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Conference Dedication
Dr. Harvey I. Scudder

Submitted by Earl Mortenson, Assistant Chief, Retired
California Department of Health Services, Vector Surveillance and Control
MVCAC Honorary Member, Trustee of Contra Costa County MVCD

Mr. President and the members of the conference, I have the honor and privilege to participate in the dedication of the 69th conference of the MVCAC in recognition of Dr. Harvey Scudder for his contributions and many years of service to mosquito and vector control.

My first contact with the name Scudder was in 1950 when I was first introduced to using the Scudder Fly Grill, (a three foot square framework of 1/2 inch wooden slates) to count and identify adult domestic fly populations during a joint State Health Department and USPHS project to determine the role of flies as vectors in a Shigella disease outbreak in Fresno County.

I had an opportunity to meet and work with Harvey in 1951 when he was a USPHS Entomologist assigned to the Bureau of Vector Control, California State Health Department to serve as technical coordinator of the then CMCA operational mosquito research program at the newly established Fresno Field Station.

Members of the Archives Committee had an opportunity to interview and record the oral history of Dr. Scudder this past December 8 at the Alameda MAD office. I would like to share with you some of the interesting highlights of Dr. Scudder's very active and extensive professional career.

Dr. Scudder spent his early years on a dairy farm near Elmira, New York, and it was in this setting he developed an active interest in entomology. He received his B.S. degree in entomology and several years later a Ph.D. in Public Health from Cornell University.

In 1942 he joined the commission corps of the USPHS. His first assignment was to investigate and develop a control program for a dogfly outbreak in southwestern Florida. (The dogfly in California is called the biting stable fly, *Stomoxy calcitrans*). In Florida this fly propagates in piles of seaweed that is washed along the coast. Because of the severe biting habits of this fly, the military, during the early war years, had difficulty in keeping the guard stations manned and the horseback patrols operating along the beaches to watch for possible landing parties coming ashore from German U-boats that were often sighted along the Florida coast.

He was then sent to Alabama to establish a malaria-mosquito surveillance and control program around military bases.

In 1947, the national concern for oil supplies caused the USPHS to send Dr. Scudder to Sumatra, Indonesia to work with petroleum companies to develop and direct a malaria control program in the region.

During the early fifties, in his position as Mosquito Research Coordinator at the Fresno Field Station, he also developed and operated in conjunction with Bureau of Vector Control Staff a training program in fly biology and control for local health department sanitarians throughout the state. After the Fresno assignment, he was transferred in 1954 to the CDC technical Development Center in Savannah, Georgia to carry out studies in the use of DDT for *Anopheles* control.

From 1956 to 1966, Dr. Scudder was assigned to the National Institutes of Health, Bethesda, Maryland, as senior administrator and research analyst in the Research and Training Grants Division.

He retired from the PHS commission corps as Scientist Director in 1966 and joined the faculty at California State University, Hayward as Professor of Entomology and Dean of Science Department until 1980.

He became a board member of the Alameda MAD in 1982 to the present time, representing the City of Dublin. Harvey served on various committees of the association and the Society of Vector Ecologists. He was Chairman of the MVCAC Trustee Corporate Board and received the Association's Annual Trustee Achievement Award in 1997.

Dr. Scudder's current interest is as an Associate of the California Academy of Sciences, exploring the fossil beds of Scotts Valley in Nevada, collecting and identifying 15 million year old mosquitoes and other aquatic insects in slate deposits.

Surveillance for Mosquito-Borne Encephalitis Virus Activity and Human Disease in California, Including West Nile Virus, 2000

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The California Mosquito-Borne Encephalitis Virus Surveillance Program is a cooperative effort of the California Department of Health Services' (CDHS) Division of Communicable Disease Control, the University of California at Davis and Berkeley, the Mosquito and Vector Control Association of California, local mosquito and vector control agencies, local health departments, physicians, veterinarians, and other interested parties.

In the year 2000, the surveillance system was expanded to include testing for the detection of introduced mosquito-borne viruses, such as West Nile virus (WN) utilizing some funding from the Centers for Disease Control and Prevention (CDC). The enhanced surveillance system involved collaboration with several other agencies, including the California Departments of Food and Agriculture (CDFA), and Fish and Game (CDFG), and the United States Fish and Wildlife Service (USFWS).

In 2000, the program included the following components:

1) Testing of diagnostic specimens from human patients exhibiting symptoms of viral meningitis or encephalitis.

