throughout July, but WNV has not been detected in this species until the second week of August. In the ten years since 2003, we have tested approximately 2,000 *Cx. stigmatosoma* in the month of July compared with nearly 31,000 *Cx. tarsalis*. Despite *Cx. stigmatosoma* feeding on corvids earlier in the season and, with the exception of one week, seasonally maintaining a higher MIR than *Cx. tarsalis*, WNV was detected in *Cx. tarsalis* two weeks earlier than in *Cx. stigmatosoma*.

The work that has been done in Lake County with *Cx. stigmatosoma* suggests that this species contributes to local WNV transmission and may also be a vehicle for WNV overwintering. A portable trapping method based on the olfactory stimuli of *Cx. stigmatosoma*, a method akin to CO_2-baited traps for *Cx. tarsalis*, needs to be developed. Its use may provide insights into the biology and distribution of *Cx. stigmatosoma* and potentially improve the number and seasonal range of specimens available for WNV testing. Additional information as to when and to what degree *Cx. stigmatosoma* serves as a WNV vector would facilitate a better understanding of WNV transmission in regions where this mosquito is found.

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Control of Chironomid Midges (Diptera: Chironomidae) in Urban Retention Basins

Tianyun Su and Min-Lee Cheng

West Valley Mosquito and Vector Control District
1295 E. Locust St., Ontario, CA 91761
tsu@wvmvcd.org

ABSTRACT: Chironomid midges are important members in aquatic invertebrate communities, and they play a critical role in sustaining the trophic web and in depleting the organic material from their habitats. However, the midges can be a great public nuisance when they appear in excessively large numbers. They are a significant allergens for people with bronchial asthma and other allergic disorders. In response to complaints of large numbers of fly insects (midges) from the general public in Ontario, CA, initial samplings of larval and adult midges were conducted. Control efficacy of immature stages of midges in water with an insect growth regulator, diflubenzuron (Dimilin® 25W), was evaluated. Excellent control was achieved when this formulation was applied at 16 oz per acre.

INTRODUCTION

Chironomid midges, also known as non-biting midges (the midges) belong to order Diptera, Suborder nematoceran, infraorder Culicomorpha, subfamily Chironomoidea and family Chironomidae which has 11 subfamilies. Among all aquatic macro-invertebrates, chironomid midges are the most ubiquitous and diverse group of insects with over 20,000 estimated species; there are ~4,000 described species of which approximately 100 are considered pestiferous. Spatially, chironomid midges breed in all aquatic (marine, brackish and fresh water) as well as semi-terrestrial habitats across all continents (Ali 1991, 1996). Most species adapt to a wide variety of aquatic environment, ranging from potable water storages to highly eutrophied waste water lagoons. Most of the species are poorly studied, partially because of difficulties in their identification and colonization in the laboratories. Chironomid midges play an important role in various aquatic ecosystems. The presence (or absence) and populations sizes of various midge species are often indicators of certain pollutants in the aquatic habitats. Furthermore, fossil records of midges are widely used as an indicator of past environmental and climatic changes. As an important component of the macro zoobenthos, larval midges can live under almost anoxic conditions by having reduced trachea systems and polymorphic hemoglobins of high oxygen affinity; this latter feature may have some biomedical implication. However, nuisance problems and wide ranges of economic losses caused by midges are widespread making them significant pests (Ali 1991, 1996). Worldwide public health concerns of the midges have also been documented since the 1980s, when midge-derived allergens were incriminated as cause of bronchial asthma and other allergic disorders.

In response to the complaints of nuisance flying insects from the residents and businesses located in Ontario, CA in 2007, the West Valley Mosquito and Vector Control District initiated this project to locate the breeding sources, to assess the larval and adult population densities and to evaluate the control efficacy of an insect growth regulator against the midges.

MATERIALS AND METHODS

Experiment Sites. Based on the locations of complaints, sampling by sweep nets during daytime and New Jersey light traps overnight, as well as on-site observations, were conducted in the neighborhoods adjacent to the complainer's property. "Large numbers of flying insects" were believed to be non-biting midges (Diptera: Chironomidae) (Figure 1). Two groups of retention basins located in the City of Ontario were assumed to be the breeding sources of the midges. The Ely Basins consist of three basins, approximately 25 acres each, located at East Philadelphia Street between South Walker Avenue and South Carlos Avenue in Ontario, CA (Figure 2). Water depth varied from 0.5

to 3 feet. The Turner Basins has five individual basins with a total surface area of about 50 acres and is located at North Archibald Avenue and East 4th Street, Ontario, CA (Figure 3). Water depth in this group of basins ranged from 2 to 4 feet. Both groups of basins were designed to collect storm-water and urban surface runoff or to hold the water purchased from the Metropolitan Water District. The water in these basins is allowed to percolate for recharging the aquifer. In each basin, a number of ramps were constructed along the edge to facilitate maintenance, which enabled our access to sample the benthic community.

Pesticide Treatment. The pesticide used for controlling chironomid midges was Dimilin® 25W (Crompton Manufacturing Company, Inc., Middlebury, CT. Lot# BA2D12P001), a wettable powder containing 25% (w/w) diflubenzuron. This formulation is registered under special local need (SLN) in California (SLN CA-970021) for controlling chironomid midges breeding in non-crop lakes, ponds, channels, ditches and percolation basins at 6.4 - 16 oz /acre (1.6 - 4.0 oz a.i.), not to exceed 6 Lbs. per acre per year. The wettable powder was diluted and applied from a ground-spray rig at the minimum volume of 10 gallons per acre for good coverage. The dose used in these studies was 16 oz/acre suspended in 10 gallons of water.

Efficacy Evaluation. *Ely Basins* – To sample adult midges, on day 12 pre-treatment and days 4, 17, 24 and 31 post-treatment, three New Jersey light traps were set up in the adjacent neighborhood around Ely Basins at 2090 South Baker Avenue, 1730 East Philadelphia Street and 2077 South Vineyard Avenue, Ontario, CA (Figure 2). Traps were operated overnight, adult midges caught were collected and processed and the major species were identified to genus. To sample the immature aquatic stages, a scoop sampler measured 8"x 4" x1" was fabricated in-house. The top layers of the sediment were sampled using this scoop; about 32 cubic inches of sediment were collected in each sample. Larval sampling was conducted

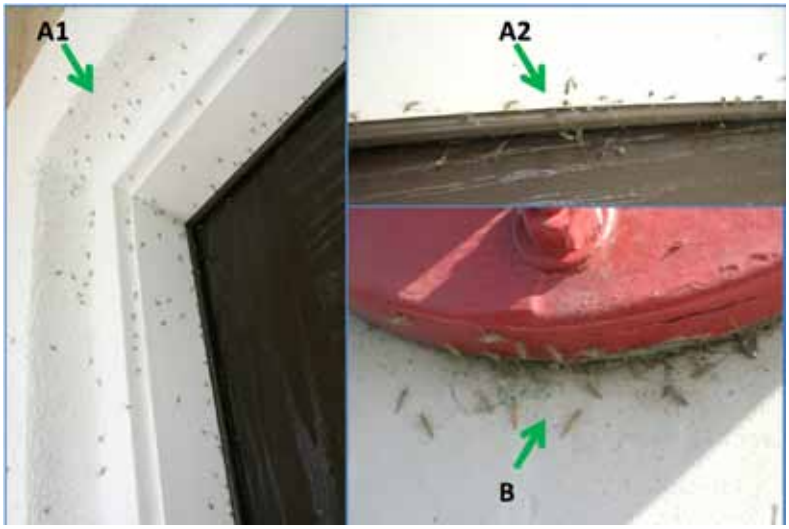

Figure 1. Infestation of chironomid midges – Adults resting on doorways (A) and fresh paint on the wall (B) (Ontario, CA, 2007).

Figure 2. Overview of Ely Basin location and sampling sites (Upper - New Jersey traps: Yellow pins; Lower - Larval sampling: Red pins) (Ontario, CA, 2007).

Figure 3. Overview of Turner Basin location, layout and sampling sites (Upper - New Jersey traps: Yellow pins; Lower - Larval sampling: Red pins) (Ontario, CA, 2007).

pre-treatment and at 11 days post-treatment. On each sampling day, eight samples were collected three feet from the shoreline of each of the three basins (Figure 2). Samples were transferred to a five gallon bucket containing three gallons of water from the basin; samples were thoroughly mixed with water to dislodge the midge larvae. Supernatant containing light debris and larvae was poured through a modified handheld fish net with mesh size of 300 micrometers. Retained larvae and light debris were transferred to a one liter plastic jar containing 0.5 liter of water from the basin. Samples were stored in a 20 gallon Igloo with blue ice refrigerant during transportation. Samples were processed on the same day if schedule allowed or stored in the refrigerator (40-45°F) and processed on the following day. Midge larvae were sorted, identified to genus and instar.

Turner Basins - The same adult sampling technique was used in the neighborhood around Turner Basins, where sampling was conducted on day 11 pre-treatment and days 5, 16, 22 and 29 post-treatment. Four sampling locations were 1009, 1015 and 1019 South Archibald Avenue, as well as 2503 East Smiderle Loop, Ontario, CA (Figure 3). Larvae in Turner Basins were sampled in the same manner as in Ely Basins, except six samples (Figure 3) were collected from each of five basins pre-treatment and day 18 post-treatment.

RESULTS

In the neighborhood immediately adjacent to the Ely Basins, species in two chironomid genera, *Chironomus* and *Crocotpus*, were the most abundant midges. Other genera of non-biting midges, as well as other arthropods, were occasionally encountered in very small numbers in the light traps; data on these organisms were excluded from our analysis. Adult *Chironomus* spp. were found at the highest densities (n = 290.7 per trap night) on day 12 pre-treatment. densities declined substantially to 15.0, 35.0, 29.7 and 26.0/trap night, with corresponding number reductions of 94.8, 88.0, 89.8 and 91.1% on days 4, 17, 24 and 31 post-treatment, respectively. Adult *Crocotpus* spp. were found in lower numbers in the light traps: 84.3/trap night pre-treatment, and 9.3, 13.7, 3.7 and 8.0/trap night on the same sampling days post-treatment. Population reduction was between 83.7 and 95.6% post-treatment (Figure 4). The same two midge genera, *Chironomus* and *Crocotpus,* were found in larval samples. Larval populations declined significantly on day 11 post-treatment for both *Chironomus* and *Crocotpus*. The former declined from 89.2 to 2.9/scoop for 1st and 2nd instars (96.7% reduction), and from 106.5 to 1.8/scoop for 3rd and 4th instars (98.3% reduction). The latter declined from 7.0 to 1.6/scoop for 1st and 2nd instars (78.9% reduction), and from 7.3 to 0.6/scoop for 3rd and 4th instars (91.8% reduction) (Figure 5). Pupal counts were not considered here as their numbers were very low throughout test period.

The same two genera of chironomids dominated adult midge samples collected by New Jersey light traps in the neighborhoods around Turner Basins in Ontario, CA. The counts, however, were much higher than those in Ely Basins, particularly for *Crocotpus* spp. On day 11 pre-treatment, the population of *Chironomus* spp. was as high as 410.3/trap night, but declined considerably to 32.0, 72.5, 57.0 and 56.8/trap night with population reductions of 92.2, 82.3, 86.1 and 86.2% on days 5, 16, 22 and 29 post-treatment, respectively. Populations of *Crocotpus* spp. were also reduced significantly from 226.3/trap night to 21.3, 44.4, 42.8 and 38.3/trap night with population reductions of 90.6, 80.4, 81.1 and 83.1% on days 5, 16, 22 and 29 post-treatment, respectively (Figure 6). Larval populations in Turner Basins pre-treatment were higher than in Ely Basins: 155.1/scoop for 1st-2nd instars and 244.9/scoop for 3rd-4th instars for *Chironomus* spp.; 105.2/scoop for 1st-2nd instars and 200.1/scoop for 3rd-4th instars for *Crocotpus* spp. Populations of species in both genera were reduced significantly 18 days post-treatment: the early instars of *Chironomus* spp. declined to 25.1/scoop (83.8% reduction) and the late instars to 34.9/scoop (85.7% reduction); the early instars of *Crocotpus* spp. declined to 25.7/scoop (75.6% reduction) and late instars to 30.0/scoop (85.0% reduction) (Figure 7). As in Ely Basins, pupal counts were negligible; therefore, they are not included in this paper.

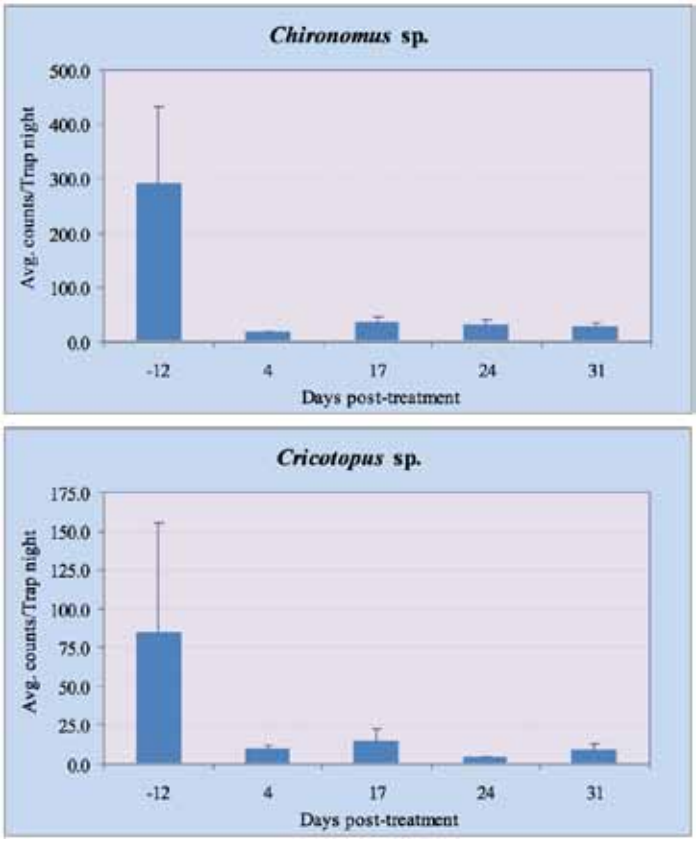

Figure 4. Efficacy of Dimilin® 25W against Chironomid midges in Ely Basins (Ontario, CA. 2007) as indicated by adult populations.

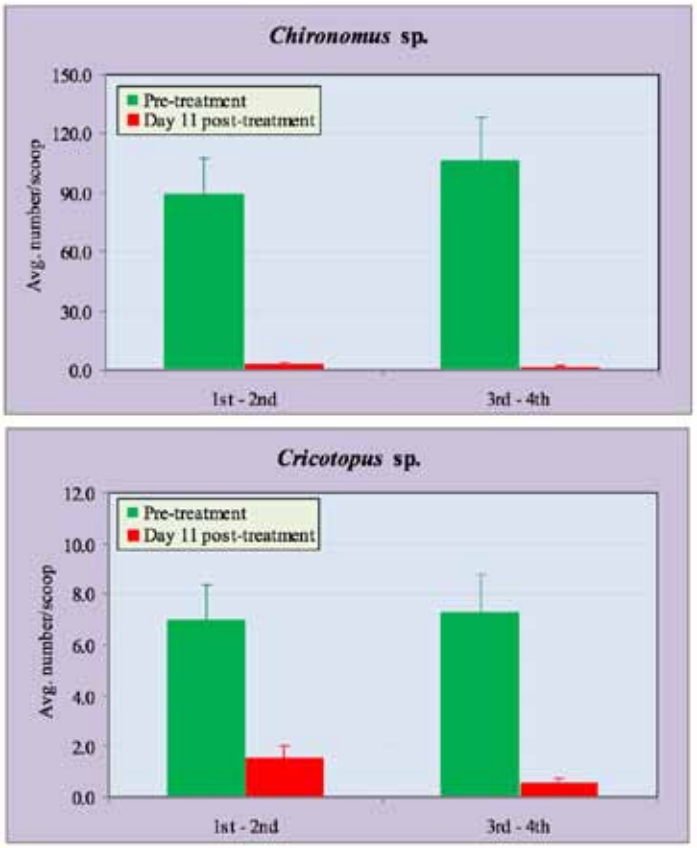

Figure 5. Efficacy of Dimilin® 25W against Chironomid midges in Ely Basins (Ontario, CA. 2007) as indicated by larval populations.

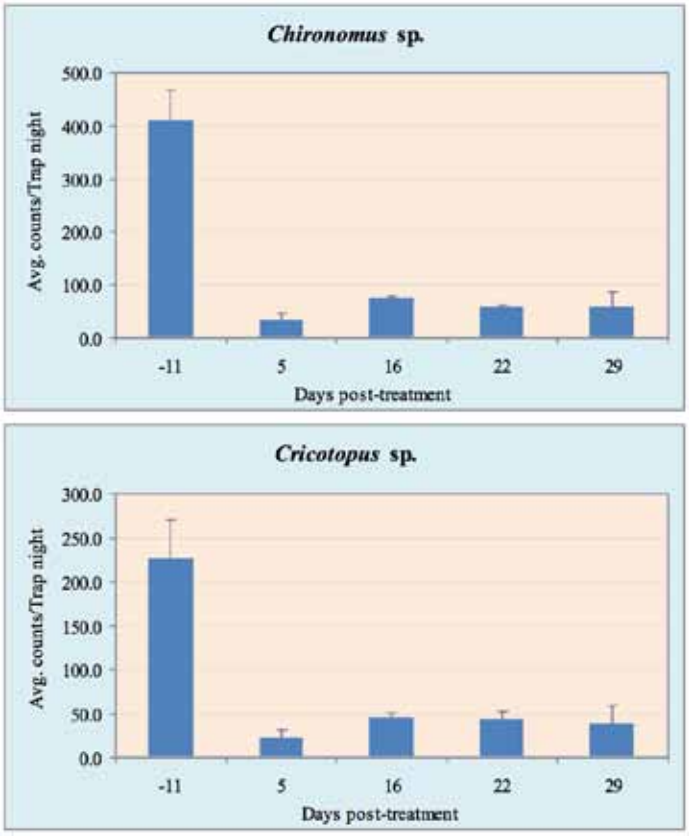

Figure 6. Efficacy of Dimilin® 25W against Chironomid midges in Turner Basins (Ontario, CA. 2007) as indicated by adult populations.

