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ABSTRACT In response to an epidemic amplification of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV), the Sacramento and Yolo Mosquito and Vector Control District (SYMVCD) sprayed ultralow-volume (ULV) formulations of pyrethrin insecticide (Evergreen EC 60-6: 6% pyrethrin insecticide, 60% piperonyl butoxide; MGK, Minneapolis, MN, applied as 0.003 kg/ha [0.0025 lb/acre]) over 218 km^2 in north Sacramento and 243.5 km^2 in south Sacramento on three consecutive evenings in August 2005. We evaluated the impact of this intervention in north Sacramento on the abundance and WNV infection rates of *Culex pipiens* L. and *Culex tarsalis* Coquillett. Mortality rates of caged *Cx. tarsalis* sentinels ranged from 0% under dense canopy to 100% in open fields. A comparison of weekly geometric mean mosquito abundance in CO$_2$-baited traps in sprayed and unsprayed areas before and after treatment indicated a 75.0 and 48.7% reduction in the abundance of *Cx. pipiens* and *Cx. tarsalis*, respectively. This reduction was statistically significant for *Cx. pipiens*, the primary vector of WNV, with highest abundance in this urban area, but not for *Cx. tarsalis*, which is more associated with rural areas. The infection rates of WNV in *Cx. pipiens* and *Cx. tarsalis* collected from the spray zone were 8.2 and 4.3 per 1,000 female mosquitoes in the 2 wk before and the 2 wk after applications of insecticide, respectively. In comparison, WNV infection rates in *Cx. pipiens* and *Cx. tarsalis* collected at same time interval in the unsprayed zone were 2.0 and 8.7 per 1,000, respectively. Based on the reduction in vector abundance and its effects on number of infective bites received by human population, we concluded that the aerial application of pyrethrin insecticide reduced the transmission intensity of WNV and decreased the risk of human infection.

KEY WORDS West Nile virus, vector-borne disease, mosquitoes, California, control

The intensity of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) transmission to humans is dependent upon the level of enzootic amplification, which, in turn, is related to mosquito abundance, infection rates, and feeding patterns as well as local ecology and behavior that influence human exposure (Komar 2000, Hayes 2005). In California, practically all mosquito species found naturally infected with WNV are within the genus *Culex*, with *Culex pipiens* L. and *Culex tarsalis* Coquillett infected most frequently in the Sacramento Valley (Hom et al. 2005, Hom et al. 2006). Other California species have been found to be competent laboratory vectors (Goddard et al. 2002), but they rarely are infected in nature; therefore, they are presumed to be of minimal epidemiological importance. Based on previously published host selection studies (Tempelis et al. 1965, Tempelis and Washino 1967), *Cx. pipiens* and *Cx. tarsalis* likely function as maintenance, amplifying, and bridge vectors.

WNV first was detected in California during 2003, but it was restricted to areas south of the Tehachapi Mountains (Reisen et al. 2004). The next year, WNV amplified to epidemic levels in southern California and spread northward to all 58 counties, including Sacramento County where it was associated with low-level transmission to humans and horses (Hom et al. 2005; Armijos et al. 2005). Subsequently in 2005, a severe WNV outbreak occurred in Sacramento County, with 177 human infection cases (incidence of 14.5 cases per 100,000), 40 equine cases, 16,900 reported dead birds, and a 53% seroconversion rate in