2) Enrolling patients diagnosed with encephalitis into the California Encephalitis Project (CEP) that evaluates demographics, exposure to arthropods, and laboratory analyses to determine etiology. Testing for antibody to WN was added to the CEP protocol in 2000.

3) Diagnostic testing of specimens from domestic animals that exhibited clinical signs of viral neurologic disease compatible with arboviral infection (western equine encephalomyelitis [WEE], St. Louis encephalitis [SLE], eastern equine encephalomyelitis [EEE], and WN as appropriate).

4) Monitoring and testing mosquitoes for SLE and WEE virus infection by in situ enzyme immunoassay. Because of antigenic similarity, pools of mosquitoes positive for SLE also were tested for WN using reverse transcription-polymerase chain reaction (RT-PCR).

5) Monitoring of sentinel chickens for seroconversion to SLE and WEE in areas of California where encephalitis virus historically has been active. Some of the chickens from each flock that were SLE seropositive also were tested for WN using an end point titration plaque reduction neutralization assay.

6) Surveillance and diagnostic testing of dead birds, especially crows, for WN.

7) Weekly reporting in the CDHS Arbovirus Surveillance Bulletin of surveillance data from California and the United States.

HUMAN DISEASE SURVEILLANCE

The CDHS Viral and Rickettsial Disease Laboratory (VRDL) tested 226 sera and/or cerebrospinal fluid specimens from patients exhibiting symptoms of viral meningitis or encephalitis for antibodies to SLE and WEE viruses. Neither elevated IgM antibody nor a four-fold rise in total antibody between paired sera was observed in specimens from any of the suspect cases.

The CEP enrolled 370 patients from June 1998 to December 2000 (170 in 2000). For each patient enrolled, a core battery of tests was conducted, including polymerase chain reaction, serology, and isolation for 15 agents. Testing for additional etiologic agents was pursued as clinical symptomatology and exposure history warranted; extensive testing for arboviruses was conducted for cases with known mosquito exposure. No cases of SLE or WEE were identified through the CEP. Assays for antibody to WN were performed for all patients enrolled in 2000, as well as 36 patients enrolled in 1998 or 1999. Of these patients, six had traveled to the eastern United States within the incubation period consistent with arboviruses, and two of the six were bitten by mosquitoes during their travels. An additional seven patients had a history of recent mosquito bites. No cases of WN were identified.

EQUINE SURVEILLANCE AND A CASE OF EASTERN EQUINE ENCEPHALOMYELITIS

Sera and brain tissue specimens from a total of 15 horses displaying neurological signs were submitted to VRDL for arboviral testing in 2000. Testing failed to detect antigen or antibody for WEE, EEE, or WN.

In late April, a 16-month-old horse in Ventura County was euthanized after developing progressive neurologic signs. Eastern equine encephalomyelitis virus (EEE) was isolated from the horse's brain at the National Veterinary Service Laboratory, U.S. Department of Agriculture in Ames, Iowa. Virus isolation was attempted and subsequently confirmed by VRDL and the UC Davis Arbovirus Research Unit (DARU). The horse had traveled to shows in Utah and southern California in the month preceding onset of illness. This horse and 27 others at the barn were vaccinated with a commercial four-way multi-dose EEE/WEE/rhinopneumonitis/tetanus vaccine seven days prior to the case's illness onset.

CDHS coordinated an investigation of this case with the Ventura County veterinarian, Ventura County health officer, City of Moorpark Vector Control District, and the Ventura County Environmental Health Vector Control Program. Sera from a sentinel chicken flock on the farm where the horse was stabled were negative for EEE, SLE, and WEE antibodies. Five battery-operated CDC-EVS (Encephalitis Virus Surveillance) carbon dioxide baited traps were placed immediately on and within a five-mile radius of the farm for a total of 23 trap nights, but only a small number of mosquitoes were collected. Several mosquito pools were submitted to DARU for virus isolation. New Jersey light trap collections were also sent to DARU for testing by reverse transcriptase polymerase chain reaction (RT-PCR). All the mosquitoes tested negative for EEE as well as WEE and SLE. Although attempts to isolate viruses from residual and archived samples of vaccine were unsuccessful, the paucity of evidence for mosquito transmission in the area indicated that an incompletely inactivated vaccine was the most likely source of infection for this horse. Sequencing studies showed close concordance between the horse isolate and the strain used to produce the vaccine.