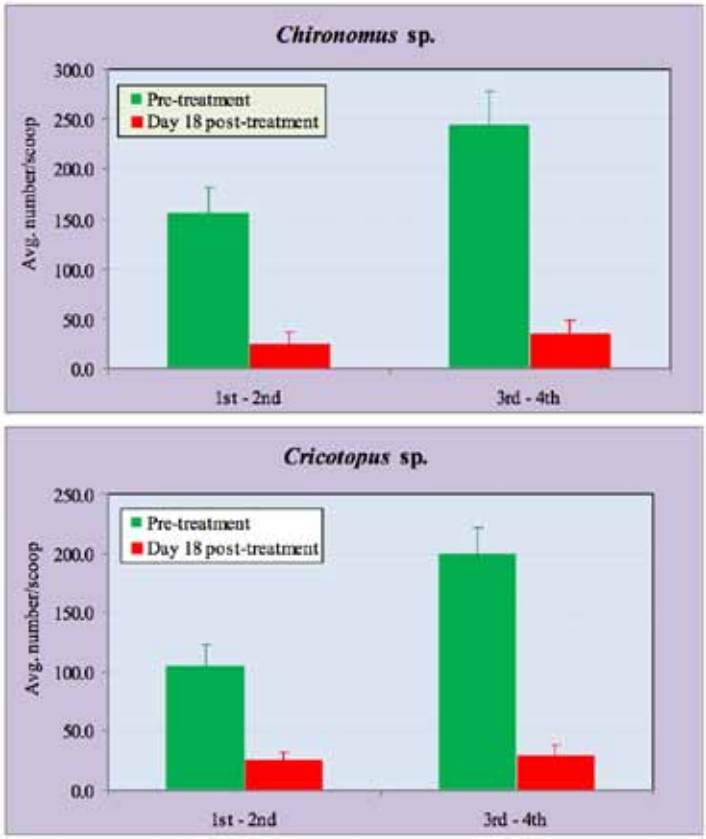

Figure 7. Efficacy of Dimilin® 25W against Chironomid midges in Turner Basins (Ontario, CA. 2007) as indicated by larval populations.

DISCUSSION AND SUMMARY

Chironomid midges are highly diverse aquatic macro-invertebrates in terms of species and distribution. They are beneficial in many ways such as serving as food sources for other beneficial aquatic invertebrates, as an indicator of water quality in the habitats they colonize and providing hints for historical climatic and environmental changes. However, public nuisance problems and economic losses caused by midges are worldwide. For instance, male midges swarm in the millions at dusk and dawn and may find their way into human mouth, ears and eyes or even respiratory system. Large adult populations can limit outdoor recreational and leisure activities, particularly for human populations residing in the areas adjacent to ponds and lakes (M. S. Mulla, personal communication). Midges may enter homes and buildings and rest on surfaces, attracting spiders and other predators into human dwellings and business structures. Dead midges are undesirable for their unpleasant, fishy odor which either impacts the quality of life in households or causes economic loss in tourism businesses. Large numbers of resting and dead midges on automobile windshields and headlights may block the view of the drivers and create unsafe driving condition (Mulla and Su, unpublished data). Massive numbers of live or freshly dead midges on the road may also pose a slippery driving hazard. In urban areas with large open aquatic habitats, outdoor lighting fixtures are often surrounded by swarming midges or covered by resting and dead midges, impairing their function and increasing maintenance costs. For unknown reasons, midges are also attracted to fresh paint on automobiles, homes and buildings (Su and Cheng, Personal observations) and other surfaces. Paint jobs are often ruined if the midges land on freshly painted surfaces. Contamination of food and medicine by midge body parts also occurs, and there are often complaints from areas around sewage treatment plants where high densities of larval midges live in the eutrified waters. Moreover, midge larvae can clog the filters and increase maintenance costs (Mulla, Chaney and Su, Personal observations).

Since the mid 1980s, clinical studies have attempted to link allergic disorders such as respiratory asthma, rhinitis and contact dermatitis with chironomid midge infestations worldwide, particularly in Japan and Latin America (Baur 1992, Cabrerizo et al. 1996). Both larval and adult stages contain significant allergens (Matsuoka et al. 1990). Positive hypersensitivity test results have been reported against midges (McHugh et al. 1988, Galindo et al. 1998, Eriksson et al. 1989, Matsubara et al. 1989, Fernández et al. 1996), and allergies to larvae have been reported for people who fish recreationally (Wu et al. 2005). Occupational allergy to adult chironomid midges has also been documented among environmental researchers, aquarists and fish food handlers (Liebers et al. 1993, Teranishi et al. 1995, López et al. 1996, Aldunate et al. 1999, Cabrerizo Ballesteros et al. 2006).

Bronchial asthma is the most common of all allergic disorders related to midges (Igarashi et al. 1987, Sakai et al. 1993), and a connection between midge-derived allergens and asthmatic cases has been established by ELISA (Kimura et al. 1990), skin test and radioallergosorbent (Ito et al. 1986). Ig G and Ig E antibodies against the midges were detected in asthmatic patients (Matsuoka et al. 1988, Yamashita et al. 1989a, 1997, Witteman et al. 1995). Ig E-mediated release of histamine (Yamashita et al. 1989b, Pascual et al. 1997) is involved in the subsequent process of allergic reaction. Supportive spatial epidemiological evidence of bronchial asthma induced by chironomid midges was established by Hirabayashi et al. (1997) around a hypereutrophic lake in Japan. Further studies revealed that the hemoglobins (*Chi t* I) are common antigenic determinants relevant to environmental, occupational, and hobby-related allergens causing bronchial asthma (Cranston et al. 1983, Tee et al. 1985, Cranston 1988, Kitani et al. 1989, Baur and Liebers 1992, Van Kampen et al. 1994, Liebers and Baur 1994). *Chironomus thummi* expresses 12 homologous hemoglobin components, I, IA, IIbeta, III, IIIA, IV, VI, VIIA, VIIB, VIII, IX and X, each consisting of 136 - 151 amino acids with molecular weights of 16,000 - 32,000 kDa. These non-cell bound chromo-proteins are synthesized in the larval fat body and account for approximately 80% of the total proteins in the hemolymph (Liebers and Baur 1994). Other allergic disorders related to the midges are rhinitis (Ogino et al. 1990) and dermatitis (Galindo et al. 1996, Brasch et al. 1992, Eriksson and Schou 1992).

Considering the significance of Chironomid midges discussed above, initial studies on sampling and control of the midges were conducted in response to the complaints from the general public in City of Ontario, CA in 2007. In the neighborhoods immediately adjacent to the Ely Basins and Turner Basins, two predominant genera of chironomid midges (Diptera: Chironomidae) were found in the adult and larval samples: *Chironomus* and *Crocotpus*. Midge populations were more abundant in Turner Basins than in Ely Basins, especially *Crocotpus* spp., which might be attributable to the sandy sediment, deeper water, lower organic load and less active eutrophication as compared with Ely Basins. *Chironomus* spp. seem to be able to thrive in a wide range aquatic environments, even in semi-aquatic habitats; in contrast, *Crocotpus* spp. are mostly found in habitats with relatively clean water (Mulla, Su and Chaney, unpublished data). Midge larvae are benthic dwellers, accurate sampling of populations can be challenging and laborious. The in-house made scoop sampler provided us with a method for quantifying samples which allowed us to compare the efficacy of pesticide control. Midge larvae must be dislodged thoroughly from the sediment by active agitations as they mostly dwell inside tubes in the substrate through which water flows and carries food to the larvae. Pupal counts were not considered in this communication since their numbers were very low throughout test period. The pupal stage of the midges is very brief, and individuals move to the water surface after pupation to emerge as adults. Both adults and larvae can be used as indicators of control efficacy if populations are accurately sampled. Proper sampling of the benthic sediments pre- and post-treatment provides data of the control efficacy of pesticide applications. Sampling adults in areas immediately adjacent to larval habitats is effective because adults rarely fly long distances.

The Dimilin 25W containing 25% chitin synthesis inhibitor diflubenzuron, when applied at 16 oz/acre, provided excellent control as shown by reductions in both larval and adult populations. It seemed that comparable control efficacy was achieved against *Chironomus* spp. and *Crocotpus* spp. Larvicidal efficacy in Ely Basins was slightly higher than in Turner Basins, which may be related to the deeper water and greater pesticide dilution in Turner Basins. Diflubenzuron was more effective than methoprene (Tabaru et al. 1987) and is recommended for controlling chironomid midges. Some non-target fresh water macro-invertebrates such as Notonectidae and Corixidae were noticed in larval samples, but no quantitative studies were conducted with this regard.

In summary, chironomid midges are an important group of aquatic macro-invertebrates that play beneficial roles in the habitats, but they also can be a public nuisance and generate significant allergens linked with bronchial asthma and other allergic disorders. Locating and sampling breeding sources of these benthic invertebrates can be challenging and time-consuming. Although, other products (i.e., *Bacillus thuringiensis israelensis* [*Bti*]) and insect growth regulator such as juvenile hormone analog methoprene are also labeled for midge control, the concentration of each needed to achieve proper control of midge populations can be very costly, and the efficacy may not reach the same level as the chitin synthesis inhibitor diflubenzuron. It appears that the diflubenzuron-based product is cost-effective and provides operationally acceptable levels of control both initially and residually.

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Impacts of Heat on Product Efficacy

Gabriela Perezchica-Harvey and Jennifer A. Henke

Coachella Valley Mosquito and Vector Control District
43-420 Trader Place, Indio, California, 92201 USA
gharvey@cvmvcd.org, 760-342-8287

ABSTRACT: Chemical products used for mosquito control were assayed to determine if they maintained the efficacy claimed by the product label after being subjected to outdoor storage conditions for certain periods of time. Samples of VectoBac G® and VectoMax CG® were placed in the chemical lock box of Coachella Valley Mosquito and Vector Control District vehicle and exposed to local daytime temperatures for a 6 hour period for 1, 2, 3, 4 or 5 days. Bioassays were performed in glass trays with 1.5 liters of water. Twenty-five, laboratory reared, second-instar *Culex quinquefasciatus* larvae were treated using the low-range field application rate according to the label. The bioassay results showed an average of 100% larval mortality, and no significant difference between the products that were stored up to 6 hours at a time and up to 5 days in the heat of a chemical lock box of a vehicle and those kept in the cold storage.

INTRODUCTION

Maintaining a cool, dry place is essential for storing most chemical control products. Yet, the summer daytime temperatures in the Coachella Valley are extreme, often as high as 49ºC (120ºF) when vector control technicians are returning to the District. While in the field, vector control technicians keep products locked in chemical lock boxes of their vehicles, and at the end of the day the products are returned to cold storage (11ºC [52ºF]). Given that heat can reduce the effectiveness of several types of microbial insecticides (Weinzierl et al. 2005), the goal was to determine if the District's operational procedure was impacting the efficacy of control products. This study evaluated the efficacy of VectoBac G and VectoMax CG after being exposed to outdoor lock box storage temperatures in the Coachella Valley.

MATERIALS, METHODS AND RESULTS

Aliquots were prepared from a new package of VectoBac G (Bti – Lot # 204-096-N8) and VectoMax CG (Bti and Bs – Lot # 215-202-N8) that the District received. Each aliquot was made by weighing 500 mg of each product and placing the samples into individually labeled plastic Ziploc® bags. Aliquots were placed in the chemical lock box of a District vehicle for 6 hours each day and returned to cold storage at the end of each work day. The products were stored in the chemical lock boxes for 1, 2, 3, 4 or 5 days, for a total of 6, 12, 18, 24 or 30 hours, respectively. Three bags of VectoBac G and three of VectoMax CG were kept in the cold storage for the duration of the experiment as controls. The temperature inside the chemical lock box was recorded at 1300 and 1500 hours daily (Table 1).

	1300 hours		1500 hours	
Date	ºF	ºC	ºF	ºC
9/26/12	112	44.8	102	39.2
9/27/12	110	43.7	98	37.0
9/28/12	102	39.2	100	38.1
10/1/12	120	49.3	116	47.0
10/2/12	114	45.9	108	42.6

Table 1. Daily recorded temperatures inside the truck lock box at 1300 (1:00 PM) and 1500 (3:00 PM).

A bioassay was done in the laboratory rearing room with air temperature at 27.8ºC (82ºF) and a relative humidity at 40%. Each glass tray was filled with 1.5 liters of reverse osmosis (RO) water and twenty-five, 2nd-instar *Culex quinquefasciatus* larvae were pipetted into each tray. Eighteen trays each received 35 mg of VectoMax CG, eighteen trays each received 17 mg of VectoBac G and six trays received no product (controls). The rates used were label-recommended low rates (Valent BioSciences Corporation 2012). The larvae were fed ¼ teaspoon of larval food, and mortality was recorded at 24 hours.

The temperatures inside the chemical lock box ranged from 36.7 – 48.9°C (98 – 120ºF) at 1300 and 1500 hours, and the temperature was higher at 1300 hours than at 1500 hours daily. There was 100% mortality with the aliquots that were exposed to the outdoor temperatures for 6 hours at 1, 2, 3, 4, and 5 days and with those that were kept in the cold storage (Figure 1).

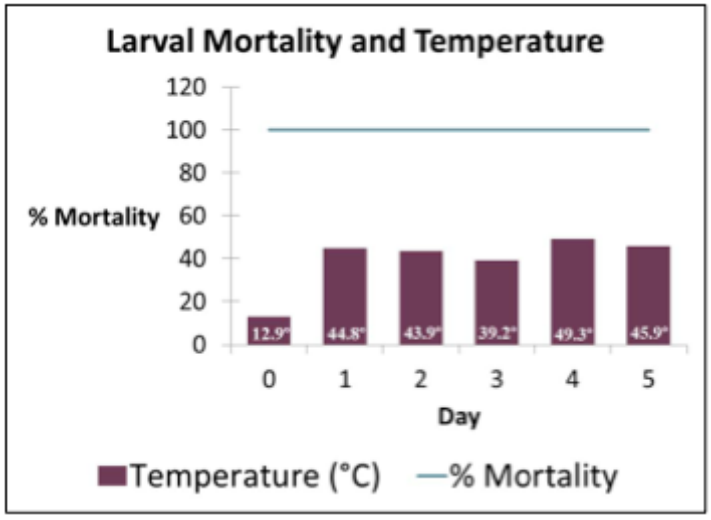

Figure 1. Larval mortality of VectoBac G and VectoMax CG when control products were exposed to daytime temperatures for 0 to 5 days in the Coachella Valley in September 2012. Both products were 100% effective at all exposure times.

CONCLUSIONS

VectoMax CG and VectoBac G, two microbial larvicides that were exposed for up to 5 days for 6 hours per day in the lock box to the Coachella Valley outdoor temperatures, remained effective at controlling mosquito larvae although the labels for both products require storage in a cool, dry place (Valent BioSciences Corporation 2012). Vector Control Technicians will continue to store the two tested control products in the chemical lock box of District work trucks throughout the work day, since the local high temperatures of the Coachella Valley did not impact their effectiveness. Future work will consider how storage overnight in the lock box may impact product efficacy.

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ULV Ground Larviciding with VectoBac WDG®: *Aedes sierrensis* Project – Year Two

Valkyrie P. Kimball[1] and Bill Reynolds[2]

[1] *Marin/Sonoma Mosquito & Vector Control District, Cotati, CA 94931, piper@msmosquito.com,* [2]*Leading Edge Associates, LLC, 1073 Lentini Drive, Waynesville, NC 28785, breynolds@leateam.com*

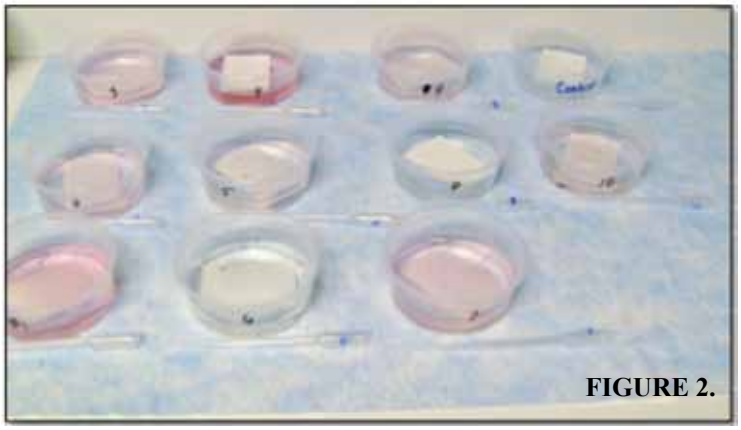
ABSTRACT: Over the past several years, area-wide larviciding methods have been developed to control container breeding *Aedes* species, particularly *Aedes aegypti* and *Aedes albopictus*. In 2010 a three-year project was launched to determine if these same application techniques could be efficacious against the Western Treehole Mosquito, *Aedes sierrensis*. After two years of several field applications using VectoBac WDG®*, the Marin/Sonoma Mosquito and Vector Control District (MSMVCD) was able to reduce the droplet spectrum from 190-409 microns (µ) to a range of 40 - 80 µ using two modified Curtis Dyna-Fog® sprayers – the truck mounted LV8™ and the Twister™ backpack sprayers**. The reduction of droplet size allowed for increased availability and probability of a droplet to penetrate individual treeholes, thereby increasing the amount of larvicide available for controlling larval populations.