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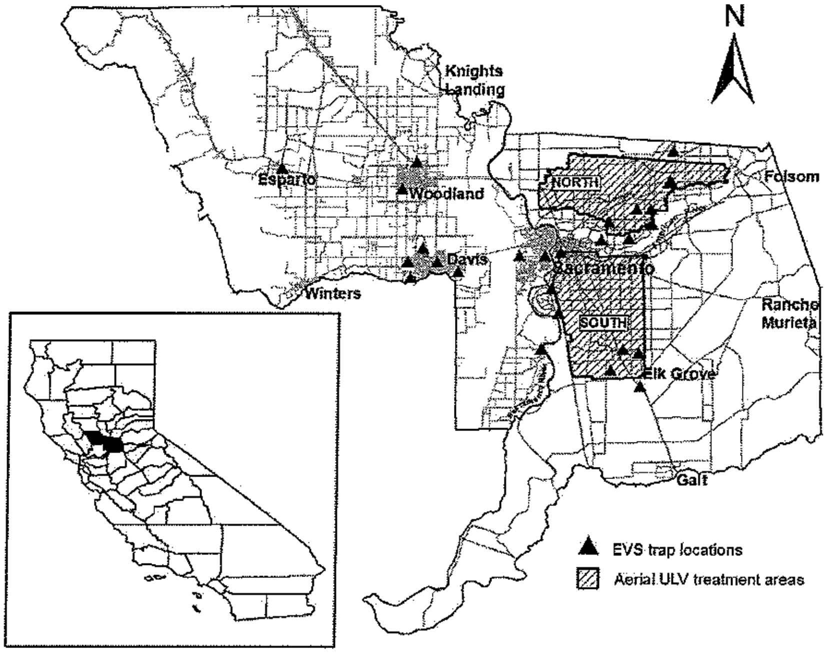

Fig. 1. Map of Sacramento and Yolo counties, CA, showing location of mosquito trapping sites (▲) and area subjected to aerial spraying of pyrethrin insecticide in north Sacramento (north) and south Sacramento (south). Inset, location in California.

110 sentinel chickens (Elnaiem et al. 2006). During this outbreak, WNV infection was detected in 139 of 1,896 pools (7.3%) containing 34,386 female mosquitoes. *Cx. pipiens* and *Cx. tarsalis* made up 68.3 and 28.8% of the infected pools, respectively. Other mosquito species found infected were *Culex erythrothorax* Dyar (0.7% of the infected pools), *Culex thriambus* Dyar (0.7% of the infected pools), and *Culex stigmatosoma* Dyar (1.4% of the infected pools) (Elnaiem et al. 2006).

During the early phase of the 2005 outbreak, the Sacramento and Yolo Mosquito and Vector Control District (SYMVCD) used intensive larviciding and public education to suppress vector abundance and limit human exposure, respectively. In response to very high focal mosquito infection rates, the clustering of dead American crows (AMCR), and an elevated risk for human infection, and following the guidelines of California Mosquito-Borne Virus Surveillance and Response Plan (Barker et al. 2003; Kramer 2005), SYMVCD intervened by applying adulticides. Mosquito adult control initially was attempted with 5% pyrethrin/25% piperonyl butoxide (PBO) applied by ground ultralow-volume (ULV) equipment at scattered sites in Sacramento and Yolo counties. As it became clear that epidemic transmission of WNV was occurring over large urban–suburban areas in north (218.5 km^2) and south Sacramento (243.5 km^2), SYMVCD contracted two aircraft to spray ULV formulations of the pyrethrin insecticide Evergreen over these two areas on 8–10 and 20–22 August 2005, respectively. Although these applications initiated debate over the effectiveness and the environmental and health risks of aerial spraying of insecticides against WNV transmission in an urban setting (Weston et al. 2006), these spray events effectively interrupted epidemic transmission (Carney et al. 2008). In the current article, we describe the impact of aerial spraying of pyrethrin insecticide on *Cx. tarsalis* and *Cx. pipiens* abundance and infection rates with WNV in north Sacramento Spray zone. The evaluation study was limited to the north Sacramento Spray zone, because of lack of adequate mosquito trapping data in the south Sacramento Spray zone.

Materials and Methods

Study Area. Located in the middle of the Central Valley of California, Sacramento County covers 2,578 km^2 and supports a human population of 1,223,499 (Fig. 1). The climate is Mediterranean, characterized by a mild wet winter and hot dry summer. In 2005, this area experienced above-average summer temperatures, reaching daily averages of 26.4 and 24.9°C for July and August, respectively. During the 2 wk before and after the application of insecticide in north Sacramento spray area, the daily minimum-maximum temperatures were 17–37°C, 16–38°C, 16–36°C, and 14–31°C, respectively (Sacramento International Airport weather station).