MOSQUITO TESTING

Local agencies initiated mosquito collections by New Jersey light traps in April 2000. Data from these sources were forwarded to CDHS and collated weekly in the Adult Mosquito Occurrence Summary Report (AMOR) from April 13 to November 2, 2000.

Twenty-eight mosquito control agencies in California in the year 2000 submitted a total of 160,947 mosquitoes (3,901 pools) collected from carbon dioxide traps (Tables 1, 2, 3). Mosquitoes were tested for arboviruses at DARU by an in situ enzyme immunoassay using Vero cell culture. Thirty mosquito pools collected from the Coachella Valley were positive for SLE, but none were positive for WEE (Figure 1). The 30 SLE positive pools were negative for WN. Six isolates were sequenced and found to be a genome new to southeastern California.

CHICKEN SEROSURVEILLANCE

In 2000, 43 local mosquito and vector control agencies in California maintained 170 sentinel chicken flocks. Blood specimens were collected and tested biweekly from each flock. A total of 18,560 chicken sera from California, and 2,225 sera from Nevada, Oregon, Utah, Washington and Arizona, were tested for antibody to WEE and SLE.

A total of 49 seroconversions to SLE were recorded among nine sentinel chicken flocks in Imperial (11 seroconversions), Riverside (36), and San Bernardino (2) counties (Table 4, Figures 2 & 3). The first SLE seroconversion was detected in five chickens bled on July 3 in Riverside County (North Shore, CA). The last seroconversion for 2000 was in Riverside County (Blythe, CA) on October 12. Because chicken IgG antibody to SLE cross-reacts with WN, SLE-positive chickens were re-tested for WN by end point titration PRNT; all were negative. There were no seroconversions to WEE in 2000. This was only the second year since the chicken serosurveillance program was initiated in 1979 in which no seroconversions to WEE were detected.

DEAD BIRD SURVEILLANCE FOR WEST NILE VIRUS

A dead bird surveillance program for WN (Figure 4) was initiated in September 2000 in conjunction with a grant received from the Centers for Disease Control and Prevention (CDC). In September, CDHS notified approximately 600 agencies and organizations of the program and requested that they contact CDHS when dead birds, especially crows, were sighted. Included in the mailing were the CDFA, CDFG, USFWS, wildlife rehabilitation and refuge centers, veterinarians, the Audubon Society, animal control and environmental health officers, local health departments, and mosquito and vector control districts. The CDFA California Animal Health and Food Safety Laboratory performed necropsies of submitted carcasses. Kidney, brain, and heart samples were forwarded to DARU for testing via cell culture and when appropriate, PCR.

A total of 40 dead birds were reported to CDHS from Los Angeles, Tehama, Kern, San Joaquin, San Bernardino, Placer, San Francisco, Riverside, Fresno, and Santa Barbara Counties. Twenty of these met criteria for testing; all were negative for WN.

Table 1. Mosquitoes (*Culex* spp. and *Aedes melanimon*) tested for WEE and SLE viruses by submitting county & agency, 2000

		Ae melanimon		*Cx pipiens*		*Cx quinquefasciatus*		*Cx stigmatosoma*		*Cx tarsalis*		*Total*	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Contra Costa	CNTR	29	1,432							284	14,153	313	**15,585**
Fresno	CNSL									10	500	10	**500**
Fresno	FRNO									7	264	7	**264**
Glenn	GLEN									20	1,000	20	**1,000**
Kern	KERN	148	6,314							364	9,696	512	**16,010**
Kings	KNGS									23	1,129	23	**1,129**
Lake	LAKE	6	300					3	52	111	5,140	120	**5,492**
Los Angeles	GRLA					363	13,836			72	2,784	435	**16,620**
Los Angeles	LONG					368	14,374	3	49	201	7,742	572	**22,165**
Madera	MADR			9	450					2	100	11	**550**
Merced	TRLK									5	226	5	**226**
Orange	ORCO					117	3,267			9	230	126	**3,497**
Placer	PLCR									19	788	19	**788**
Riverside	COAV	19	869							544*	23,928	563*	**24,797**
Riverside	NWST					181	8,936	4	166	128	6,266	313	**15,368**
Sacramento	SAYO	6	177							63	2,188	69	**2,365**
San Bernardino	SANB					13	377	4	84	73	3,342	90	**3,803**
San Diego	SAND									50	2,477	50	**2,477**
San Joaquin	SJCM									114	5,495	114	**5,495**
Santa Barbara	SBCO					21	919	5	194	49	2,368	75	**3,481**
Shasta	SHAS									64	3,175	64	**3,175**
Stanislaus	TRLK	1	50							32	1,457	33	**1,507**
Sutter	SUYA	14	556							147	6,466	161	**7,022**
Tulare	DLTA					4	163			10	226	14	**389**
Ventura	MOOR			2	3	1	18	4	58	5	58	12	**137**
Ventura	VENT									20	954	20	**954**
Yolo	SAYO	1	50							71	3,212	72	**3,262**
Yuba	SUYA									15	617	15	**617**
Grand Total		**224**	**9,748**	**11**	**453**	**1,068**	**41,890**	**23**	**603**	**2,512**	**105,981**	**3,838**	**158,675**