INTRODUCTION

On May 22nd and June 8th, 2012 Leading Edge Associates, in cooperation with Marin/Sonoma Mosquito & Vector Control District (MSMVCD), deployed equipment, personnel, techniques and technologies required to monitor and measure the deposition of droplets applied by a Curtis Dyna-Fog® LV8™ ULV truck mounted fogger (May 22nd) and a Curtis Dyna-Fog® Twister™ backpack sprayer (June 8th). The applications were made within the treehole canopy habitat known as Rainbow Ranch, Healdsburg, CA. Efficacy was also monitored through standard mortality protocols. These applications were conducted to capitalize on data derived from the previous two years' work and to complete the following objectives: (1) monitor droplet deposition and dispersal throughout the application site [i.e., treehole breeding sites], (2) measure volume of deposition in gallons per acre, (3) apply a more efficient droplet spectrum as compared to previous years' applications [Year 1 $DV._5$ = 190-409 µ, Year 2 $DV._5$ = 35-80 µ], (4) measure the droplet spectrum as calculated by the $DV._1$, $DV._5$ & $DV._9$, (5) determine if the application techniques, methods and equipment successfully applied the active compound in a uniform and required manner needed to achieve efficacious results, (6) measure the droplet l and deposition at or in the treehole and (7) measure larval mortality associated with droplet deposition.

MATERIALS AND METHODS

Field sampling for larval bioassay and droplet analysis. An array of kromekote cards was deployed for the purpose of sampling deposition and dispersal of the spray cloud. At each treehole location, droplet spectrum, droplet density and volume deposition were evaluated on passive sampling cards (cards placed in plastic cups) and on active samplers (rotating impingers) as these values varied among treehole locations (see Figure 1). Following the application, the cups were collected with the droplet exposed kromekote cards left inside the cup. 120 ml of filtered water and nine to ten colony reared *Aedes sierrensis* larvae (third and fourth instar) were added to the cups in the laboratory to measure mortality associated with the droplet deposition (see Figure 2). Standard efficacy protocols were followed and included controls that measured the mortality associated with an unexposed kromekote card to larvae. All kromekote cards were collected 30 minutes subsequent to the spray off time. The following image is a sample of a kromekote card exposed during the application (Figure 3). All cards were digitally scanned, processed and analyzed using auto-imaging and droplet measuring technology, DropVision® AG (Figure 4).



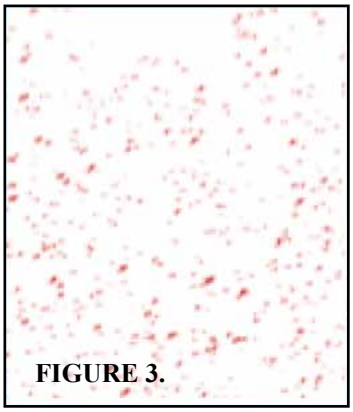
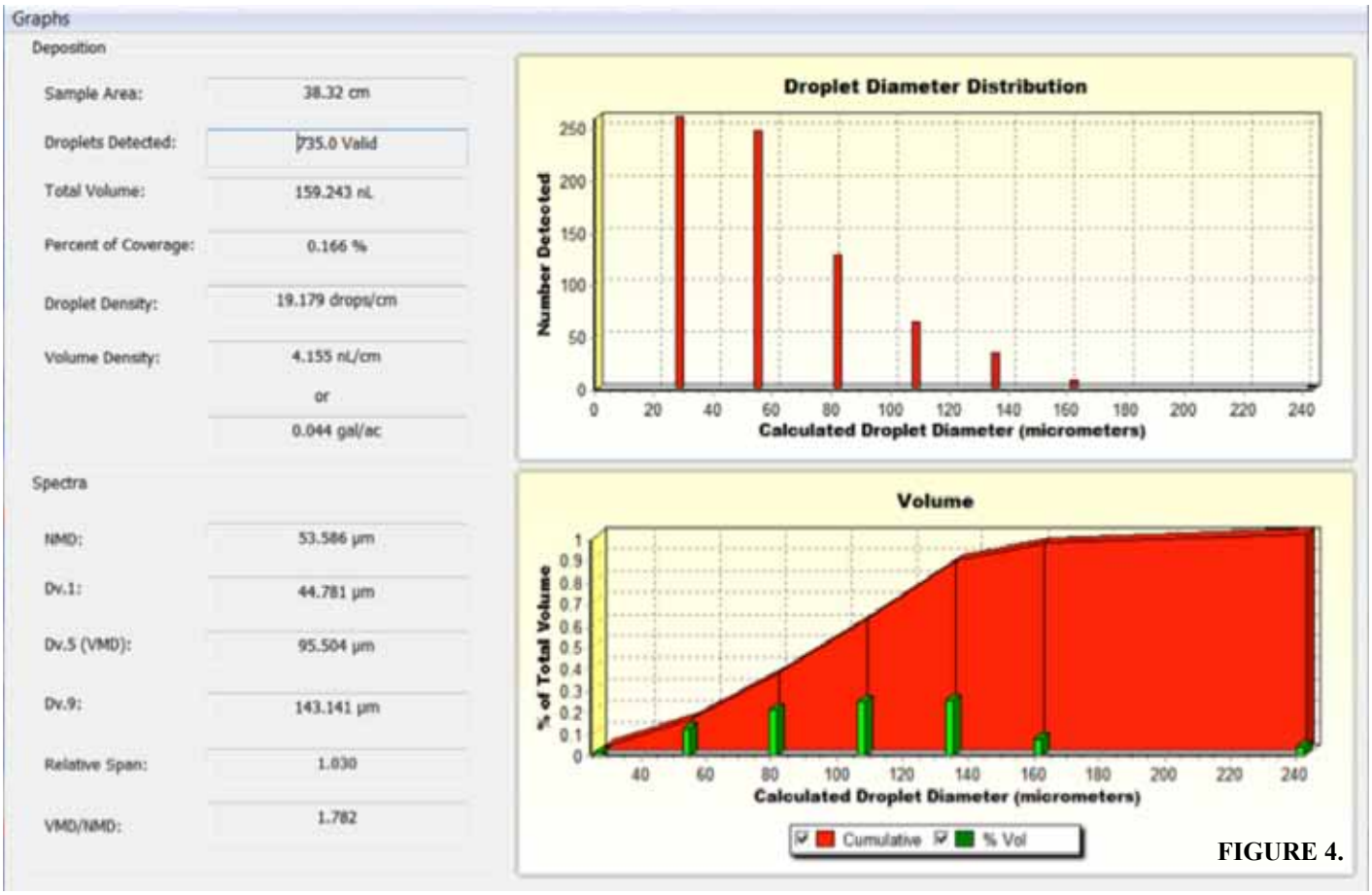


Figure 1. Spinning impinger placed at treehole with bioassay cup inside.

Figure 2. Bioassay cups with Kromekote cards stained with red dyed droplets.

Figure 3. Kromekote cards with droplets.

Figure 4. DropVission AG™ software for droplet analysis.



Equipment configuration of LV8 truck mounted sprayer. The Curtis Dyna-Fog LV8 truck mounted sprayer was calibrated and configured according to the following settings:

- RPM setting: Max RPM
- Flow control: Needle valve max setting
- Flow valve setting: 244 ozs./min
- Boom Pressure setting: 9.0 psi
- Application speed: 5 mph
- Acres treated/minute: 3.03
- Tank mix: 3,791 g VectoBac WDG: 9 gal. water: 758 g #40 red dye
- Application rate:

Ounces applied per minute	244
Acres treated per minute	3.03030303
Grams WDG/ounce	3.290798611
Grams applied/minute	802.9548611
Grams applied/acre	264.9751042

- Nozzle type: LV8 ULV (new boom, 2012)
- Number of nozzles: 8
- Swath width: 300’
- Lane separation: 300’
- Nozzle baffle: not applicable

LV8 truck mounted application. The following table (Table 1) reflects the meteorological conditions during the single spray pass using the Curtis Dyna-Fog LV8 ULV truck mounted fogger. Figure 4 is a diagram which reflects the test site location for the application using the LV8. A pre-determined swath width of 300 feet was used for the single spray run (as represented by the one red line at the 0’ location). The applicator followed the personal protection practices and methods according to the product label. All other personnel were repositioned outside the application swath widths.

Spray Run #	Spray Run Direction (degrees)	Time (pm)	Wind Vector (degrees)	Wind Speed (MPH)	Temperature (°F)	Humidity (%)
1	168	11:20	285	3.4	72.6	41

Table 1. Weather conditions during LV8 application.

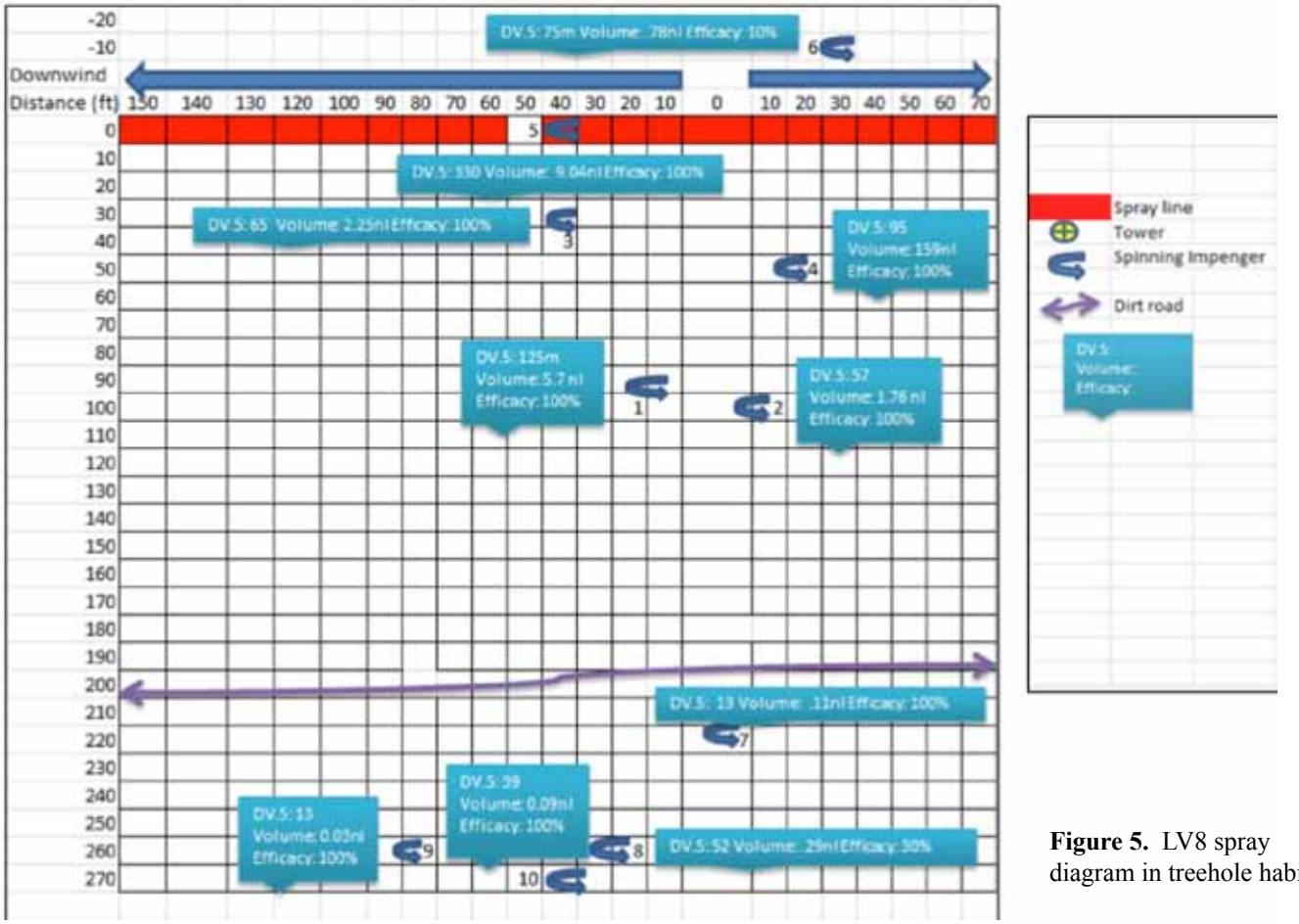


Figure 5. LV8 spray diagram in treehole habitat.

Equipment configuration of Twister backpack sprayer. The Curtis Dyna-Fog Twister backpack sprayer was calibrated and configured according to the following settings:

- RPM setting: Max RPM
- Flow control: Venturi
- Flow valve setting: No restrictor valve used - full flow rate achieved
- Application speed: 1 mph (restricted to 1 mph based on max flow rate of Twister)
- Acres treated/minute: 0.101 acres (1 mph*50'/495)
- Tank mix: 1,238 g VectoBac WDG: 6.35 l. water: 154.8 g #40 red dye
- Application Rates Chart:

Ounces applied per minute	6.6
Acres treated per minute	0.101010101
Grams WDG/ounce	3.437080122
Grams applied/minute	22.6847288
Grams applied/acre	224.5788152

- Nozzle type: Twister ULV
- Number of nozzles: 1
- Swath width: 50'
- Lane separation: 50'
- Nozzle baffle: not applicable

Due to a pre-determined swath width of 50 feet, several spray passes were necessary to cover the same amount of acreage as the truck mounted LV8 sprayer at the same treatment location. Meteorological measurements recorded during the five spray passes from the backpack application are noted in Table 2.

Application Date: June 8, 2012 Replication: 1 Spray on time: 12:21 pm / Spray off time: 12:30 Passes: 5

Location: Rainbow Ranch, Sonoma County, California

Spray pass:	Wind Dir °	Wind Speed (MPH)	Temp F °	RH%	Total Spray Time (min.)
1	168	3.5	71	36%	2
2	346	2.0	71	36%	3
3	168	3.5	71	36%	3
4	346	3.0	71	36%	3
5	168	3.6	71	36%	3

Table 2. Weather conditions during Twister application

Twister backpack application. The following diagram (Figure 4) reflects the test site location for the application using the Curtis Dyna-Fog Twister backpack spayer. Five spray passes were applied at 50'

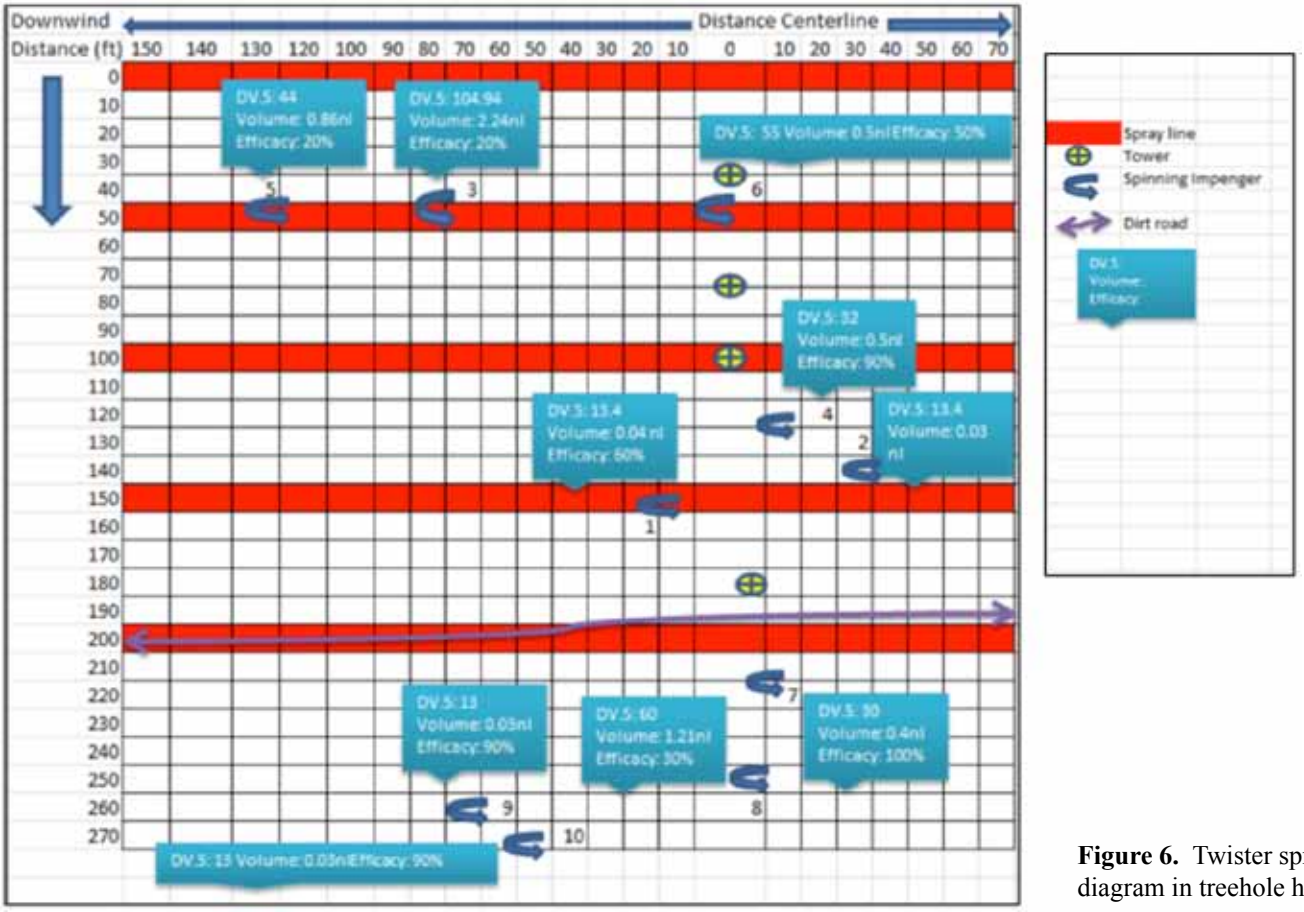


Figure 6. Twister spray diagram in treehole habitat.

intervals (as represented by the red lines at 1, 50, 100, 150 and 200 feet). Again, the applicator followed the personal protection practices and methods according to the product label. All other personnel were repositioned outside the application swath widths.

RESULTS

LV8 sprayer. The following table (Table 3) provides the deposition results from the spray application using the LV8 sprayer at each treehole location using the passive sampling method of the kromekote cards placed in the plastic cups. Table 4 reflects the bioassay efficacy associated with the deposition on the kromekote cards within the plastic cups at 24 & 48 hours post application.