Aerial Spraying. SYMVCD contracted with ADAPCO Vector Control Services (ADAPCO, Inc., Sanford, FL), which used two Piper Aztec aircraft (flight speed 130 knots, elevation 61 m [200 feet]) to apply Evergreen Crop Protection EC 60-6 (6% pyrethrin insecticide, 60% PBO, MGK, Minneapolis, MN), over a 218.5-km^2 area in north Sacramento and a 243.5-km^2 area in south Sacramento (Fig. 1). Using AU 4000 Micronair nozzles (Micron Sprayers Ltd., Bromyard Industrial Estate, Bromyard, Herefordshire, United Kingdom), the insecticide was applied at 0.003 kg/ha (0.0025 lb/acre), the maximum rate permitted by the label. The spraying in north Sacramento was conducted on three consecutive nights during 8–10 August 2005. Ground level wind speed ranged from 4 to 10 knots, temperature averaged 27°C, and the dew point was 24°C. The application in south Sacramento was conducted during 20–22 August 2005.

Mosquito Abundance and Infection with WNV. Efficacy of insecticide spraying was measured by mortality of sentinel *Cx. tarsalis* from a laboratory colony with known susceptibility for pyrethrins. Mosquitoes were exposed from 20 to 2400 hours within sentinel cages (Townzen and Natvig 1973) placed at replicate sites representing an open field, an apartment complex and a creek (Brook Tree Park and Coyle Creek). Cages were removed 30 min after the completion of spray, examined for immediate mosquito mortality, placed in plastic bags, transported to the SYMVCD laboratory, held for 12 h, and then examined for mortality. Results were expressed as percentage of mortality for each cage of 12–28 mosquitoes.

Mosquito abundance was measured by CO$_2$-baited traps (Rohe and Fall 1979), placed within sprayed areas in north Sacramento and unsprayed control zones in other urban–suburban locations in Sacramento and Yolo counties (Fig. 1). Data were summarized for 1-wk intervals pre- and postspray. Total trap nights were 26 and 20 in the spray zone and 26 and 29 in the unsprayed zone during the week before and the week after spray, respectively. Apart from three trapping records obtained from the data base of SYMVCD, all mosquito trapping in the spray zone was done in fixed locations that were used consistently in the week before and the week after spraying. In contrast, all data from the unsprayed zone were obtained from the routine mosquito and encephalitis virus surveillance done at the same period by technicians at SYMVCD. In this surveillance, CO$_2$-baited traps were placed randomly in different locations within control zones in Sacramento and Yolo counties. For the purpose of our study, we used all unsprayed zones' trapping data that occurred in urban–suburban locations that had a similar habitat as the north Sacramento Spray zone. All data were expressed as mosquito number per trap night. These numbers were either retrieved directly from the records of the sites that had one trap per night or obtained by dividing total number of mosquitoes by number of traps used per site per night. For analysis, mosquito numbers per trap per night were transformed by $\ln(y + 1)$ to normalize the distribution and control the variance and expressed as geometric or back transformation mean of weekly numbers for *Cx. tarsalis* and *Cx. pipiens* in sprayed and unsprayed zones. The formula described by Mulla et al. (1971) was used to calculate percent reduction of *Cx. tarsalis* and *Cx. pipiens* abundance in the week after intervention. In addition, factorial two-way analysis of variance (ANOVA) was used, within SPSS version 14 software (SPSS Inc., Chicago, IL), to test for significant changes in mosquito abundance in sprayed and unsprayed zones, before and after the spraying.

Mosquitoes from the traps described above and from traps placed in the spray zone and unsprayed areas at 2 wk before and 2 wk after the application of insecticide were pooled into lots of ≤50 females each, and then they were tested for WNV, St. Louis encephalitis, and western equine encephalomyelitis virus RNA by using a real-time multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) (Brault et al., unpublished). WNV infection rates in mosquitoes were estimated using the bias-corrected maximum likelihood estimate (MLE) described by Biggerstaff (2006). Methods described by Biggerstaff (2008) were used to compute 95% confidence intervals (CI) for the differences of infection rates in the two areas before and after the application of insecticide.