* 30 pools positive for SLE

Table 2. Mosquitoes (Other *Aedes* spp.) tested for WEE and SLE viruses by submitting county & agency, 2000

		Ae hexodontus		*Ae sierrensis*		*Ae taeniorhynchus*		*Ae washinoi*		*An franciscanus*		*An hermsi*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Contra Costa	CNTR	1	50											1	**50**
Sacramento	SAYO			9	233									9	**233**
Santa Barbara	SBCO					14	633	6	194	1	50	10	441	31	**1,318**
Total		**1**	**50**	**9**	**233**	**14**	**633**	**6**	**194**	**1**	**50**	**10**	**441**	**41**	**1,601**

Table 3. Mosquitoes (*Culiseta* spp. and *Culex erythrothorax*) tested for WEE and SLE viruses by submitting county and agency, 2000

		Cs incidens		*Cs inornata*		*Cs particeps*		*Cx erythrothorax*		*Cx restuans*		Total	
County	Agency	Pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	Pools	mosqs.	pools	mosqs.
Santa Barbara	SBCO	2	39	1	14			14	573	1	19	18	**645**
Ventura	MOOR					1	2	3	24			4	**26**
Total		**2**	**39**	**1**	**14**	**1**	**2**	**17**	**597**	**1**	**19**	**22**	**671**

Table 4. Chicken seroconversions to SLE by location and date bled, 2000.

County	Location	City	7/3	7/6	7/17	7/31	8/14	8/28	9/7	9/11	9/25	10/9	10/10	10/12	11/28	**Total**
Imperial	Cady	Brawley		1			5		1							**7**
Imperial	Campbell	Seeley		1			3									**4**
Riverside	4th Avenue	Blythe												1		**1**
Riverside	Adohr	Mecca						1		3	2	2				**8**
Riverside	Desert	North Shore	5		2	2	1								1	**11**
Riverside	Gordon	Mecca					2			7						**9**
Riverside	Mecca	Mecca									1					**1**
Riverside	SSSP	North Shore			2	1	1			1					1	**6**
San Bernardino	Treatment Pl.	Needles								1			1			**2**
SLE Totals			**5**	**2**	**4**	**3**	**12**	**1**	**1**	**12**	**3**	**2**	**1**	**1**	**2**	**49**

REPORTING ARBOVIRUS SURVEILLANCE DATA

Mosquito pool and sentinel chicken test results were summarized in weekly arbovirus surveillance bulletins from May 11 to December 19. Reports were distributed to all surveillance program participants and other interested parties. Positive serologies and mosquito pools were reported immediately by telephone to the submitting agencies. Surveillance results were depicted spatially on the California website http://mosqnet.ucdavis.edu sponsored by the University of California Mosquito Research Program, CDHS, and the Mosquito and Vector Control Association of California.

CALIFORNIA STATE MOSQUITO-BORNE VIRUS SURVEILLANCE AND RESPONSE PLAN

CDHS, the Mosquito and Vector Control Association of California, and the University of California developed an enhanced surveillance and response program for the State of California to ensure that local and state agencies are prepared to detect and respond in a concerted effort to a mosquito-borne disease outbreak. Efforts to develop this plan were initiated by Charles Beesley, Contra Costa Mosquito and Vector Control District, and an ad hoc committee of the Mosquito and Vector Control Association of California. This plan includes a mosquito-borne virus risk assessment model that defines three response levels and the conditions indicative of each level. A final version of the plan document is expected in early 2001.