Twister sprayer. The following table (Table 5) provides the deposition results at each treehole location using the passive sampling method of the kromekote cards placed in the plastic cups for the backpack sprayer. Table 6 reflects the larval efficacy associated with the deposition on the kromekote cards within the plastic cups at 24 & 48 hours post application.

Droplet Data (Cards placed inside plastic cups at treehole) from LV8 ULV truck mounted sprayer					
	# droplets	Volume (nL)	VMD	Droplet Density (drops/cm2)	Volume Density (gal/ac)
Cup 1	33	5.7	125.49	0.86	0.00
Cup 2	44	1.76	57.06	1.15	0.00
Cup 3	47	2.25	64.67	1.23	0.00
Cup 4	735	159.24	95.5	19.18	0.04
Cup 5	155	904.3	330.26	4.4	0.25
Cup 6	7	0.78	74.94	0.18	0.00
Cup 7	11	0.11	13.4	0.29	0.00
Cup 8	3	0.29	51.6	0.08	0.00
Cup 9	3	0.03	13.4	0.08	0.00
Cup 10	2	0.09	38.52	0.05	0.00

Table 3. Droplet data for LV8 application.

Bioassay mortality counts with VectoBac WDG following application with LV8			
Number dead out of 9 to 10 3rd and 4th instar *Aedes sierrensis* colony raised larvae			
Set up date: May 22, 2012 3:20 pm	Read date & time: May 23, 2012 3:20 pm	Read date & time: May 24, 2012 3:20 pm	% Mortality
Cup 1	10	10	100%
Cup 2	10	10	100%
Cup 3	9 out of 9	9 out of 9	100%
Cup 4	10	10	100%
Cup 5	10	10	100%
Cup 6	0	1	10%
Cup 7	10	10	100%
Cup 8	3	3	30%
Cup 9	10	10	100%
Cup 10	10	10	100%
Average % Mortality			**84%**
Control	0	0	0%

Table 4. Larval mortality after LV8 application.

Droplet Data (Cards placed inside plastic cups at treehole locations) from Twister ULV backpack sprayer					
	# droplets	Volume (nL)	VMD	Droplet Density (drops/cm2)	Volume Density (gal/ac)
Cup 1	4.0	0.04	13.4	0.10	0.00
Cup 2	3.0	0.03	13.4	0.08	0.00
Cup 3	3.0	2.27	104.94	0.08	0.00
Cup 4	23.0	0.51	32.24	0.6	0.00
Cup 5	17.0	0.86	44.93	0.44	0.00
Cup 6	10.0	0.5	55.57	0.26	0.00
Cup 7	7.0	0.14	30.14	0.18	0.00
Cup 8	14.0	1.21	60.53	0.37	0.00
Cup 9	3.0	0.03	13.4	0.08	0.00
Cup 10	3.0	0.03	13.4	0.08	0.00

Table 5. Droplet data for Twister application.

Bioassay mortality counts with VectoBac WDG following application with LV8			
Number dead out of 9 to 10 3rd and 4th instar *Aedes sierrensis* colony raised larvae			
Set up date: May 22, 2012 3:20 pm	Read date & time: May 23, 2012 3:20 pm	Read date & time: May 24, 2012 3:20 pm	% Mortality
Cup 1	10	10	100%
Cup 2	10	10	100%
Cup 3	9 out of 9	9 out of 9	100%
Cup 4	10	10	100%
Cup 5	10	10	100%
Cup 6	0	1	10%
Cup 7	10	10	100%
Cup 8	3	3	30%
Cup 9	10	10	100%
Cup 10	10	10	100%
Average % Mortality			**84%**
Control	0	0	0%

Table 6. Larval mortality after Twister application.

Mortality and ffficacy comparison of the Twister backpack sprayer and the LV8 truck mounted sprayer. The following table (Table 7) and bar graph (Figure 5) compare the efficacy of the two methods of applied engineering using the Curtis Dyna-Fog ULV LV8 truck mounted sprayer and the Curtis Dyna-Fog ULV Twister backpack sprayer. The environmental conditions, application techniques, application rates and all other variables were within reason the same, allowing this comparison to be unbiased.

Distance	LV % Mortality	Twister % Mortality
50	100%	30%
120	100%	30%
130	100%	70%
160	100%	60%
220	100%	100%
260	30%	55%
270	100%	90%

Table 7. Mortality comparison of Twister backpack sprayer and LV8 ULV truck mounted sprayer based upon distance from spray line.

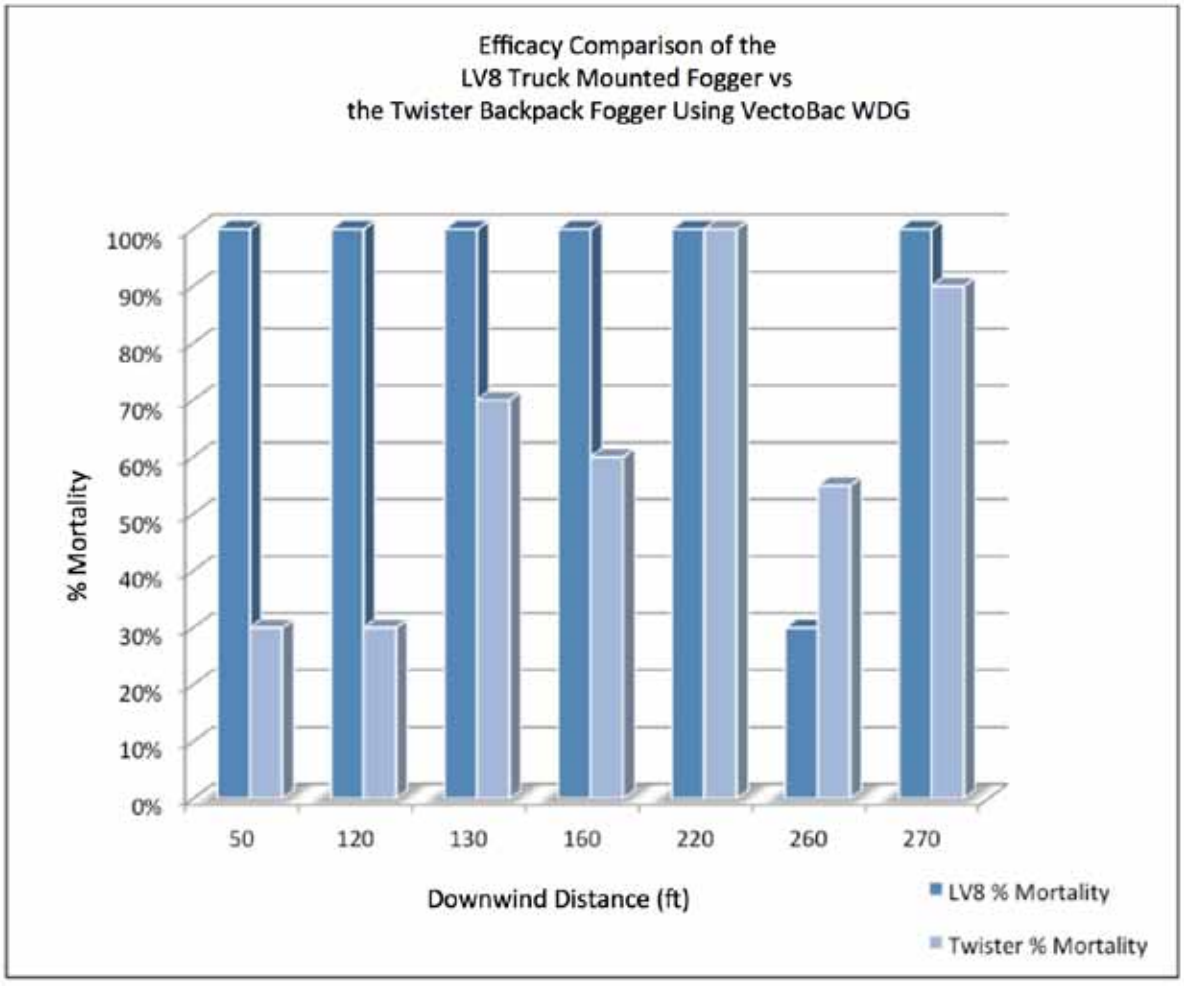


Figure 7. Efficacy comparison of LV8 and Twister sprayers.

DISCUSSION

2011 field assessments. In 2011, spray applications using both a Stihl®**** backpack sprayer and the Curtis Dyna-Fog LV8 sprayer (supplied with the original fixed boom) were assessed and used for comparision for the 2012 field trials. A general review of the results uncovered several problems during these earlier field trials. Most remarkably noted was excessively large deposition of the product at or close to the spray line which ultimately produced an insufficient coverage of the intended treatment area. Additionally, the average droplet size was larger than desired ($DV._5$ = 190-409 microns). These large sized droplets were ineffective in dispersing through the desired application site, with most depositing on the ground and not into the targeted treeholes. Finally, the effective swath width (100 feet) achieved in open field characterization with the Stihl sprayer was inconsistent with an effective swath width (< 50 feet) as determined in actual field applications (canopied area). Less than desirable results from the 2011 trials prompted recommending reducing droplet spectrum size, increasing atomization efficiency, comparing efficacy to equipment configuration and droplet specturm and measuring efficacy and droplet deposition by pairing kromekote cards inside individual treeholes.

2012 field assessments. Efficacy results from the 2012 field trials exceeded expectations. Capitalizing on the recommendations of the 2011 report, efficacy results exceeded not only our local expectations, but the results of applications occuring in other locations within the United States. By reducing the droplet spectrum from an average range of 190 - 409 microns to a range of 40-80 microns, the volume, size and availability of droplets dispersing through the entire three dimensional target site greatly increased the availability and probability of droplets penetrating the individual treeholes. The dramatic decrease in average droplet size was achieved through applied engineering techniques by altering the type and directional position of the eight atomization nozzles of the LV8 ULV fogger. Additionally, a true ULV droplet spectrum was also achieved by using a modified Curtis Dyna-Fog Twister backpack sprayer.

CONCLUSION

Although challenging, this project has proven to be extremely beneficial based on the discoveries in 2011 and the results in 2012. The positive impacts to the District from this field work will be in the deployment of the applied technologies developed in operational settings. Recommendations to transition from field trials to operational use of this innovative and successful ULV larvaciding application would be to: (1) identifiy a neighborhood[s] where adulticiding is used and apply larvacides using ULV methods, (2) measure and track the ULV larvaciding droplet spectrum during the neighborhood ULV application using active, passive and deposition sampling techniques, (3) measure the efficacy of neighborhood ULV larvaciding applications using the same bioassay protocols as developed in previous years' trials and (4) test methoprene and other larvaciding compounds identified by Marin/Sonoma MVCD in test plots using identical protocols. It is hoped the incorporation of ULV larvaciding techniques into the current adulticiding program employed by the District will result in better, more effective results, ultimately better serving the general public and the environment.

** VectoBac WDG is a registered trademark of Valent BioSciences Corp., Libertyville, IL 60048*

*** LV8 and Twister are trademarks of Curtis Dyna-Fog Ltd, Westfield, IN 46074*

**** DropVision AG is a registered trademark of Leading Edge Associates, Waynesville, NC 28785*

***** Stihl is a registered trademark of STIHL Incorporate, Virginia Beach, VA 23452*

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Pre- and Post-Adulticide EVS Trapping to Compare Effectiveness of Evening versus Morning ULV Application

John Albright, Vector Ecologist

Shasta Mosquito and Vector Control District, 19200 Latona Rd., Anderson, CA 96007

ABSTRACT: From May 4, 2011 through August 31, 2012, the Shasta Mosquito and Vector Control District set CO_2-baited traps one to two days before and after 80 ULV adulticide site treatments (160 total trap-nights). Detailed records were kept of the sex, species and counts of mosquitoes captured. The trial included 39 evening ULV events and 41morning applications. Three adulticide products were used. A total of 17,460 mosquitoes (17,104 females and 356 males) of 17 species were counted. Weather observations for all trap and treatment nights were kept as well. This preliminary report will give a rough comparison of the relative efficacy of morning versus evening ULV adulticide applications by comparing the reduction in total mosquito populations following adult mosquito control products using truck-mounted application equipment. This study has so far failed to demonstrate a significant difference in the efficacy of evening versus morning ULV treatments within the Shasta MVCD area.

INTRODUCTION

It is widely accepted dogma in mosquito control that ULV adulticide operations conducted in the evening are more effective than morning applications. This assumption is largely based upon data collected from timed mosquito-trapping devices (rotator traps) that generally show higher trap counts in post-sunset hours compared to pre-dawn. It can be hypothesized that significantly more adult mosquitoes will be killed if ULV treatments are timed to coincide with time frames associated with high trap rotator counts. However, an arguably better way to assess the effectiveness of any pest control strategy is actually to compare populations of the target organisms pre- and post-treatment and assess the relative change in adult mosquito populations associated with the treatment. This project began in May of 2011 and utilizes CO_2-baited traps set before and after ULV adulticide treatments as a way to compare the efficacy of different adult mosquito control regimens under normal field conditions. This paper compares differences between reductions in mosquito populations within Shasta Mosquito and Vector Control District depending on whether adulticiding took place post-dusk or pre-dawn.

MATERIALS AND METHODS

Products applied were Anvil® 10+10 and Zenivex® E20 at maximum label rates using truck-mounted Beecomist Pro-Mist 25HD ULV application equipment with SmartFlow® using radar ground-speed sensors. Applications were made along normal adulticiding routes and scheduled based upon normal mosquito population thresholds and weather criteria used by Shasta Mosquito and Vector Control District as part of its normal operational practices.

Bioquip® CO2-baited EVS traps were set overnight on the night directly preceding and the night directly following ULV applications of mosquito adulticide products in the direct vicinity of sites within the ULV treatment areas. Sites were selected within ~ 30 minute drive from the District office and in areas where previous EVS trapping had collected moderate to high numbers of a variety of local mosquito species. Mosquitoes were removed from the traps, identified to sex and species, counted and data recorded in an Excel spreadsheet. Post-treatment counts were compared to pre-treatment counts for all sites to determine the percent reduction in the adult mosquito population resulting from each treatment event. A single recorded treatment event would encompass three consecutive overnight activities. These consisted of setting a trap at a site, running an adulticide route that included the trapped site and follow-up trapping at the same site the night after the application. In subsequent treatments at the selected sites the time of the ULV applications was alternated between nighttime applications (~9:00 - 11:00 PM) and morning applications (~4:00 - 6:00 AM).

Data were collected from May 4, 2011 through August 31, 2012 for a total of 80 treatment events (39 nighttime treatments and 41 morning treatments). Data were compiled in a Microsoft Excel workbook for analysis of the changes in mosquito populations and the generation of graphs. Fifteen minute interval weather information for the nights of trapping and application was gathered at the District using Weatherview® software and exported to Microsoft Excel for correlation with the mosquito population statistics. Some trap counts were intentionally left out of the data analysis for this report. These exclusions include treatment events where the mosquito populations actually appeared to increase post-treatment. These data was excluded from this analysis because these results could be readily correlated to either poor pre-treatment weather conditions (e.g., wind and/or rain) compared to post-treatment conditions, or a treatment problem such as the application equipment running out of product prior to reaching the trap site location.

RESULTS

Post-treatment trap counts were noticeably lower than pre-treatment counts for all mosquito species at all locations for the great majority of application events, regardless of whether adulticiding applications took place in the pre-dawn hours or post-sunset. Data in Figure 1 represent 67 trapping treatment events (35 evening and 32 morning applications) at 21 trap sites. A total of 17,460 adult mosquitoes were caught and identified, representing 17 of the 23 common mosquito species known to occur within Shasta Mosquito and Vector Control District. Data collected to date shows a slightly higher percent reduction of mosquito populations following nighttime adulticide applications compared to pre-dawn applications at 52.43 and 51.17% respectively (Figure 1).

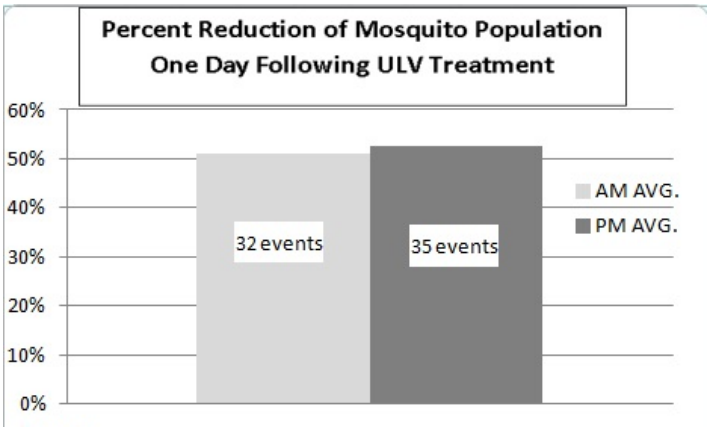

Figure 1. Percent reduction in mosquito populations one day following ULV treatment.

DISCUSSION AND CONCLUSIONS

Taken as a whole, this project has not provided support for the idea that evening applications of adulticide products show significantly greater efficacy than morning applications. Despite the large number of variables inherent in this methodology, trapping pre- and post-ULV generally showed consistent reductions in overall adult mosquito populations following normal adulticide practices in typical field conditions, regardless of the timing of the adulticide applications. Our methodology for determining efficacy has advantages over some other techniques such as cage trials because it assesses normal adult mosquito control activities using naturally occurring wild mosquitoes in a variety of environmental, climatological, and demographic circumstances.

Much more data were collected in this project than were analyzed in this report. In particular, no attempt has been made so far to compensate in a sophisticated way for fluctuations in the data caused by minor differences in trapping conditions (e.g., weather) between the pre- and post-treatment trapping nights or on the different nights that adulticide treatments took place. Some outlying data could be readily correlated to dramatic differences between pre- and post- trapping weather conditions, but these correlations were not rigorously analyzed. Recording a large number of treatment events has been somewhat helpful in compensating for more subtle positive or negative influences on trap numbers such as minor weather variations, conditions at the time the adulticide applications were made or which product was used. As more data are collected in the future and current data are analyzed more thoroughly, a model may be developed to identify less obvious factors that may be influencing the data.