Results

Sentinel mosquitoes placed under different levels of canopy and wind shadow conditions during the first aerial spray showed variable mortality (Fig. 2). Greatest mortality was encountered in cages placed in open fields (100% in each cage), whereas the lowest rates occurred in sentinel cages placed along the bank of a dry creek under dense canopy and between buildings of a residential site. The overall mortality among mosquitoes placed in exposed or partially exposed sites (172/223 = 77.1%) was significantly higher than mortality of mosquitoes placed in protected places (62/250 = 24.9%; $\chi^2 = 129.1$, df = 1, $P < 0.001$). Although the actual counting of dead and live mosquitoes was performed at 12 h after spraying, we noticed that in the nine cages with 100% mortality rates all mosquitoes were dead 30 min after the spraying. This represented 78.2% (183/234) of the total number of dead mosquitoes in all cages. Immediate mortality at 30 min after spraying also was observed in the remaining cages with partial mortality rates. However, it was difficult to estimate the level of early mortality in these cages, because of the presence of live mosquitoes.

Comparing mosquito abundance measured during 1 wk before with 1 wk after spray, *Cx. pipiens* and *Cx. tarsalis* abundance was reduced by 75.0 and 48.7%, respectively (Table 1). The reduction in both species combined was 57.5%. Two-way ANOVA showed that mean *Cx. pipiens* abundance was significantly affected by the spray, as indicated by the significant interaction between time before and after treatment in the sprayed and unsprayed zones ($F = 4.965$; df = 1, 47; $P = 0.031$). In contrast, mean *Cx. tarsalis* abundance in the spray zone was not significantly reduced compared with the unsprayed zone ($F = 0.754$; df = 1,47; $P = 0.390$).

WNV infection rates in *Cx. pipiens* and *Cx. tarsalis* in the 2 wk before and the 2 wk after the insecticide application are shown in Table 2. Because of the small number of mosquito pools tested we were not able to