WEST NILE VIRUS IN THE EASTERN UNITED STATES

In 1999, WN activity was reported in New York, New Jersey, Connecticut, and Maryland. In 2000, in addition to these states, WN was detected in Delaware, Massachusetts, New Hampshire, North Carolina, Pennsylvania, Rhode Island, Vermont, Virginia, and the District of Columbia. WN surveillance included humans, equids, other mammals, birds, mosquitoes, and sentinel chicken flocks. In 2000 there were 21 confirmed human cases (including two fatalities) of WN encephalitis. This represents a decrease in case numbers from 1999 when 62 confirmed cases with 7 fatalities were detected.

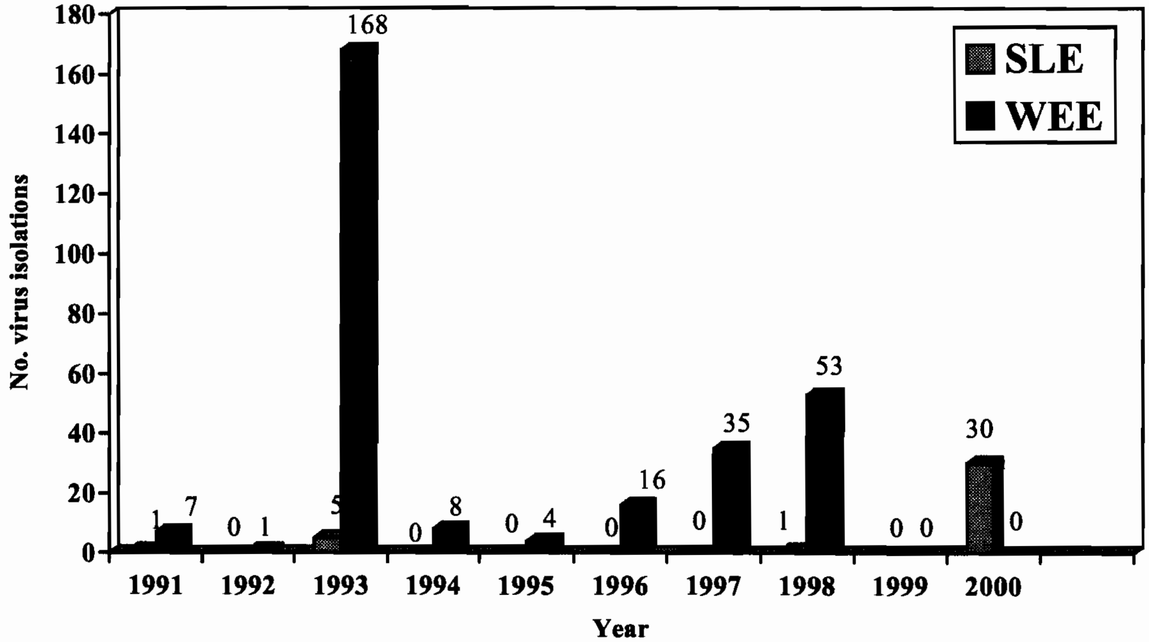


Figure 1. Isolations of St. Louis encephalitis (SLE) and Western equine encephalomyelitis (WEE) viruses from pooled *Culex tarsalis* in California, 1991-2000.