No analysis has yet been done of the control efficacy for individual mosquito species. Looking at individual treatment events, some differences in control effects on the different species were seen when multiple species were caught at a given location (data not shown). However, without further analysis it is hard to say whether these preliminary conclusions are related to any combination of factors such as the proximity or direction of sources, other recruitment

factors, or differences in the pesticide susceptibility of different mosquito species.

In addition to high wind and rain, another weather factor that may shift the presence and timing of mosquito activity is extreme nighttime temperature variations. Some rotator trapping data indicated that temperature extremes may shift mosquito activity forward or backward in time due to a physiological need for mosquitoes to function within certain temperature parameters. Shasta Mosquito and Vector Control District plans to do more rotator trapping to quantify this phenomenon and see if it might have affected the relative efficacy of post-dusk versus pre-dawn adulticiding during the time frame covered in this report. This temperature effect may be an important consideration when timing ULV treatments for optimal efficacy results during midsummer in an area like Shasta MVCD where overnight temperatures often stay very high.

ACKNOWLEDGEMENTS

Conducting this project created substantial logistical problems in coordinating with Shasta Mosquito and Vector Control District staff who deserve a great deal of credit for their cooperation and assistance: Peter Bonkrude, District Manager; Audie Butcher, Operations Supervisor; Kendra Angel-Adkinson, Assistant Vector Ecologist; and Field Technicians Mike Alexander, Corey Boyer, Kelly Cleland, Catherine Hasher, Tim Mickela, Joe Mimbs, Kevin Pearson, Al Shabazian and Geoff Taylor.

The Law of Unintended Consequences: Stormwater Detention Basins as Suburban Foci of WNV Activity

Steve Schutz, Greg Howard and Sheila Currier

Contra Costa Mosquito and Vector Control District,
155 Mason Circle, Concord, CA 94520 sschutz@contracostamosquito.com, *(925) 771-6105*

ABSTRACT: Under the Clean Water Act, property developers are required to install features to hold stormwater and residential runoff temporarily in order to reduce pollutant discharge into natural waterways. Unfortunately, there is often little or no planning or budgeting for the long term maintenance of these structures to prevent them from becoming sources of nuisance or vector mosquitoes. Therefore, a law designed to reduce environmental risk can inadvertently lead to the creation of a public nuisance or public health risk. We found two locations in suburban neighborhoods in Brentwood, California where where poorly maintained stormwater detention basins repeatedly produced West Nile Virus-infected *Culex pipiens* and *Culex tarsalis* and CO_2 trap counts above our adult mosquito treatment thresholds during 2012. Larvicide treatments of these sources met with limited success due to dense vegetation that prevented effective inspections and treatments. The resulting high public health risk levels necessitated multiple adulticiding events in the surrounding communities. It was initially difficult to determine who was responsible for maintaining the sites. Late in the season and after extensive discussion, the local flood control district brought in goats which were quite efficient at removing the excess vegetation, enabling more effective larvicide treatments, at least temporarily. However, vegetation re-growth was already significant as of January 2013. We are currently attempting to work with local authorities to develop a long-term maintenance plan that will reduce or prevent future issues at these and other similar sites.

Evaluation of Mini Resting Box for Mosquito Collections

Min-Lee Cheng, Jennifer Thieme, Tianyun Su and Quan Vong

West *Valley Mosquito and Vector Control District*
1295 E. Locust St., Ontario, CA 91761, tsu@wvmvcd.org

ABSTRACT: A mini resting box was constructed using a polyethylene planter box and used to evaluate collection of adult mosquitoes in the field from 2011 to 2012. The compactness and durability of the mini resting box enables it to be easily deployed as an auxiliary tool to EVS and gravid traps in mosquito population monitoring and West Nile Virus surveillance.

INTRODUCTION

Mosquitoes spend most of their adulthood resting except when seeking mates, blood meals or aquatic habitats for oviposition. Most commonly used traps were designed to be used in conjunction with stimuli that mimics elements in the environment in order to attract mosquitoes of certain species or physiological ages (e.g., CO_2-baited CDC or EVS traps for collecting host-seeking females; organic infusion-baited gravid traps for luring ovipositing females; and animal baited traps for attracting mosquitoes responding to host associated olfactory, exhaled CO_2 and body temperature). Each of these types of traps may underestimate the overall density and species composition of mosquito populations because they attract mosquitoes based on their responses to specific stimuli in the environment. However, the resting boxes, either the large "walk-in" type or the small cubes (Burbutis and Jobbins 1958, Edman et al. 1968, Gusciora 1971, Meyer 1985, Crans 1989, Komar et al. 1995, Williams and Gingrich 2007, Panella et al. 2011), are deployed without lures and are non-selective traps. They provide resting places for various mosquito species of both sexes with varied physiological ages. These traps can augment the existing mosquito surveillance program to detect breeding sources and monitor population density as well as mosquito-borne disease pathogens (Williams and Gingrich 2007). The mini resting box described in this study is made of commercially available planter boxes. It offers several advantages including durability, cost-effectiveness and ease of conversion, transport, set up and storage.

MATERIALS AND METHODS

Construction of the Mini Resting Box. The construction of the mini resting box is shown in Figure 1. Specific details for the mini resting box are as follows:

Figure 1. Construction of the mini resting box.

1. Polyethylene planter box, 12" high x 14" x 14", black color inside and out, slightly tapered bottom. Inside is painted red.
2. Collection lid – 3 components: (1) A piece of foam, 3" x 14" x 14" with a 3" x 3" center hole; (2) A ⅛" x 14" x 14" plywood with a 3" x 3" center hole; and (3) A 4" x 4" nylon window mesh. The center square holes in the foam and plywood are matched, and the foam is glued to the plywood with the nylon mesh window sandwiched between the foam and plywood.
3. A 1" x 2" x 6" piece of wood with a ½" wide by 6" long slit is attached to the bottom center of the planter box with a wing nut, allowing the opening of the planter box to be leveled or tilted slightly forward.

Figure 2. Set up of the mini resting box, alone or paired with the EVS trap.

4. **Operation of the Mini Resting Box (2011-2012).** The mini resting boxes were placed in locations away from traffic and adjusted to ensure the opening was leveled or slightly tilted forward (Figure 2). Traps were collected by approaching from behind or side and covering the opening of the planter box with the foam lid. Traps were subsequently returned to the District for processing. If desired, a replacement resting box may be placed at the same location at the same time for continuous collection of mosquitoes, if desired. To retrieve mosquitoes, the entire resting box with the lid facing up was placed in a 128-quart Igloo cooler. Two Q-tips wetted with triethylamine (TEA) were placed on the Nylon screen and the lid of the cooler was closed. After 20 to 30 minutes, the cooler lid was opened to allow dispersion of the TEA, and mosquitoes were recovered from the mini resting box for processing.

Comparison with Gravid Trap on Dairies (2011). In order to compare trapping efficiencies, mini resting boxes and gravid traps were placed in pairs 20 yards apart on two dairies in southern Ontario from August 11 to November 2, 2011. Mosquito counts were determined as described above.

Testing Gravid Mosquitoes for WNV (2011-2012). Gravid females caught in mini resting box were identified by species and pooled. Gravid mosquito pools were tested for WNV infection by rRT-PCR.

RESULTS

Mosquito Collections (2011-2012). During 2011-2012, significant numbers of species of mosquitoes with different physiological states were collected in mini resting boxes from urban, suburban and rural areas. In general, more mosquitoes were caught from suburban areas, followed by urban and rural areas. In most cases, more males than females of each species were recovered from resting boxes. Among the females, "non-fed (empty)" ones were predominant. The most commonly found species was *Cx. quinquefasciatus* Say, followed by *Cx. stigmatosoma* Dyar and *Cx.*

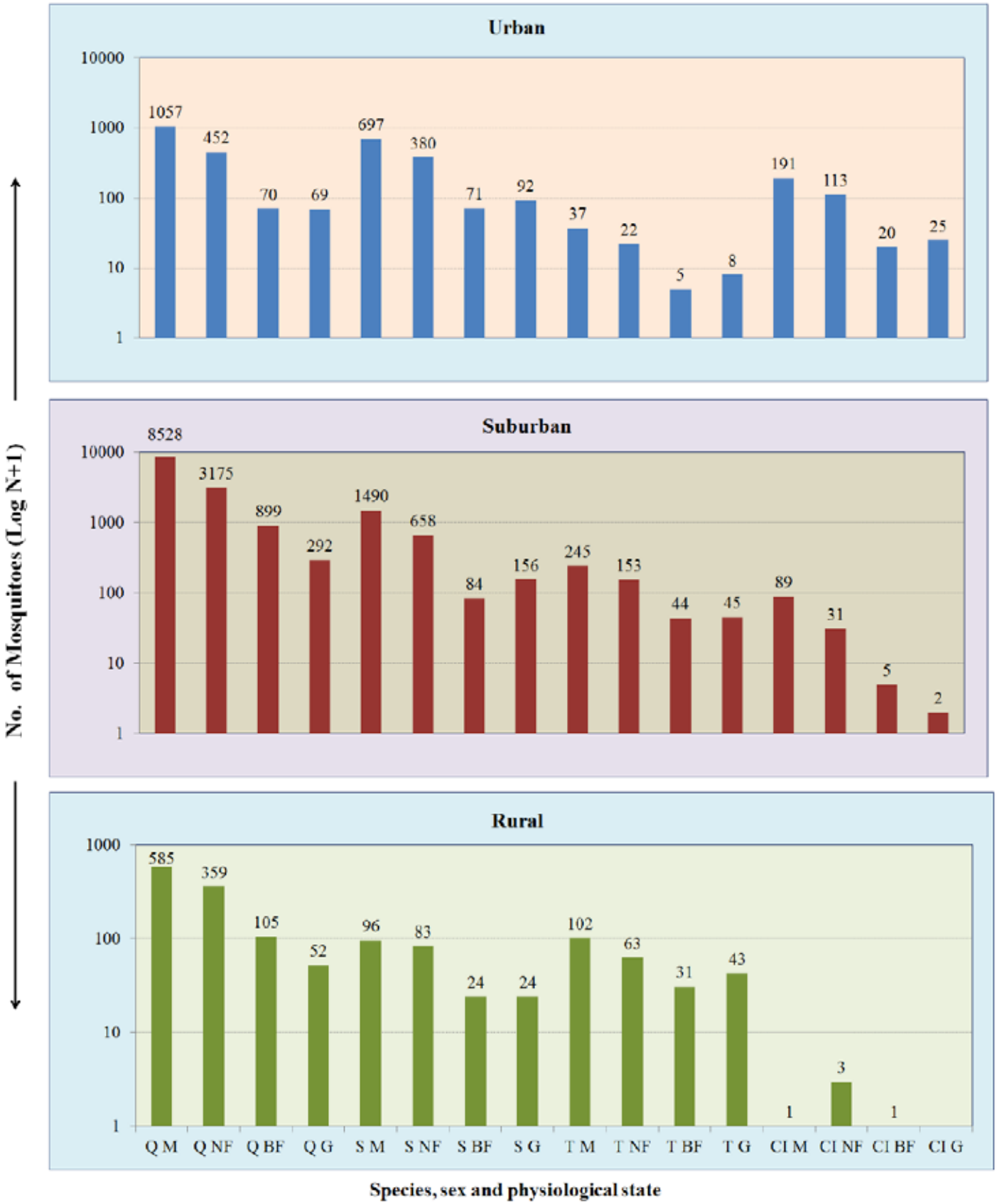

Figure. 3. Mosquitoes collected in resting boxes in different ecological environments (2010-2012) (Note: Q - *Cx. quinquefasciatus*; S – *Cx. stigmatosoma*; T – *Cx. tarsalis*; CI – *Culiseta incidens*; M – Male; NF – Nonfed female; BF – Bloodfed female; G – Gravid).

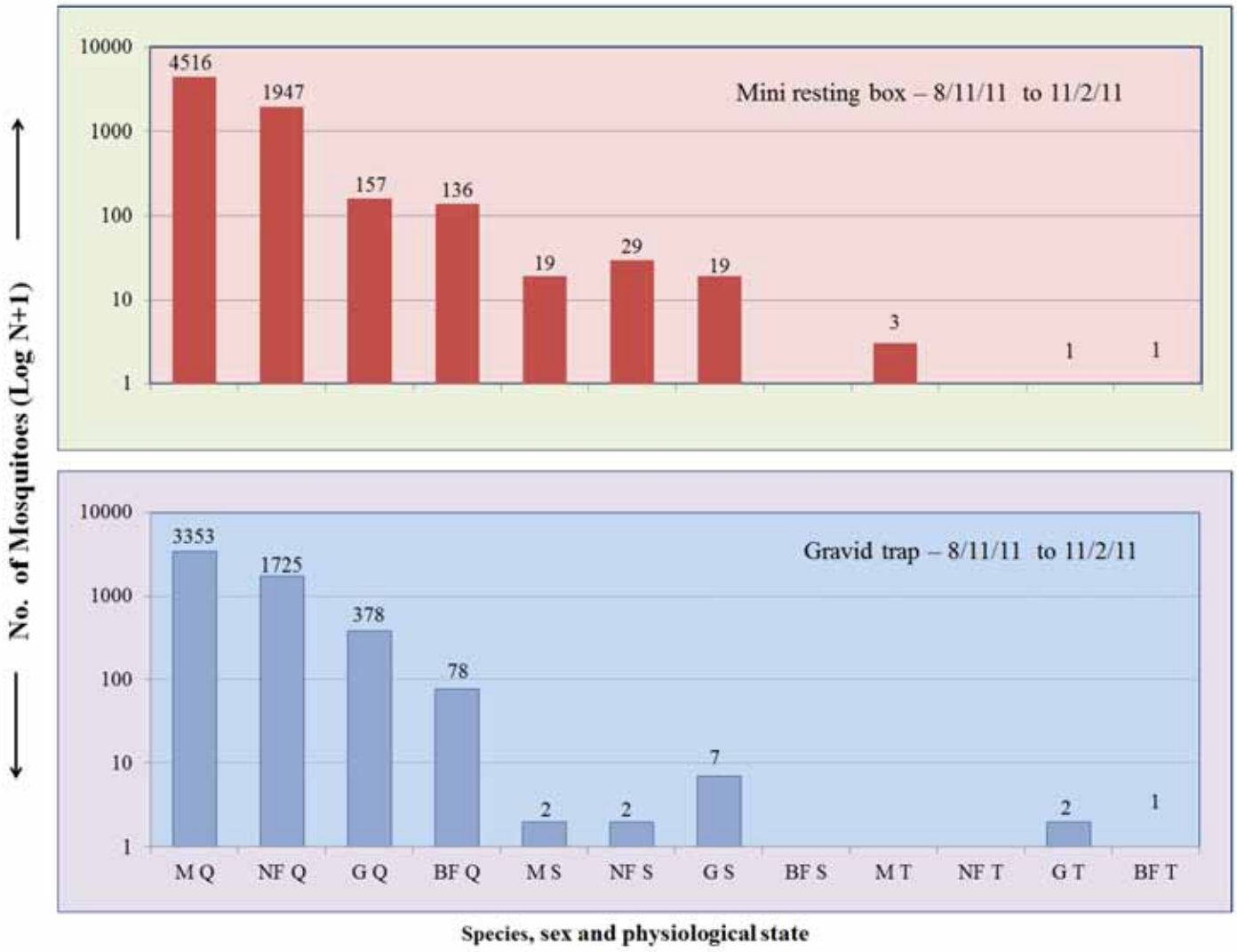

Figure 4. Composition of mosquitoes collected in mini resting boxes on dairies (2011). (Note: Q - *Cx. quinquefasciatus*; S – *Cx. stigmatosoma*; T – *Cx. tarsalis*; CI – *Culiseta incidens*; M – Male; NF – Nonfed female; BF – Bloodfed female; G – Gravid).

tarsalis Coquillett. *Culiseta incidens* (Thompson) occurred in relatively high numbers in urban areas as compared with suburban and rural areas.

Comparison with Gravid Traps (2011). Both mini resting boxes and gravid traps caught mostly *Cx. quinquefasciatus* on dairies. Mini resting boxes collected more *Cx. stigmatosoma* than gravid traps. Mini resting boxes also caught more males and blood-fed females, whereas gravid traps attracted more gravid females.

Test Results for WNV (2011-2012). In total, 7.5% (3/40) of gravid *Cx. quinquefasciatus* samples and 18.8% (3/16) of gravid *Cx. stigmatosoma* samples collected by mini resting boxes tested WNV positive.

DISCUSSION

Culex stigmatosoma was under-represented in both the EVS or the gravid traps based on larval densities found in nearby breeding habitats (e.g., dairy wastewater lagoons, water troughs). Mini resting boxes provided an additional sampling tool for *Cx. stigmatosoma* adults, as well as other genera of mosquitoes, such as *Culiseta and Anopheles*, which are less frequently found in EVS traps (without lighting). Despite the small size of the mini resting boxes, as many as 2,300 mosquitoes of different species with all physiological states have been collected in a single resting box overnight.