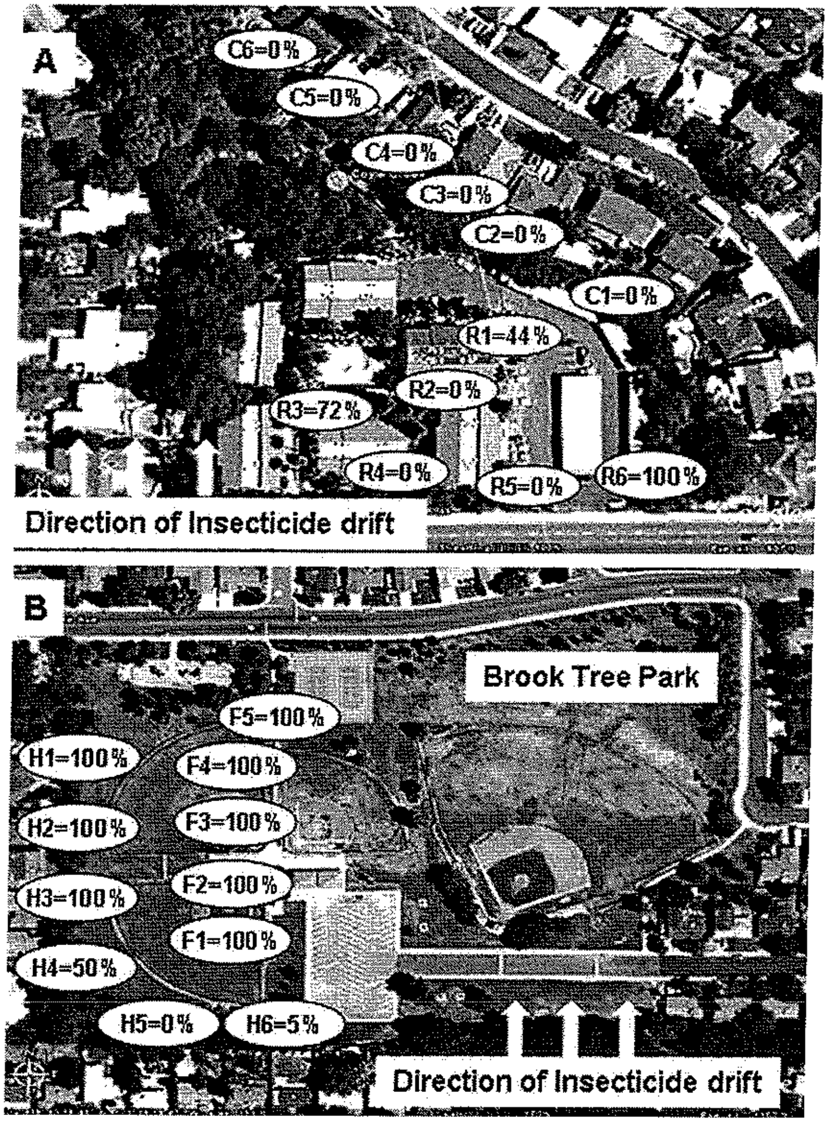

Fig. 2. Mortality rates (%) of mosquitoes held in bioassay cages (sentinel cages) and subjected to aerial spraying of pyrethrin insecticides in Coyle Creek and Brook Tree Park areas of north Sacramento, CA, 8 August 2005; (A) Cages held under dense canopy on the banks of Coyle Creek (C) and between buildings of an apartment complex (R). (B) Cages held under hedges of trees (H) and an open field (F) in Brook Tree Park. Arrows show direction of insecticide spraying. Maps were based on screenshots from Google Earth Mapping Service (http://earth.google.com).

determine the infection rates for each species independently. Using data for the two species combined, the overall infection rates in the spray zone were 8.2/1,000 (95% CI, 3.1–18.0/1,000) before spray and 4.3/1,000 (95% CI, 0.3–20/1,000) female mosquitoes after spray. Only a single positive pool was collected from the intervention zone, in the second week after spraying. In contrast, WNV infection rates in the same time intervals in the unsprayed areas were 2.0/1000 (95% CI, 0.1–9.7/1,000) and 8.7/1000 (95% CI, 3.3–18.9 /1000) females, respectively. It seemed that the infection rate in the spray zone decreased by 3.9/1,000 females (95% CI of prepost spray difference, −12.9–15.2/1,000), whereas it increased by 6.7/1,000 females (95% CI of prepost spray difference, −17.3–2.6/1,000) in the unsprayed areas. However, these differences were not statistically significant, as indicated by the overlap of the null value 0 by the 95% confidence intervals.

Discussion

Although most guidelines for protecting the public during outbreaks of mosquito-borne encephalitis recommend aerial adulticiding as the most effective method of rapidly eliminating infective mosquitoes

Table 1. Effects of aerial spraying of pyrethrin insecticide on abundance of *Cx. pipiens* and *Cx. tarsalis* in north Sacramento, CA, during the week before and after spray in August 2005

Sampling area	Sampling period in relation to spraying	No. trap nights	Geometric mean no. (confidence intervals) of mosquitoes per trap night		
			Cx. pipiens	*Cx. tarsalis*	Total
Sprayed	Before	26	7.4 (5.2–10.2)	3.4 (1.8–5.9)	11.0 (7.4–16.1)
	After	20	3.7 (1.7–7.1)	1.1 (0.3–2.4)	4.6 (2.0–9.5)
Unsprayed	Before	26	2.0 (0.6–4.4)	4.8 (3.1–7.0)	8.1 (5.3–12.3)
	After	29	4.0 (1.8–7.8)	2.9 (1.3–5.7)	8.1 (4.2–14.9)
% control[a]			75.0	48.7	57.5

[a] The % control value was calculated using the formula described by Mulla *et al* (1971). Values in parentheses show 95% CI of the mean.

and interrupting transmission (Mount et al. 1996, Moore et al. 2002, California Department of Health Services 2007), there are surprisingly few published studies measuring the impact of this control method on transmission in residential areas, especially in the United States. Our results indicated that the aerial spraying of pyrethrin in north Sacramento significantly reduced mosquito abundance and the number of infective bites received by human population. These results may explain the significant reduction of human cases and the interruption of the WNV epidemic in Sacramento that was reported by Carney et al. (2008). The analysis conducted by these authors indicated that the aerial spraying of north and south Sacramento resulted in an approximately six-fold decrease in the relative risk of infection in humans. They showed that after spraying, there were no new human WNV cases in either of the treated areas, whereas 18 new cases occurred in adjacent untreated areas in Sacramento County. In each of the sprayed areas, the proportions of pretreatment versus posttreatment cases were also significantly lower than untreated areas (Carney et al. 2008).