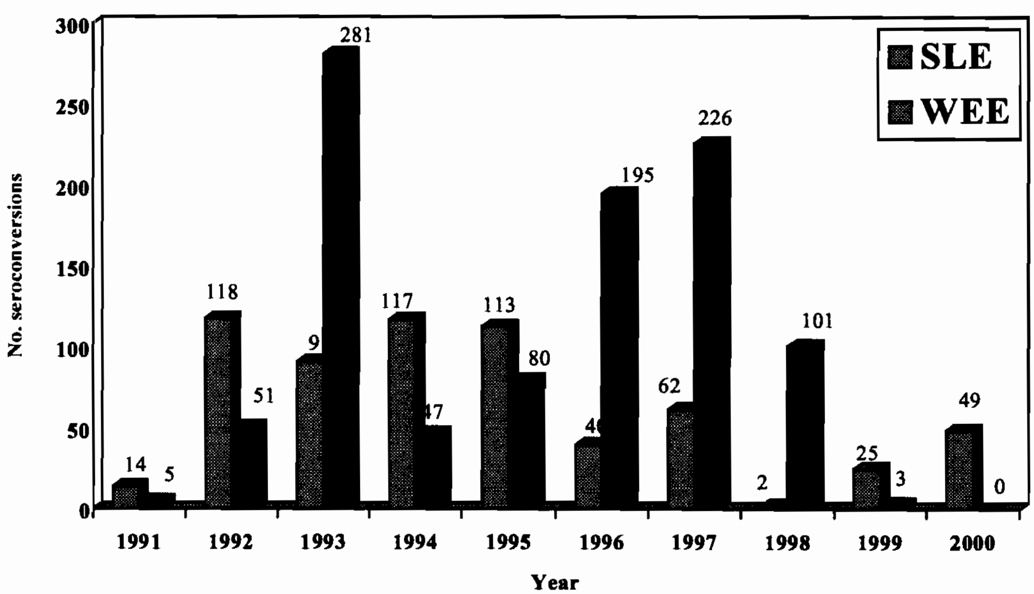
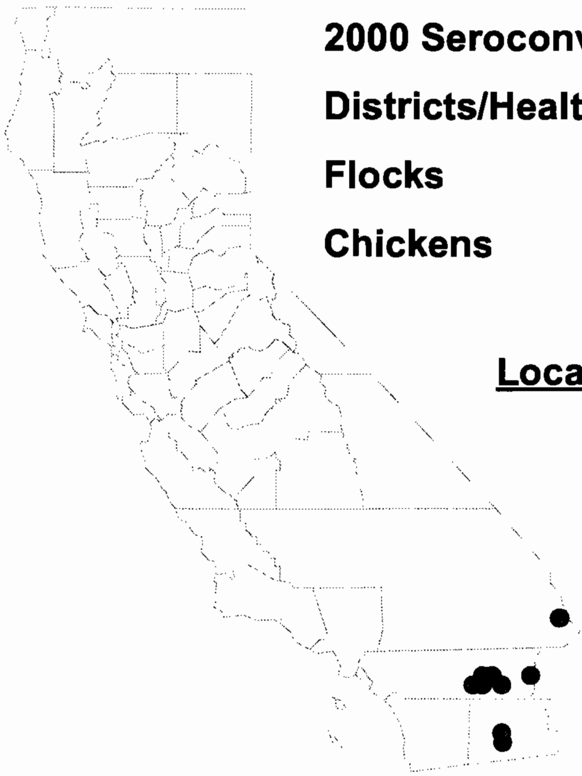


Figure 2. Seroconversions to St. Louis Encephalitis (SLE) and Western equine encephalomyelitis (WEE) viruses in sentinel chicken flocks in California, 1991-2000.



2000 Seroconversions	WEE	SLE
Districts/Health Depts	0	4
Flocks	0	9
Chickens	0	49

Location of Positive Flocks

SLE •

Figure 3. Sentinel chicken flocks with at least one seroconversion to St. Louis encephalitis (SLE), California 2000.