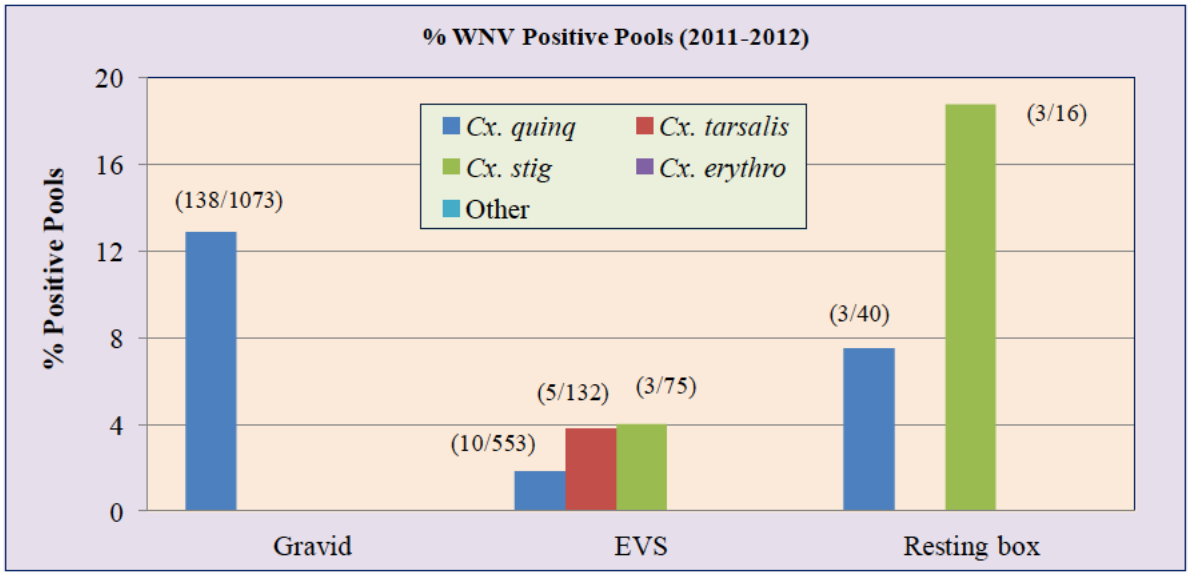

Figure 5. Comparison of WNV positive samples collected by gravid traps, EVS traps and mini resting boxes (2011 – 2012).

Culex stigmatosoma has been demonstrated as a very competent WNV vector in the laboratory, but its epidemiological importance in WNV transmission in nature has not been fully elucidated. In some densely pupulated urban neighborhoods, relatively more gravid and freshly blood-fed female mosquitoes of this species have been collected in the mini resting box.

Mini resting boxes are useful in urban, suburban and rural environments. These trapping devices can be used alongside EVS or gravid trap at the same location to boost the collection of adult mosquitoes of different species and physiological ages. Mosquito collections can be augmented with the use of specific stimuli in the gravid traps. Based on the presence of large numbers of freshly emerged males and females, the resting box also can be used as a monitoring tool for detecting mosquito emergence from breeding sources. The materials for constructing the mini resting box described here are readily obtainable, inexpensive, weather resistant, and durable, and the boxes are easy to operate.

ACKNOWLEDGMENTS

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Where are the *Culex pipiens* in Lake County, CA?

Nicole Breuner, Bonnie M. Ryan, Michelle L. Koschik, Brittany M. Nelms, Jamesina J. Scott

Lake County Vector Control District, 410 Esplanade St., Lakeport, CA 95453, 707-263-4770, nbreuner@lcvcd.org

ABSTRACT: Members of the *Culex pipiens* complex occur throughout the world and are competent vectors of several viruses, including West Nile virus and St. Louis encephalitis virus. Although the *Cx. pipiens* complex has a widespread distribution in California, it is conspicuously uncommon in Lake County, CA. Despite an efficient mosquito surveillance program at the Lake County Vector Control District, no member of the *Cx. pipiens* complex was collected in Lake County for more than 50 years. It was detected in Clearlake in 2006, and has been collected each year since, but in low numbers. This paper will review the history of collections of *Cx. pipiens* complex mosquitoes in Lake County, discuss improvements to the District's surveillance program for this species complex, and explore possible explanations for its scarcity in Lake County.

BACKGROUND

A History of the Lake County Vector Control District. The Lake County Vector Control District (LCVCD) serves all of Lake County, a relatively rural community of 65,000 people in the coastal range of northern California (Fig. 1). Central to the county is Clear Lake, a 65-square mile lake that was once infamous for the Clear Lake gnat (*Chaoborus astictopus* Dyar and Shannon), but is now well-known for its bass fishing and recreational boating activities. Lake County has become a popular vacation destination. The District was established in 1948 as the Lake County Mosquito Abatement District chiefly for the control of the Clear Lake gnat. The Clear Lake gnat emerged from Clear Lake in tremendous numbers, causing both health and economic problems for the county's residents. Although the LCVCD has worked on both Clear Lake gnat and mosquito surveillance from its inception, the control of the Clear Lake gnat occupied much of the LCVCD's resources until the mid-1970s when Clear Lake gnat populations declined, statewide mosquito-borne disease activity increased, as did local complaints about bites from *Culicoides* spp.

A Review of Mosquito Surveillance and *Culex pipiens* Collections in Lake County. The District relied on New Jersey light traps (NJLTs) to assess adult Clear Lake gnat, mosquito and *Culicoides* spp. populations until 1985 when it incorporated carbon dioxide-baited Fay traps into its surveillance program. Between 1990 and 1992, LCVCD replaced its CO_2-baited Fay-Prince

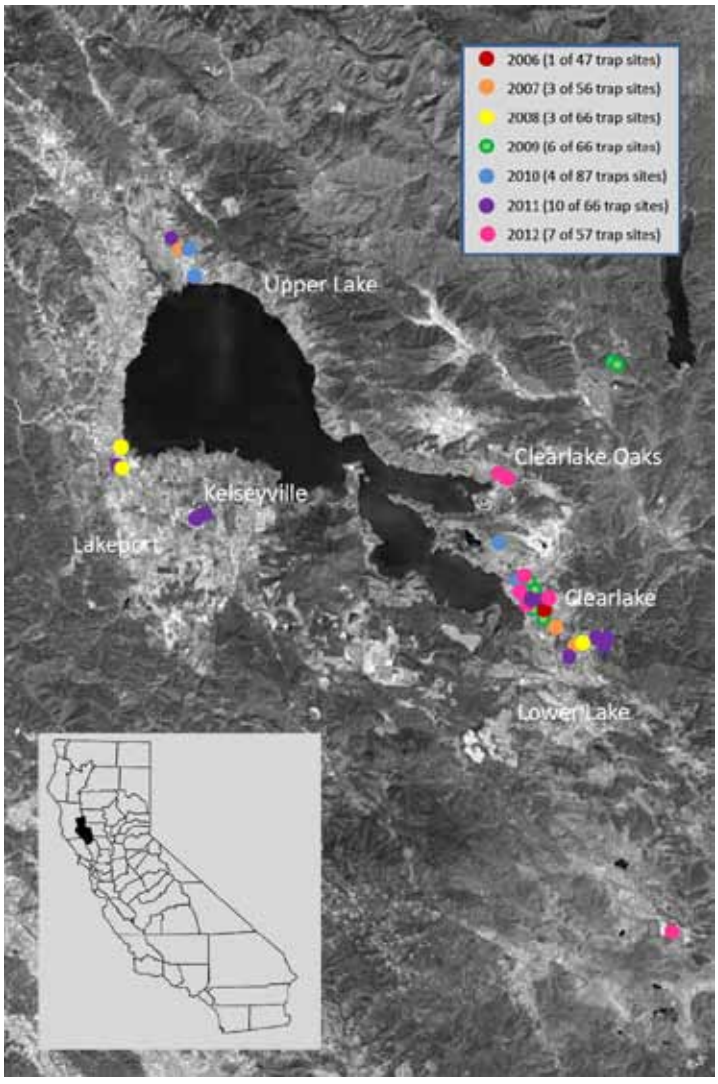

Figure 1. Map of Lake County showing sites where *Cx. pipiens* complex mosquitoes have been collected using CO_2 gravid traps, New Jersey light traps and large resting boxes.

traps (John W. Hock Co., Gainesville, FL) with CO_2-baited CDC traps that were painted black and white ("Black and White CO_2 traps"). Large resting boxes (Meyer 1985) were incorporated into LCVCD's mosquito surveillance program in 1995; data from the large resting box (LRB) collections are available starting in 2001. Mosquitoes collected from CO_2 traps, NJLTs and LRBs are identified to species and sex and enumerated.

The District has surveillance data from the early 1990s to present, but data became increasingly sparse moving back in time. We have no written record of *Culex pipiens* Linnaeus collected in Lake County before 2006. However, three *Cx. pipiens* specimens labeled with collection dates from the 1950s are in the District's reference collection: the first was collected in 1950 from Lucerne, the second collected in 1951 from Upper Lake and the third in 1954, again from Upper Lake. The collection method was not recorded for these specimens; however, NJLTs were the only traps used by the District in the 1950s and 1960s. The NJLTs were maintained to monitor the Clear Lake gnat population, and it is likely that the specimens were found in these trap collections.

Culex pipiens remained undetected in Lake County until 2006. Following the report of a human West Nile virus (WNV) case that year, a CO_2 trap was set outside the patient's residence in the city of Clearlake. Traps were set four times at this location during September. A single *Cx. pipiens* female was collected during the initial trap night, along with three *Culex tarsalis* Coquillett. There was an insufficient number of mosquitoes collected from these traps to test for the presence of WNV. Since 2006, seven additional female *Cx. pipiens* have been collected from this site, two from CO_2 traps and five from gravid traps. This location has been the most consistent site for *Cx. pipiens* complex collections in the county.

Presently, it is not known whether the *Cx. pipiens* mosquitoes found in Lake County are *Cx. pipiens* form *molestus* Forskäl, *Cx. pipiens* form *pipiens*, or hybrids. Throughout the remainder of this paper they will be referred to as "*Cx. pipiens* complex mosquitoes."

INTRODUCTION

The *Cx. pipiens* complex has a worldwide distribution (Farajollahi et al. 2011), and populations are broadly distributed throughout California (Urbanelli et al. 1997) with the apparent exception of Lake County. Between 1954 and 2006, no collection of a *Cx. pipiens* complex mosquito was recorded in Lake County, despite regular surveillance by the LCVCD. In 2006 a single female *Cx. pipiens* was identified from a CO_2 trap set in the city of Clearlake (population 15,250), one of the county's more urbanized and populated areas. Since 2006 low numbers of adult *Cx. pipiens* complex mosquitoes have been detected each year throughout the county using a variety of trapping methods including NJLTs, Hock gravid traps, CO_2 traps and LRBs. Members of the *Cx. pipiens* complex are competent vectors of WNV (Goddard et al. 2002, Turell et al. 2005, Hayes et al. 2005) and may serve as bridge vectors between birds and humans (Hamer et al. 2008). A persistent *Cx. pipiens* complex population may contribute to an increase in human WNV cases. Determining if there is an established population of *Cx. pipiens* in Lake County or if its sporadic collection is merely the result of repeated local introductions will help the District to target its limited resources efficiently.

TRAPPING METHODS

CO_2 Traps. The LCVCD has used CO_2 traps extensively for mosquito and biting fly surveillance since the early 1990s. Trapping begins in March and continues through October when temperatures drop and trap collections wane as mosquitoes stop host-seeking and enter diapause/dormancy. The annual number of CO_2 trap nights ranged from 289 in 2006 to 717 in 2010.

Culex pipiens complex mosquitoes were collected from numerous CO_2 trap sites around the county from both urban and rural areas. However, the majority were found in relatively urban areas such as Clearlake, Lower Lake, Clearlake Keys and Kelseyville (Fig. 1). Countywide, 8 male and 62 female *Cx. pipiens* complex mosquitoes were collected from CO_2 traps between 2006 and 2012. Of the 3,458 CO_2 trap nights, only 50 trap nights included a *Cx. pipiens* complex

mosquito collection (Table 1). Between 2001 and 2005 the number of CO_2 trap nights ranged from 220 and 332, yet no *Cx. pipiens* complex mosquitoes were collected from these traps.

New Jersey Light Traps. Since 1986 two New Jersey light traps have been maintained continuously at two locations in Lake County. The Upper Lake trap, set near the north end of Clear Lake, monitors mosquitoes produced in the wild rice fields where the District devotes much time and energy to control *Cx. tarsalis* and *Anopheles* spp. The southern NJLT is in the City of Clearlake and monitors the biting black gnat (*Culicoides occidentalis* Wirth and Jones) and mosquitoes associated with the alkaline Borax Lake.

A *Cx. pipiens* complex mosquito was collected in the Upper Lake NJLT in 2007. They were subsequently collected from the Clearlake NJLT in 2009, 2010 and 2012 and again from Upper Lake in 2010. The NJLTs run an average of seven nights before the sample is retrieved. Nine weekly NJLT collections (representing 64 total trap nights) between 2006 and 2012 (5,118 trap nights) accounted for the collection of a total of 10 *Cx. pipiens* complex mosquitoes (Table 1).

Large Resting Boxes. The District has routinely used large resting boxes (LRBs) since 1995, but *Cx. pipiens* complex mosquitoes were collected in only two years, 2008 and 2011. All collections were made with a hand-held or backpack aspirator between 8AM and 10AM. In 2008 a single *Cx. pipiens* complex female was collected in a LRB at a private residence in Lakeport. In 2011 multiple members of the *Cx. pipiens* complex were collected from LRBs at the private residence in Lakeport, at a winery in Kelseyville and a private residence in Lower Lake. Seventy-one *Cx. pipiens* complex mosquitoes were collected from LRBs in 2008 and 2011. Between 2006 and 2012, a total of 2,348 collections were made from LRBs, and only 28 of these included *Cx. pipiens* complex mosquitoes (Table 1).

Gravid Traps. Hock gravid traps (John W. Hock Co., Gainesville, FL) were set at specific locations in 2007 and 2011 in an attempt to determine if members of the *Cx. pipiens* complex were present in the area.

Year	Trap Type(s)	Total # Trap Nights	# *Cx. pipiens* complex positive trap nights	# *Cx. pipiens* complex collected
2006	CO2	289	1	1
	NJLT	737	0	0
	Rest	246	0	0
	Gravid	Not Set	N/A	N/A
2007	CO2	337	2	2
	NJLT	734	7	1
	Rest	204	0	0
	Gravid	10	7	33
2008	CO2	438	2	3
	NJLT	707	0	0
	Rest	270	1	1
	Gravid	Not Set	N/A	N/A
2009	CO2	481	5	10
	NJLT	775	7	1
	Rest	290	0	0
	Gravid	Not Set	N/A	N/A
2010	CO2	717	2	2
	NJLT	709	14	2
	Rest	476	0	0
	Gravid	Not Set	N/A	N/A
2011	CO2	527	29	42
	NJLT	728	0	0
	Rest	588	27	70
	Gravid	9	6	26
2012	CO2	669	9	10
	NJLT	728	36	6
	Rest	274	0	0
	Gravid	Not Set	N/A	N/A
Total		10943	155	210

Table 1.

Following the collection of a WNV-positive American Crow in 2007, CO_2 surveillance at Yuba College Clear Lake Campus yielded one female *Cx. pipiens* complex mosquito. Gravid traps were subsequently set at four locations in an attempt to collect additional *Cx. pipiens* complex mosquitoes: the community college and a residence in Clearlake, a residence in Lower Lake and one location in Lakeport. An alfalfa cube/Brewer's yeast/lactalbumin media was used. In mid-August, a gravid trap collected 21 females and two male *Cx. pipiens* complex mosquitoes. All other gravid trapping yielded only ten additional *Cx. pipiens* complex mosquitoes. Seven of the 17 gravid trap nights that occurred in 2007 collected *Cx. pipiens* complex mosquitoes (Table 1).

Gravid trap surveillance was initiated again in 2011 following the collection of a unusually high number of *Cx. pipiens* complex mosquitoes in LRBs and CO_2 traps from a winery in Lake County. A Brewer's yeast/rabbit chow/fish food infusion was used. Traps were set at sites in Kelseyville, Lower Lake and Clearlake, and all locations resulted in *Cx. pipiens* complex collections. A total of nine trap nights occurred in 2011, and six of these nights resulted in 9 female and 17 male *Cx. pipiens* complex mosquitoes. In all 37 females and 22 males were collected from gravid trap surveillance during 2007 and 2011.

RESULTS AND DISCUSSION

A total of 210 mosquitoes in the *Cx. pipiens* complex have been collected in Lake County since 2006. The *Cx. pipiens* complex mosquitoes have been detected annually in low numbers in Lake County since 2006, with the greatest number of individuals collected in 2011 (138 individuals from 1,852 trap nights).

The number of locations where *Cx. pipiens* complex mosquitoes has been collected has increased since 2006 (Fig. 1). The only location where the *Cx. pipiens* complex has been detected consistently is in a neighborhood in Clearlake, the same area where the first *Cx. pipiens* complex mosquito was discovered in 2006. *Culex pipiens* complex mosquitoes have been collected every year in the city of Clearlake with the exception of 2008. However, in 2008 a CO_2 trap in Lower Lake, a community bordering the City of Clearlake, collected a single *Cx. pipiens* complex female.

While trapping sites varied from year to year, three sites that were trapped consistently prior to 2006 resulted in *Cx. pipiens* complex mosquitoes only after 2006. The lack of *Cx. pipiens* complex mosquitoes at these well-trapped sites until after the first female was discovered in Clearlake suggests that *Cx. pipiens* complex mosquitoes are spreading throughout Lake County.

Three trapping methods have been used consistently for mosquito surveillance: CO_2 traps, NJLTs and LRBs. In terms of the total number of individuals collected, CO_2 traps have been the most successful trapping method for *Cx. pipiens* complex mosquitoes. LRBs have attracted only slightly higher numbers of *Cx. pipiens* complex mosquitoes but were successful for only two years, 2008 and 2011. NJLTs collected *Cx. pipiens* complex mosquitoes in 2007, 2009, 2010 and 2012, but in lower numbers than either LRBs or CO_2 traps (Table 1).

Gravid traps, which are generally the most effective trapping methods for *Cx. pipiens* (Kesavaraju et al. 2011) were used in 2007 and 2011. Overall, gravid traps appeared to be the most successful trapping method, as measured by the total number of mosquitoes per trap night, with a total of 59 *Cx. pipiens* complex mosquitoes collected. Out of 26 total trap nights, 13 resulted in *Cx. pipiens* complex collections (Table 1). However, only one trap night in 2007 yielded what could be described as a "high" count of 21 females and 2 males. Following this uncommonly successful trap night, several attempts were made to replicate this result using the media, gravid trap type and location of the first night. However, the largest number of *Cx. pipiens* complex mosquitoes collected from a gravid trap was 7 in a single trap night in both 2007 and 2011. Other media were used at various trap locations, but all yielded few or no C*x. pipiens* complex mosquitoes.