It is interesting that the aerial spraying of the insecticide significantly reduced the abundance of *Cx. pipiens* but not *Cx. tarsalis.* As suggested by Nielsen et al. (2007), these differences may be due to the location of the larval development sites of these mosquito species. *Cx. pipiens* usually breeds in urban–suburban locations, whereas *Cx. tarsalis* develops in rural agricultural sites such as the rice, *Oryza sativa* L., fields adjacent to Sacramento (Wekesa et al. 1996) and immigrates into town. Alternatively, these differences may be due to differences in their abundance in the sprayed and unsprayed areas and natural changes in their population densities during the time of spraying. It is noteworthy that the two species have marked differences in their seasonality in Sacramento County. After a decline in July, *Cx. pipiens* abundance usually continues to increase through August, reaching a peak in September (SYMVCD, unpublished data). In contrast, the population of *Cx. tarsalis* typically declines sharply by the end of July. Therefore, the insecticide application in the second week of August was impacting an increasing population of *Cx. pipiens* and an already declining population of *Cx. tarsalis.* Interestingly, *Cx. pipiens* was the primary vector of the 2005 WNV epidemic in the area (Elnaiem et al. 2006). In 2005, the total WNV infection rate in this species in Sacramento and the neighboring Yolo counties (5.3/

Table 2. Weekly infection rates of WNV in *Culex* mosquitoes collected from areas that were subjected to aerial spraying of pyrethrin insecticide and other unsprayed areas in Sacramento and Yolo counties, CA, July–August 2005[a]

Location	Sampling period	No. females	No. pools	No. +ve pools	% +ve pools	MLE[b] (95% CI)
North Sacramento spray area	Pretreatment					
	24–31 July	354	12	4	33.3	11.9 (4.2–28.3)
	1–7 Aug.	297	23	1	4.3	3.4 (0.2–16.9)
	Total	651	35	5	14.3	8.2 (3.1–18.0)
	Posttreatment					
	11–15 Aug.	145	19	0	0	0
	16–23 Aug.	85	11	1	—[c]	—[*3]
	Total	230	30	1	3.3	4.3 (0.3–20.3)
Unsprayed areas	Pretreatment					
	24–31 July	211	9	0	—[c]	—[c]
	1–7 Aug.	284	9	1	—[c]	—[c]
	Total	495	18	1	5.6	2.0 (0.1–9.7)
	Posttreatment					
	8–15 Aug.	346	21	4	19.0	12.1 (4.2–28.6)
	16–23 Aug.	251	28	1	3.6	3.9 (0.2–18.5)
	Total	597	49	5	10.2	8.7 (3.3–18.9)

[a] Aerial spraying on 8–10 Aug. 2005.

[b] Bias-corrected maximum likelihood estimate of infection rate/1,000 mosquitoes (Biggerstaff 2006); 95% CI based on skewness-corrected statistic.

[c] No calculation of percentage of number of positive pools or estimation of infection rates were made, due to small number of individuals and pools examined.

1,000; 95% CI, 3.8–7.2/1,000) was more than double the infection rate detected in *Cx. tarsalis* (2.03/1000; 95% CI, 1.4–2.8/1,000) (SYMVCD, unpublished data). Furthermore, *Cx. pipiens* was predominantly the most abundant urban vector of WNV, accounting for 66.8% (2,654/3,976) of all *Culex* mosquitoes captured in CO_2-baited traps placed in the residential areas of north and south Sacramento, where the epidemic occurred. Thus, control of *Cx. pipiens* was of greatest importance, and the significant reduction of the abundance of this species should have a strong impact on the WNV epidemic despite the absence of a significant reduction in *Cx. tarsalis* populations.

The sentinel mosquito protocol adopted in our study differed from protocols used in other studies in that mosquitoes were not transferred to new unexposed holding cages after the spraying (Bunner et al. 1989). Our procedure may have resulted in an overestimation of mosquito mortality rates by increasing their continued exposure to pesticide residues on the cages; however, results from previous trials (G.Y., unpublished) where a portion of the mosquitoes were transferred indicated minimal differences that were offset by the disadvantages of mosquito trauma from handling and transfer to new cages. Furthermore, the observation that most mosquitoes died immediately after spraying indicates that the effects of increased mortality due to continued exposure to the insecticide residues in the cages did not have a substantial influence on our results. Our results indicate that in some places the impact of the aerial spraying was affected by the wind shadow effects caused by residential buildings and dense vegetation. ULV particles apparently did not effectively contact sentinel mosquitoes placed within an apartment complex, under dense tree canopy or along the banks of a dry creek, areas often frequented by questing females. Similar results were reported recently for aerial applications in neighboring Davis in Yolo County (Nielsen et al. 2007).