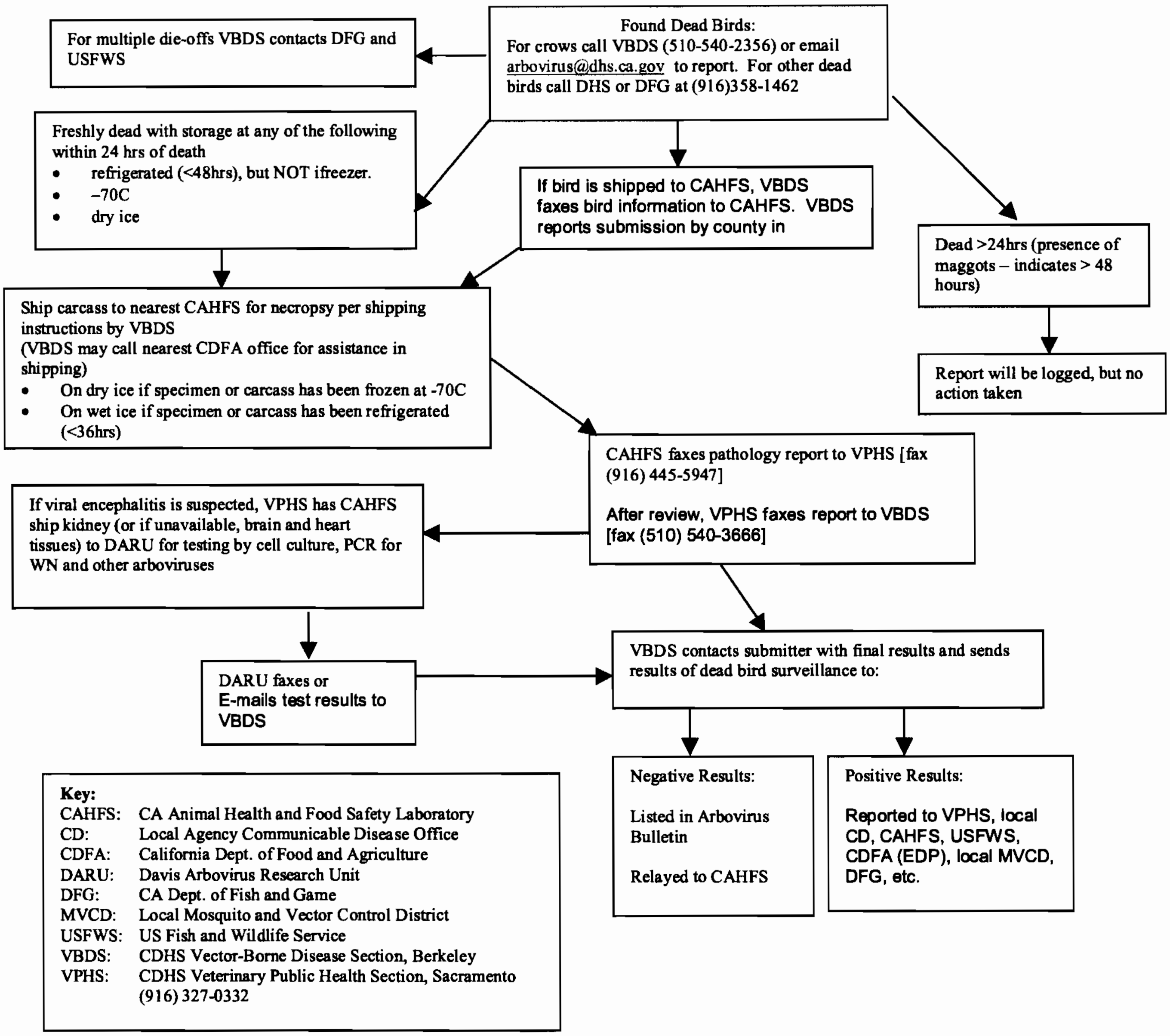


Figure 4. West Nile Virus Surveillance Program for Testing Dead Birds

In mid-2000, CDHS initiated a dead bird surveillance program in collaboration with other public agencies. CDHS notified over 600 agencies, organizations, and veterinarians involved with wildlife, including rehabilitation centers, about the program. A copy of the mailing sent to these agencies is included in this appendix along with the testing algorithm. Dead birds are usually sent first to a California Animal Health & Food Safety Laboratory for pathology testing. When appropriate, viral tests are then done on kidney, brain, and heart tissues by the UC Davis Arbovirus Research Unit (cell culture and PCR for WN and other arboviruses).

Comparison of Dry Ice-Baited CDC and NJ Light Traps for Measuring Mosquito Abundance

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ABSTRACT: Mosquito catch in NJ light traps has been declining in recent years, compromising the sensitivity of the statewide mosquito monitoring program. Research during 2000 first attempted to enhance the catch of mosquitoes in NJ light traps by augmenting these traps with dry ice. Results indicated that NJ light traps operated with and without lights and with dry ice collected significantly fewer female mosquitoes of most species [except *Culiseta inornata* and *Psorophora columbiae*] than did CDC style traps augmented with dry ice and operated without light. To provide information on comparative sensitivity and ability to measure abundance over time and space, catch of mosquitoes in NJ light traps [standard configuration with light on and without dry ice] were compared to catch in CDC-style traps [with dry ice and without light] operated concurrently at 8 sites within each of 4 mosquito and vector control districts [Coachella Valley, Kern, San Joaquin and Sac-Yolo]. CDC traps always collected more female mosquitoes than NJ traps, however, comparative differences in sensitivity varied markedly over time and space precluding the calculation of a universal conversion factor. Regression of catch in CDC traps as a function of catch in NJ traps indicated that the slopes varied markedly among districts, precluding the derivation of a universal conversion function. We concluded that districts switching from NJ to CDC traps probably will have to operate both traps concurrently at several sites to derive regression functions to convert historical NJ trap data to CDC counts for retrospective analysis.