The 2011 surveillance season was the most successful year since members of the *Cx. pipiens* complex were re-discovered in Lake County. In total 138 *Cx. pipiens* complex mosquitoes were collected in 2011; of these 103 came from a local winery. This was the only year that *Cx. pipiens* complex mosquitoes were found at this site, despite surveillance of the area in 2010 and 2012. A large number of males were collected from the LRBs at this site (Fig. 2), which suggested that the larval source was nearby. It was strongly suspected that the *Cx. pipiens* complex mosquitoes were coming from sedimentation ponds at the winery which also produced very high numbers of *Culex stigmatosoma* Dyar. In contrast no *Cx. pipiens* complex larvae were collected. The *Cx. pipiens* complex mosquitoes collected from this site were used in a separate research project and were not tested for WNV.

Future *Culex pipiens* Complex Surveillance Plans. Although *Cx. pipiens* complex mosquitoes are

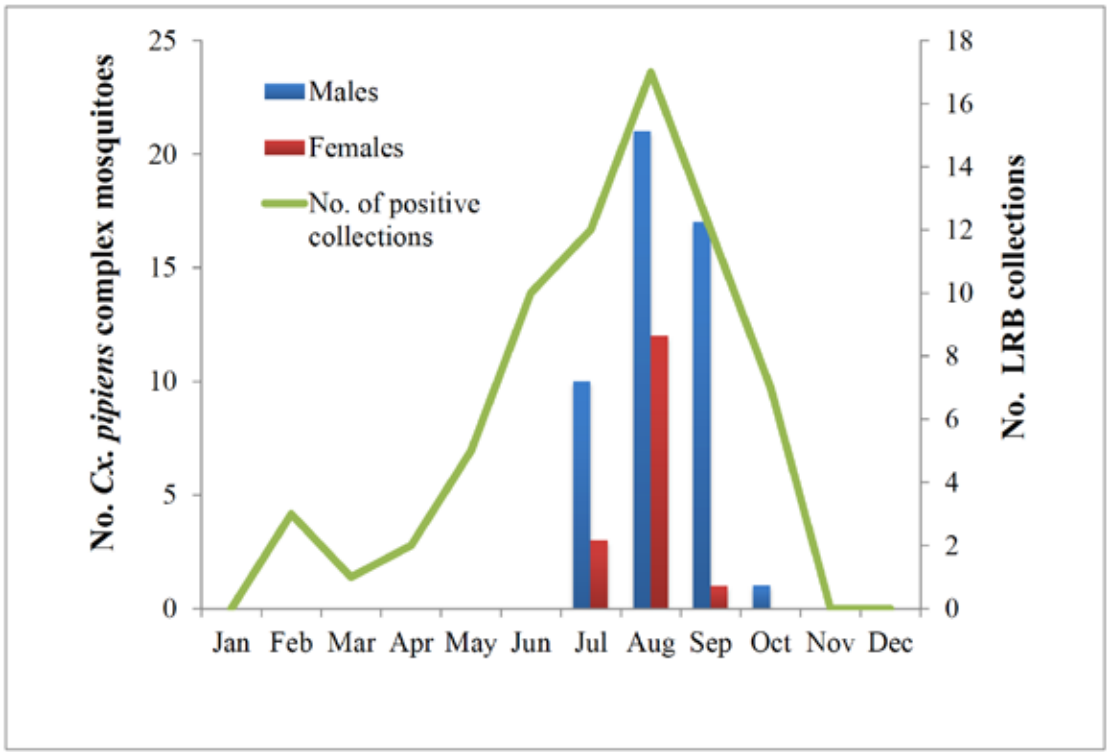

Figure 2. 2011 was the only year that the *Cx. pipiens* complex was found at this site. Given the high numbers of males, it was suspected that the larval habitat was nearby although none were discovered.

found irregularly and in low numbers in Lake County, we hypothesize that there is a small local population present. It is possible that the trapping methods currently being used are not effective at attracting large numbers of *Cx. pipiens* complex mosquitoes. Gravid traps are generally considered to be the most effective sampling method (Kesavaraju et al. 2011). We plan to increase gravid trapping, replace the Hock gravid traps with the Reiter-Cummings model and evaluate different gravid media during the 2013 season. Current trap sites being evaluated may not be near appropriate larval habitats, or there may be small, scattered populations of *Cx. pipiens* complex mosquitoes, in which case new trapping sites need to be explored. More trapping will need to be done in urban areas, focusing on the Clearlake area that has been a *Cx. pipiens* complex collection site for numerous years.

Alternatively, there may not be an established *Cx. pipiens* complex population in Lake County, and a few individuals are being introduced each year. Members of the *Cx. pipiens* complex readily utilize various human transportation methods, and importation of these mosquitoes from one area to another through these methods has been documented (Farajollahi et al. 2011). Lake County's economy is highly dependent on tourism, and the regular increase in visitors every summer between May and September to visit Clear Lake coincides with abundance data for *Cx. pipiens* complex mosquitoes (Fig. 3). Lake County is also a large agricultural import-export community, and semi-trucks regularly travel to and from the county transporting various goods. Members of the *Cx. pipiens* complex could be imported into the county either by passenger vehicle or truck, which may explain the low numbers of *Cx. pipiens* complex mosquitoes collected in the county. Lake County is geographically isolated so natural migration of the *Cx. pipiens* complex is unlikely. If the *Cx. pipiens* complex mosquitoes are being regularly introduced into Lake County, this could have important implications for control and for other introduced species such as the Asian tiger mosquito, *Aedes albopictus* (Skuse).

In order to determine whether or not there is an established population, it is crucial to investigate potential overwintering sites. *Culex pipiens* complex mosquitoes overwinter as adults (Farajollahi et al. 2011), and their presence would be a good indication that there is an established *Cx. pipiens* complex

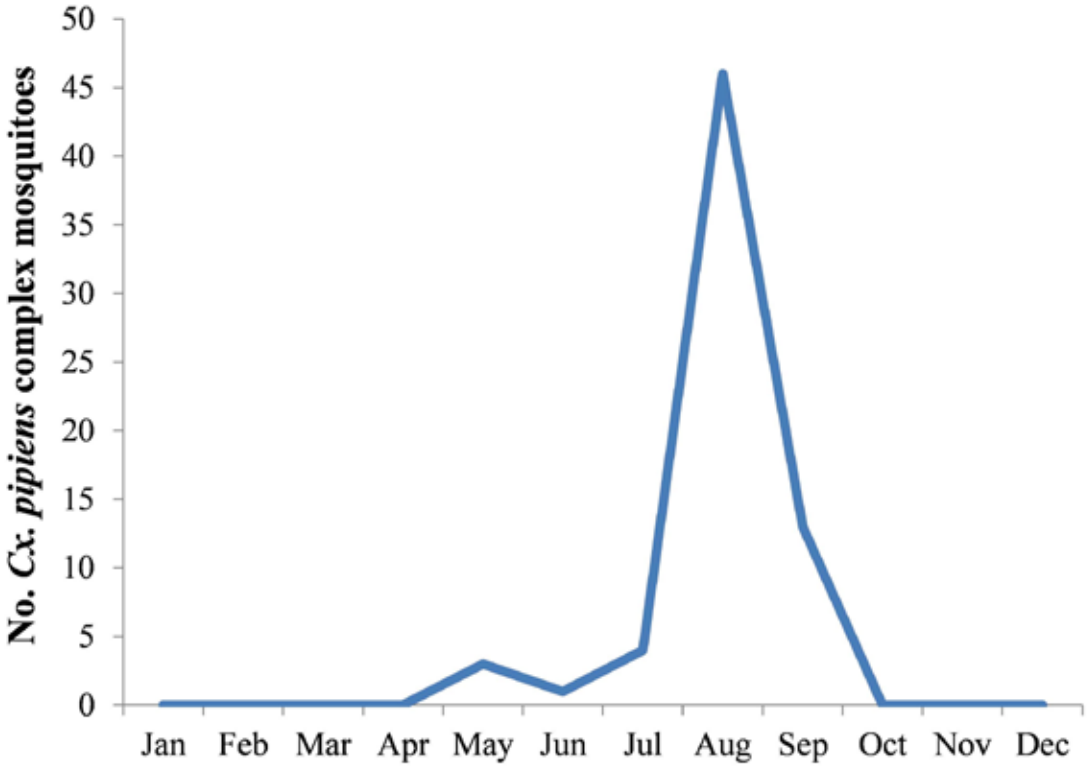

Figure 3. The seasonal 2006 - 2012 abundance of the *Cx. pipiens* complex in Lake County is similar to other counties in California, suggesting that there may be an established population. Alternatively, the population may be the result of importation by tourists as the rise in mosquito numbers coincides with increased traffic during the tourism season (May – September).

population in Lake County. If an overwintering site is discovered, it will provide strong evidence that there is a small but persistent population of the *Cx. pipiens* complex mosquitoes in Lake County.

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Mosquito and West Nile virus Surveillance at Northwest Mosquito and Vector Control District During 2012

Gregory Williams[1], Bill Van Dyke[1], Tristan Hallum[1], Nathan McConnell[1], Jonathan A. Allred[1], Bryan Haynes[1], Ryan Reneau[1], Vincent Strode[1], Lal S. Mian[2], and Major S. Dhillon[1]

[1]*Northwest Mosquito and Vector Control District, 1966 Compton Ave., Corona CA 92881-3318*
[2]*Department of Health Science & Human Ecology, California State University, San Bernardino, CA 92407*

ABSTRACT: Northwest Mosquito and Vector Control District continued its arbovirus surveillance in 2012. The district examined West Nile virus (WNV) in pools of female mosquitoes, blood samples from wild passerine birds and sentinel chickens and submitted dead birds to the state for testing. The WNV activity was detected in mosquito pools (8 of 404), wild bird seroconversions (2 of 102) and sentinel chicken seroconversions (7 of 18). *Culex quinquefasciatus* was the most frequently pooled mosquito (128 of 404), yielding the majority of positive pools (6 of 8). The House Finch and Brown-headed Cowbird constituted all of the WNV- seroconversions (2 of 2). Sentinel chicken samples totaled 192 blood samples with 7 seroconversions from 7 birds. All the positive dead birds (16) were American crows. Our district area had more viral detections in 2012 compared to the previous year.

INTRODUCTION

The Northwest Mosquito and Vector Control District (NWMVCD) has been providing mosquito surveillance and control services in the cities of Norco, Corona, Lake Elsinore, parts of the city of Riverside , Canyon Lake and several adjoining unincorporated communities for over 40 years. The NWMVCD service area encompasses approximately 245 square miles with nearly 500,000 residents living in urban, sub-urban and some near rural areas with many living in close proximity to wetlands and/or riparian habitats. West Nile virus (WNV) was first detected within the district boundaries in 2003. Since then WNV has been isolated in the laboratory every year from mosquito pools, dead birds, and/or chicken sera samples. In order to monitor arbovirus activity, mosquitoes were collected using the encephalitis virus surveillance (EVS) traps, gravid mosquito traps and resting boxes. Arbovirus surveillance was conducted for WNV, St. Louis encephalitis (SLE) and Western equine encephalomyelitis (WEE) by testing pooled female mosquitoes, blood sera from sentinel chickens and wild birds, and testing dead birds submitted to the District. In this paper, we present and discuss data generated in various facets of the District's Arbovirus Program carried out in 2012.

MATERIALS AND METHODS

New Jersey-Style Light Traps. The population dynamics of adult mosquitoes were monitored with modified New Jersey Light Traps (NJLT) (Mulhern 1942) at six fixed locations throughout the District. The traps were set at three suburban and three rural areas as described by Mian and Reed (2001) and checked on a weekly to biweekly basis throughout the calendar year. The traps were equipped with an 18 watt, 1300 lumen, 2700K energy efficient light bulb rather than the historical standard, a 25-watt incandescent light bulb (235 lumens). The bulb change was brought on by a desire to increase lumen output for chironomid surveillance; however, additional secondary benefits included monetary savings and a reduction in our carbon footprint as less energy was used as well as a small savings on time and labor from less frequent light bulb acquisitions and swaps. We also modified the collection chamber by removing the mason jar and dichlorvos (Vapona^{R}) strips and using a catch bag from a standard CO_2/EVS trap (Bio Quip Products, Inc., Rancho Dominguez, CA). In order to combat the potential escape of collected specimens, the fan was rewired to remain on constantly. These modifications were done to reduce the unnecessary use of both a toxic pesticide and exposure to a carcinogen (MSDS,

SigmaAldrich Corp., St. Louis, MO). Both of these modifications are in alignment with the goals of the NWMVCD and the mosquito control industry at large to be good environmental stewards of the land that we oversee for vector abatement activities. Mosquito data from trap collections were entered into the California Department of Public Health online reporting system.

Carbon Dioxide Baited Traps. Host-seeking female mosquitoes were monitored using the Northwest-Dever modified EVS traps (Williams et Al. 2009) sporadically throughout the calendar year from a variety of locations, especially areas known to be *Culex erythrothorax* trouble spots. All mosquitoes collected in EVS traps were anesthetized with triethylamine (TEA) and sorted out by species and sex and enumerated. Pools from 10 to 50 mosquitoes were shipped overnight on dry ice to the University of California Davis Center for Vectorborne Diseases (CVEC) for testing. Pools of female *Anopheles hermsi, Culex erythrothorax*, *Culex quinquefasciatus*, *Culex stigmatosoma*, *Culex tarsalis*, *Culex thriambus*, *Culiseta incidens* and *Culiseta particeps* were submitted for testing for arbovirus.

Gravid Traps. The Reiter/Cummings gravid female, ovipositional traps (Cummings 1992) were set in rural and suburban areas from May to October. These traps were baited with an alfalfa or Milo infusion, depending on the habitat they were operated at. All mosquitoes collected in gravid traps were anesthetized with (TEA) and sorted by species and sex and enumerated.

Resting Boxes. Six walk-in style resting red boxes were placed at established sites and monitored from January thru November (Meyer 1987). Sites were located in proximity to the Santa Ana River and usually were checked at least once per week. Mosquitoes were collected using an in-house designed battery powered aspirator. The boxes were used specifically to target and obtain blood engorged and gravid mosquitoes. All mosquitoes collected in resting boxes were anesthetized with (TEA) and enumerated before sorting by species and sex.

Sentinel Chicken Flocks. Six sentinel chicken flocks, comprised of three white leghorn birds each, were maintained at different locations throughout the District. Blood samples were collected biweekly from April through November. The samples were placed on Nuboto filter-paper strips, air dried and submitted to the CDPH Viral and Rickettsial Disease Laboratory in Richmond for testing.

Wild Birds. In 2012 we trapped and bled wild birds at two locations. Birds were trapped in Australian Crow traps (McClure 1984). Two traps were baited with wild bird feed and water to attract House Finches (*Carpodecus mexacanus*) and House Sparrows (*Passer domesticus*). Traps were checked every day during the week and were left open on the weekends. The birds were identified to species, sexed, banded, bled and released at the site. We also collected and tested blood samples of Brown-headed Cowbirds (*Molothrus ater*) obtained from modified Australian Crow traps operated by the Least Bells Vireo Conservation Project of the Santa Ana Watershed Association (SAWA) and by the Orange County Water District (OCWD). Bird blood samples (0.1 - 0.2 ml from each bird) were collected from the jugular vein with a 1-ml insulin syringe fitted with a 28 gauge, ½ inch hypodermic needle. Each sample was dissolved in 0.9 ml of 0.75% bovine serum albumin/ PBS (phosphate-buffered saline) diluent and submitted to the Orange County Vector Control District Laboratory for SLE and WEE antibody testing by serum hemagglutination inhibition as described by Gruwell at al. (2000). The samples were also tested for antibodies specific to WNV by a blocking enzyme-linked immunosorbent assay (Jozan et al. (2003).

Dead Birds. Through the participation of NWMVCD with the California Department of Public Health (CDPH) Dead Bird Surveillance Program, dead birds reported to the District were picked up and submitted to the California Animal Health and Food Safety (CAHFS) Laboratory in San Bernardino for tissue processing and to CVEC for WNV testing.

RESULTS AND DISCUSSION

Mosquito Surveillance

EVS. Over 80,000 female mosquitoes were collected in the Northwest-Dever modified EVS traps from 308 trap-nights, resulting in a mean number of mosquitoes per trap-night of 261.94 during the trapping season from January through November 2012. *Culex erythrothorax* was the predominant species collected (71,737), most of these being collected during July and August when over 15,000 per month were collected with mean per trap-night counts of 469.52 and 331.86 respectively. The highest monthly mean/trap-night count for *Cx. erythrothorax* (835.00) was in March when 4107 mosquitoes were collected in 5 trap-nights. *Culex tarsalis* was the second most trapped species (6844) with a peak monthly mean/trap-night of 33.45 during June. Relatively few *Cx. stigmatosoma* (164) and *Cx. quinquefasciatus* (351) were collected. The highest mean/trap-night count for *Cx. stigmatosoma* was 3.00 in February and 0.85 for *Cx. quinquefasciatus* in May. Average collections of *Cx. stigmatosoma* waned as the season progressed whereas collections of *Cx. tarsalis* generally increased from April through September (Figure 1). The average collection per trap night was lowest in January and November at 120 and 132 respectively.

New Jersey Light Traps. Mean trap-night values for NJLT for the 11 month period were 0.12, 0.75, 0.81 and 1.53 for *Culex stigmatosoma, Cx. quinquefasciatus, Cx. tarsalis* and *Cx. erythrothorax* respectively. Peak per trap-night collections were in July for *Cx. erythrothorax* at 8.68, February and October for *Cx. quinquefasciatus* (2.75 and 2.16 respectively), April for *Cx. stigmatosoma* (0.73), and June and July for *Cx. tarsalis* (2.47 and 2.26 respectively). Mean/trap-night counts were lowest with NJLT versus other collection methods for all *Culex* species mentioned except *Cx. quinquefasciatus* that had a lower mean/trap-night count of 0.16 in EVS traps.