The rationale for adulticiding during epidemics of mosquito-borne diseases is to reduce the number of infected mosquitoes and thus interrupt pathogen transmission. Depending on its efficacy and the number of newly emerging adults, adulticiding may also result in a reduction in mosquito infection rates by affecting the age structure of the mosquito population. Due to the small number of mosquito pools collected from the sprayed and unsprayed areas at each time interval, we were not able to determine the infection rates in each species of mosquitoes separately. This limitation may have some consequences on the interpretation of the impact of aerial spraying on the infection rates of WNV, because different species may be impacted differently and their infection rates may fluctuate depending on their ecology and behavior. Our findings that the reduction in the combined infection rates was not statistically significant are considered inconclusive however, because of the small sample sizes of the mosquito pools which generated large 95% confidence intervals.

Even without a significant change in the infection rate, we suggest that the significant reduction in the abundance of *Cx. pipiens* resulted in a decrease in the number of infective bites received by the human population and consequently impacted the transmission of the disease. It must be stressed that the vectorial capacity, or force of transmission, of vector-borne pathogens (MacDonald 1957; Garrett-Jones 1964), is highly dependent on the biting rate, which is also dependent on vector abundance. Based on this justification, we conclude that the aerial spraying of pyrethrin insecticide in north Sacramento resulted in interruption of WNV transmission and reduced the risk of human infection. Nonetheless, and considering the environmental and health hazards of pesticides, we emphasize that mosquito adulticiding should be used as part of a comprehensive intervention program, when surveillance indicates an increased risk of infection to humans.

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References Cited

Armijos, V., S. A. Wright, W. K. Reisen, K. Kelly, S. Yamamoto, and D. A. Brown. 2005. West Nile Virus in Sacramento and Yolo Counties, 2004. Proc. Mosq. Vector Control Assoc. Calif. 73: 24–27.

Barker, C. M., W. K. Reisen, and V. L. Kramer. 2003. California state Mosquito-Borne Virus Surveillance and Response Plan: a retrospective evaluating using conditional simulations. Am. J. Trop. Med. Hyg. 68: 508–518.

Biggerstaff, B. J. 2006. Pooled infection rate. Center for Disease Control and Prevention, Ft. Collins, CO. (http://www.cdc.ndidod/dvbid/westnile/software.htm).

Biggerstaff, B. J. 2008. Confidence intervals for the difference of proportions estimated from pooled samples. J. Agric. Biol. Environ. Stat. (in press).

Bunner, B. L., M. J. Perich, and L. R. Boobar. 1989. Culicidae mortality resulting from insecticide aerosols compared with mortality from droplets on sentinel cages. J. Med. Entomol. 26: 222–225.

California Department of Health Services. 2007. California State mosquito-borne virus surveillance and response plan. California Department of Health Services. (http://westnile.ca.gov/resources.php).

Carney, R. M., S. Husted, C. Jean, C. Glaser, and V. Kramer. 2008. Efficacy of aerial spraying of mosquito adulticide in reducing the incidence of West Nile virus in humans, Sacramento County, California, 2005. Emerg. Infect. Dis. 14: 747–754.

Elnaiem, D. A., K. Kelley, S. Wright, R. Laffey, G. Yoshimura, V. Armijos, M. Reed, M. Farley, G. Goodman, W. K. Reisen, and D. Brown. 2006. Epidemic amplification of West Nile Virus in Sacramento and Yolo Counties, June–

September. 2005. Proc. Mosq. Vector Control Assoc. Calif. 74: 18–20.

Garrett-Jones, C. 1964. Prognosis for the interruption of malaria transmission through assessment of a mosquito's vectorial capacity. Nature (Lond.) 204: 1173–1175.

Goddard, L. B., A. E. Roth, W. K. Reisen, and T. Scott. 2002. Vector competence of California mosquitoes for West Nile virus. Emerg. Infect. Dis. 8: 1385–1391.

Hayes, E. B. 2005. Epidemiology and transmission dynamics of West Nile virus disease. Emerg. Infect. Dis. 11: 1167–1173.

Hom, A., L. Marcus, V. L. Kramer., B. Cahoon., C. Glaser, C. Cossen, E. Baylis, C. Jean, E. Tu, B. F. Eldridge, et al. 2005. Surveillance for mosquito-borne encephalitis virus activity and human disease, including West Nile virus, in California, 2004. Proc. Mosq. Vector Control Assoc. Calif. 73: 66–77.

Hom, A., D. Bonilla, A. Kjemtrup, V. L. Kramer, B. Cahoon-Young, C. M. Barker, L. Marcus, C. Glaser, C. Cossen, E. Baylis, et al. 2006. Surveillance for mosquito-borne encephalitis virus activity and human disease, including West Nile virus, in California, 2005. Proc. Mosq. Vector Control Assoc. Calif. 74: 43–55.

Komar, N. 2000. West Nile viral encephalitis. Rev. Sci. Tech. 19: 166–176.

Kramer, V. L. 2005. California State Mosquito-borne Virus Surveillance and Response Plan. (http://westnile.ca.gov/website/pubication/2005_Ca_mosq. Response_plan.pdf).

MacDonald, C. 1957. The epidemiology and control of malaria. Oxford University Press, London, United Kingdom.

Moore, C. G., R. G. McLean, C. J. Mitchell, R. S. Nasci, T. F. Tsai, C. H. Calisher, A. A. Marfin, P. S. Moore, and D. J. Gubler. 2002. Guidelines for arbovirus surveillance programs in the United States, pp. 1–81. US Dept. Health Human Svcs., DVBID, CDC, Ft. Collins, CO.

Mount, G. A., T. I. Biery, and D. G. Haile. 1996. A review of ultra-low-volume aerial sprays of insecticide for mosquito control. J. Am. Mosq. Control Assoc. 12: 601–618.

Mulla, M. S., R. L. Norlan, D. M. Fanara, H. A. Darwazeh, and D. W. McKean. 1971. Control of chironomid midges in recreational lakes. J. Econ. Entomol. 64: 300–306.

Nielsen, C. F., W. K. Reisen, V. Armijos, S. Wheeler, K. Kelley, and D. Brown. 2007. Impacts of adult mosquito control and climate variation on the West Nile Virus epidemic in Davis, during 2006. Proc. Mosq. Vector Control Assoc. Calif. 75: 125–130.

Reisen, W. K., H. D. Lothrop, R. E. Chiles, M. B. Madon, C. Cossen, L. Woods, S. Husted, V. L. Kramer, and J. D. Edman. 2004. West Nile Virus in California. Emerg. Infect. Dis. 10: 1369–1378.

Rohe, D. L., and R. P. Fall. 1979. A miniature battery powered CO_2 baited trap for mosquito borne encephalitis surveillance. Bull. Soc. Vector Ecol. 4: 24–27.

Tempelis, C. H., W. C. Reeves, R. E. Bellamy, and M. F. Lofy. 1965. A three-year study of the feeding habits of *Culex tarsalis* in Kern County, California. Am. J. Trop. Med. Hyg. 14: 170–177.

Tempelis, C. H., and R. K. Washino. 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California, with notes on other species. J. Med. Entomol. 4: 315–318.

Townzen, K. R., and H. L. Natvig. 1973. A disposable adult mosquito bioassay cage. Mosq. News 33: 113–114.

Wekesa, J. W., B Yuval, and R. K Washino. 1996. Spatial distribution of adult mosquitoes (Diptera: Culicidae) in habitats associated with the rice agroecosystem of northern California. J. Med. Entomol. 33: 344–350.

Weston, D. P., E. L. Amweg, A. Mekebri, R. S. Ogle, and M. J. Lydy. 2006. Aquatic effects of aerial spraying for mosquito control over an urban area. Environ. Sci. Technol. 40: 5817–5822.

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