Gravid Traps. A total of 59 collections were made with gravid traps from May to October. *Culex quinquefasciatus* was predominant and accounted for 87% of all mosquitoes collected in gravid traps, with numbers peaking in September (Figure 2). Other species collected in gravid traps included *Cx. tarsalis* (8%) and *Cx. stigmatosoma* (5%); the former species peaked in September with a majority of them as host seeking. September had the highest abundance rate at 19.5 mosquitoes per trap-night. The numbers of *Cx. quinquefasciatus* collected in gravid traps were 70 times higher than those in EVS

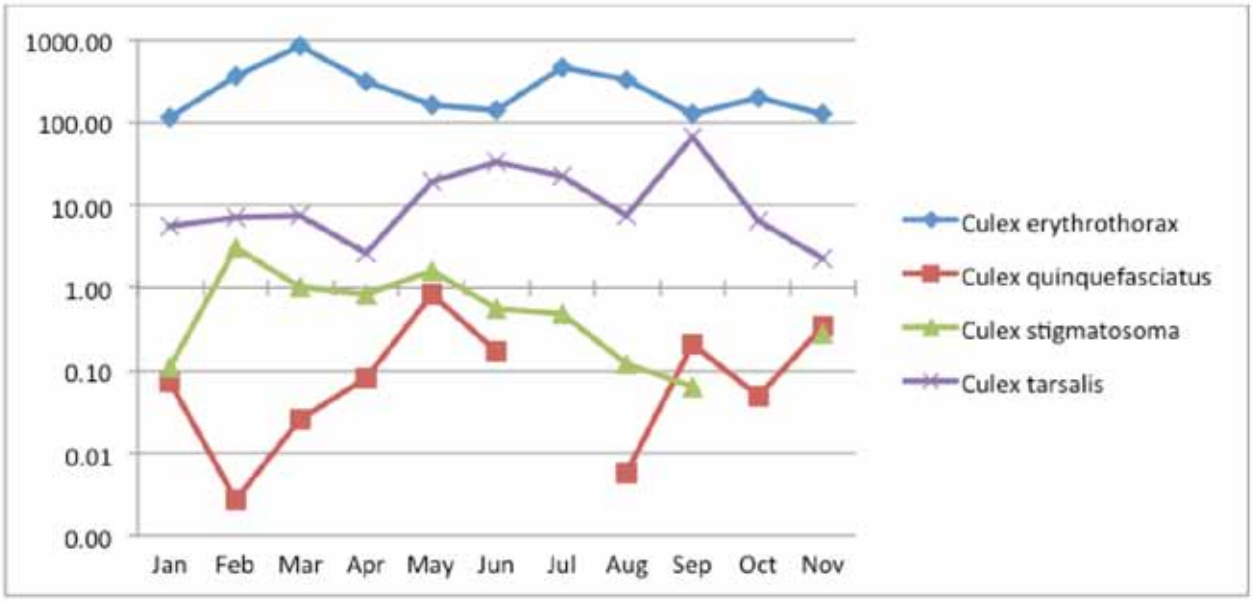

Figure 1. Monthly numbers of *Culex* spp. collected per EVS trap-night in 2012.

traps over the 6-month period; however, trailing resting boxes by one-third. The majority of positive WNV pools came from *Cx. quinquefasciatus* collected in gravid traps (4 of 8, Table 1).

Resting Boxes. A total of 244 collections were made at resting boxes. *Culex quinquefasciatus* were collected most frequently (16.67/trap-night), followed by *Cx. tarsalis* (6.16) and *Cx. stigmatosoma* (4.77,

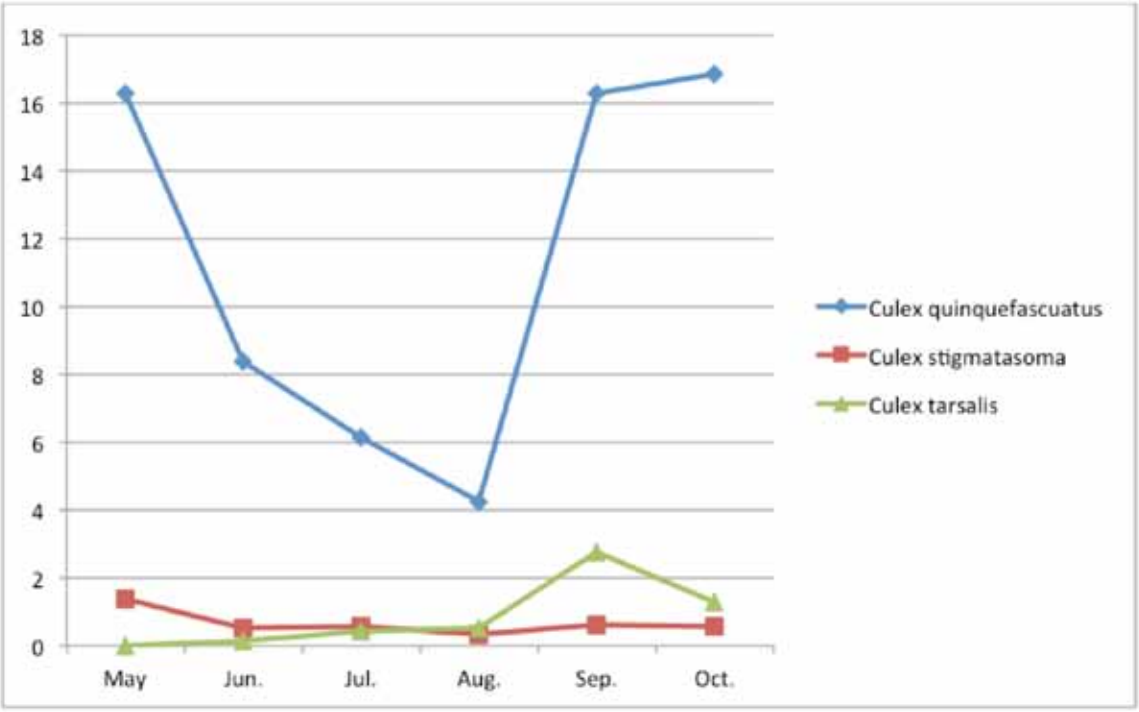

Figure 2. Numbers of *Culex* species collected per trap-night in gravid traps during 2012.

Month	*Anopheles hermsi*	*Culex erythrothorax*	*Culex quinquefasciatus*	*Culex stigmatosoma*	*Culex tarsalis*	*Culex thriambus*	*Culiseta incidens*	*Culiseta particeps*	*Total*
Jan	0	0	1 (10)	0	1 (6)	0	0	0	2(16)
Feb	0	0	0	0	0	0	0	0	0
Mar	0	0	0	0	0	0	0	0	0
Apr	0	0	3 (73)	1 (15)	0	0	1 (20)	1 (12)	6(120)
May	6 (71)*	0	37 (506)	9 (69)	13 (69)	0	10 (51)	5 (41)	80(807)
Jun	8 (72)	12 (584)	28 (516)	12 (85)[1]	44 (1080)	0	8 (35)	3 (18)	115(2390)[1]
Jul	4 (54)	3 (130)	18 (142)	8 (35)	22 (242)	0	5 (24)	3 (25)	63(652)
Aug	5 (178)	23 (1150)	14 (437)[2]**	8 (259)	8 (229)	1 (1)	0	0	59(2254)[2]
Sep	4 (36)	0	23 (240)[4]	4 (15)	26 (1136)[1]	0	0	0	57(1427)[5]
Oct	2 (24)	3 (150)	4 (64)	4 (17)	9 (102)	0	0	0	22(357)
Nov	0	0	0	0	0	0	0	0	0
Dec	0	0	0	0	0	0	0	0	0
Total	**29 (435)**	**41 (2014)**	**128 (1988)**	**46 (495)**	**123 (2864)**	**1 (1)**	**24 (130)**	**12 (96)**	**404(8023)[8]**

*Parenthetical numbers indicate the total number of mosquitoes tested.
**Red bracketed numbers represent the number of positive pools.

Table 1. Numbers of mosquito pools submitted to the UC Davis - Center for Vector-borne Diseases for surveillance of Saint Louis encephalitis, western equine

Figure 3). Monthly collections per trap-night for all species in descending order were: June, May, July, and then August. Peak abundances by species in June were 9.27, 19.23 and 33.64 mosquitoes/trap-night for *Cx. stigmatosoma, Cx. tarsalis* and *Cx. quinquefasciatus*, respectively. Resting box collections were the most efficient tool for collecting large numbers of *Cx. quinquefasciatus* (~50% more than gravid traps and >100x over EVS) as well as *Cx. stigmatosoma* (6.5x more than EVS and 7.5x more than gravid traps), yielding. 37.5% of the positive pools (2 *Cx. quinquefasciatus* and 1 *Cx. stigmatosoma*) (Table 1).

West Nile Virus Surveillance

Mosquito Pools. A total of 404 mosquito pools comprising 8023 mosquitoes were submitted to CVEC for testing (Table 1). Of the positive pools, 75% were *Cx. quinquefasciatus*; 50% of the positive pools came from gravid traps (all four pools of *Cx. quinquefasciatus*), 37.5% from resting boxes (2 *Cx. quinquefasciatus* and 1 *Cx. stigmatosoma*), and lastly 12.5% (one pool of *Cx. tarsalis*) from an EVS trap. The majority of these pools (87.5%) were collected in close proximity to the Santa Ana River.

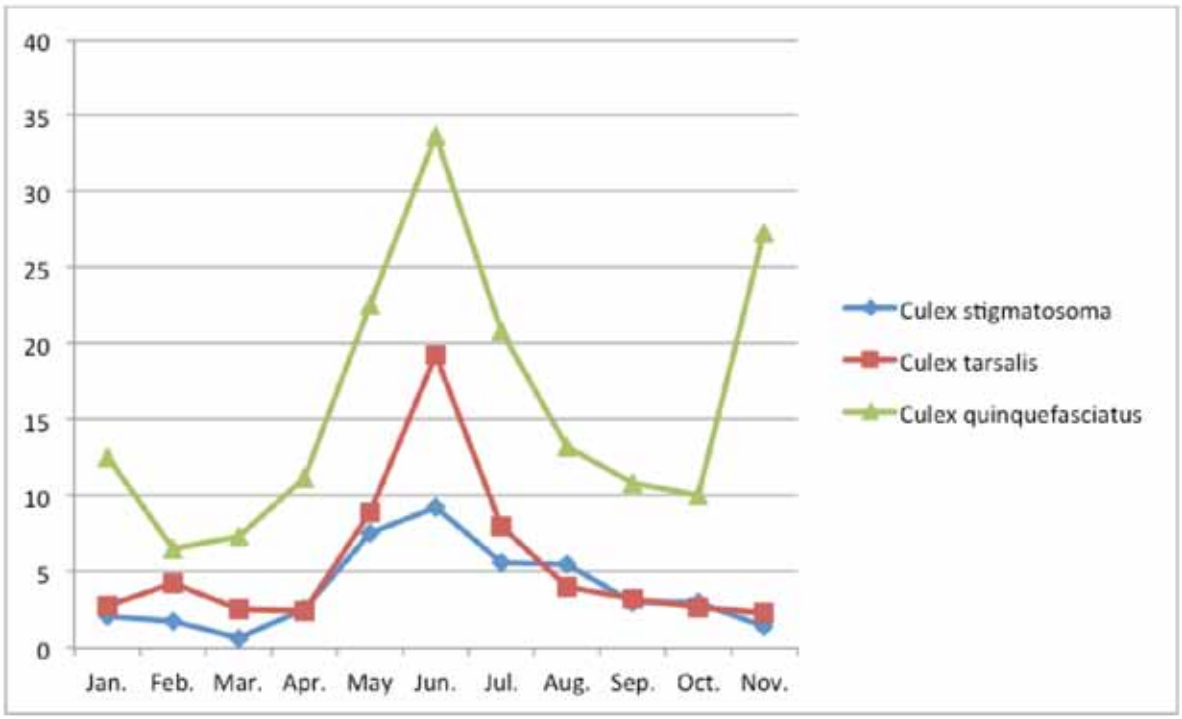

Figure 3. Mean monthly numbers of *Culex* species collected per trap-night at resting box sites during 2012.

Sentinel Chickens. Seven blood samples from our sentinel chickens tested positive for WNV. In 2012, 7 out of 18 chickens from three of six sentinel flocks throughout the District area tested positive for the virus (Figure 4). The first infected chickens, with a probable seroconversion date of September 5, were identified in two flocks, close to the Santa Ana River. One additional flock became infected on October 3, further south of the River. Transmission of WNV continued through October as shown by two additional seroconversions of chickens at the aforementioned flocks on October 17.

Wild Birds. In 2012, two birds tested antibody positive for WNV (Figure 4). The first positive bird was a House Finch bled on May 29, and an additional bird, a Brown-headed Cowbird with detectable antibody, was bled on August 3. Birds were bled once a month throughout the year (12 bleeding events) yielding a total of 102 samples. The two sites sampled were Prado (3 bleeds) and NWST (9 bleeds). Both positive birds were found at our NWST site.

Dead Birds. During 2012, 16 dead birds were submitted to the CAHFS laboratory between May and

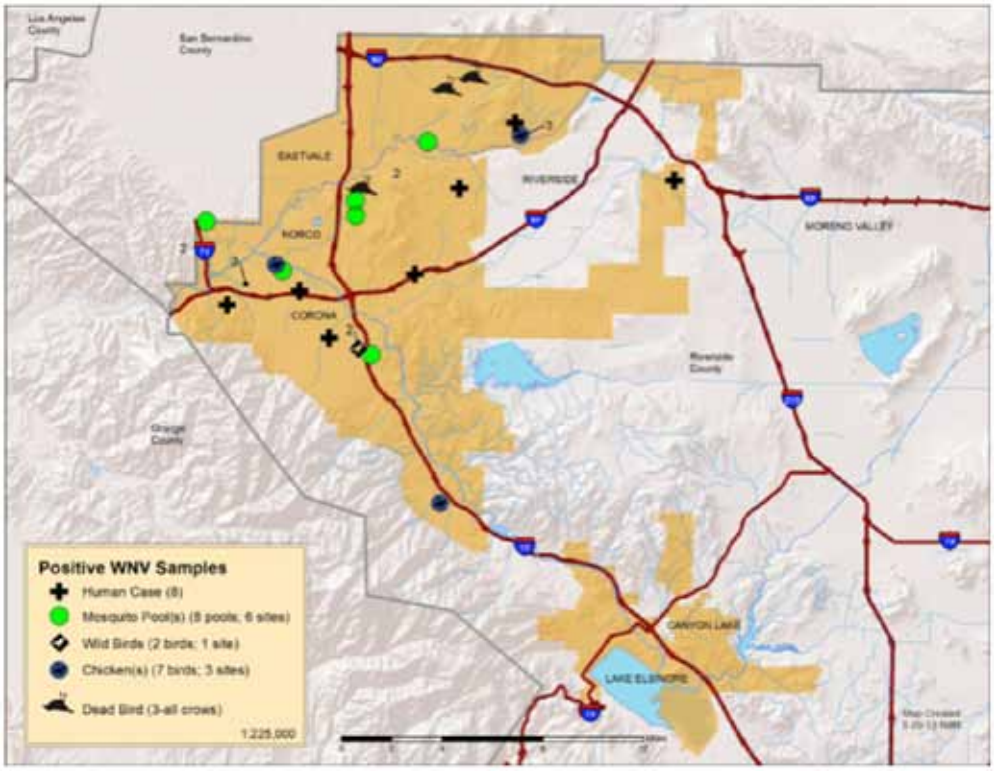

Figure 4. Map of NWMVCD West Nile virus positive samples tested at various sites in 2012.

November. Of the WNV-positive birds, three American crows were collected in June, July and August. Two of the dead birds were collected in a portion of the city of Riverside served by the District. One American crow found in close proximity to the Santa Ana River was in the City of Norco.

Significant mosquito populations can be found nearly year round at NWMVCD, likely due to the southern latitude and generally warm to mild winter climate. *Culex erythrothorax* is by far the most abundant mosquito overall; however, significant populations of other *Culex spp*. can be collected from certain areas using a variety of collection methods, notably resting boxes and gravid traps when placed appropriately. *Culex erythrothorax* is most prevalent along the Santa Ana River, particularly in mitigated riparian and wetland areas which frequently are planted with cattails (*Typha spp*.), tules and bulrush (*Schoenoplectus spp.*). This area now also has a heavy residential component, particularly since many of the historical dairies in the area have been developed into housing tracts and mitigated areas within the last 10 -15 years. These mitigation areas are usually designated for habitat restoration, Endangered Species Act designation, water quality and/or parks and recreation. Multiple land uses are typically accompanied by multiple jurisdictions that further complicate mosquito management activities. The remnant *Cx. quinquefasciatus, Cx. stigmatosoma* and *Cx. tarsalis* populations from the dairies seem to be persisting in or near their renovated environment. Larval collections from abandoned swimming pools frequently yield high numbers of *Cx. stigmatosoma* as well as *Cx. tarsalis* and *Cx. quinquefasciatus*. Previous work near this area has shown how quickly *Cx. quinquefasciatus* can recolonize areas known to be predominantly *Cx. erythrothorax* sites (unpublished data). The wide riparian corridor of the Santa Ana River and adjacent areas provide an excellent habitat for the co-mingling of birds, mosquitoes and humans which provides great potential for disease amplification and transmission. The first human WNV case in our District area occurred in late August near the Santa Ana River in the City of Riverside (Figure 4). Two additional human cases occurred in the City of Riverside. Of the eight human cases found within the boundaries of our district, five were in Corona which also had numerous houses with foreclosed swimming pools. The earliest detected WNV-positive mosquito pools, dead birds, live birds and sentinel chicken seroconversions in NWMVCD area occurred in the riparian and wetland habitats surrounding the Santa Ana River. This could only be expected since these areas provide ideal larval habitats for *Culex* mosquitoes along with a high abundance of birds along the river contributing to disease amplification in that area.

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