INTRODUCTION

Measurement of mosquito abundance throughout the state of California currently relies on the enumeration of phototactic species collected by New Jersey or American light traps (Mulhern1942). In recent years, the magnitude of change in the catch of these species over time and space has become insufficient for surveillance and may not provide measures suitable for control decisions. Dry ice-baited CDC traps (Sudia and Chamberlain 1962) operated without light at fixed locations on a systematic schedule have been recommended to augment or replace NJ light traps to enhance the sensitivity of population monitoring (Reisen et al. 1999). Before trap replacement can be finalized, comparative studies were necessary to quantify differences in trap sensitivity for a variety of vector and pest species and to provide methods of converting NJ light trap to CDC trap counts so that historical data can be used to detect anomalies in temporal abundance.

The overall purpose of the current research was to provide ways to improve the statewide mosquito monitoring program. Our research first compared the sensitivity of several NJ light trap configurations to concurrently operated dry ice-baited CDC traps to determine if the sensitivity of the current trapping system could be enhanced. These comparisons indicated that CDC traps generally were more sensitive than NJ light traps, regardless of configuration. Subsequently, catch in NJ light traps [standard configuration] was compared to CDC traps over time and space to determine if both systems provided comparable measurements of mosquito population size. Analyses then attempted to develop regression models estimating CDC trap counts from NJ light trap counts.

MATERIALS AND METHODS

NJ light trap configurations. The catch of male and female mosquitoes in NJ light traps baited with a 25-watt light bulb, 1-2 lbs of dry ice, or both, were compared to an unbaited NJ light trap and to CDC traps baited with dry ice and operated without light within 25-50 m of the NJ light trap. Traps were operated at 3 rural sites each within the Coachella Valley and

Kern MVCDs for 4 to 6 wks during the summer of 2000. Four configurations were used during 4 nights for each NJ light trap: Monday – light on [standard configuration], Tuesday – light off [negative control], Wednesday – light on and 1-2 lbs of dry ice in a Styrofoam holder suspended next to the trap opening, and Thursday – light off and the dry ice replenished. On Thursday, a CDC trap baited with 1-2 lbs of dry ice was operated with the light off within 25-50 m of the NJ light trap. Trap configurations within each week were considered to be treatments that were replicated over time.

NJ light and CDC trap comparison. A NJ light trap [standard configuration] and a CDC trap [baited with dry ice and operated without light] were paired at 8 locations each in the Coachella Valley, Kern, San Joaquin County and Sacramento-Yolo MVCDs. Traps were operated weekly for 16-21 weeks during the summer of 2000, and the catch enumerated by species and sex. Counts were transformed by ln[y+1] to normalize the distribution and control the variance (Bidlingmayer1969). Trap sensitivity was evaluated by comparing the transformed mean catch per trap type for each species using an ANOVA blocked by site and date. Patterns in catch size over time and space were compared by correlation and regression analyses using untransformed and transformed counts.

RESULTS

NJ light trap configuration. Among species and sexes collected in sufficient numbers for analyses, dry ice (CO_2) was the best attractant for all female mosquitoes, except *Cs. inornata* and *Ps. columbiae* that were attracted equally well by light and dry ice (Table 1). CDC traps operated within 25-50 m of the NJ light trap always collected more females than NJ light traps, even when NJ traps were augmented with dry ice and operated with the light off. Differences here could relate to the depressing effect of the trap lid on mosquito access and/or airflow (Cummings and Meyer 1999). Significantly more *Cx. tarsalis* males were collected in traps with the light turned on, indicating that males detected light from the 25-watt bulb above background illumination levels. Reduced catch of females in NJ light traps operated with the light on was unexpected, because we anticipated that light would act in an additive or synergistic way with the dry ice. These data indicated that light actually might have decreased the catch of females.

NJ light and CDC trap comparison. To provide data on trap sensitivity and comparative catch over time and space, paired NJ light and CDC traps were operated concurrently at 8 sites for 16-21 weeks within each of 4 districts. In agreement

Table 1. Mean number of mosquitoes collected per trap night in 4 configurations of NJ light and CDC traps, Coachella Valley and Kern MVCD, 2000.

		New Jersey light trap				
Species	District	light	no light	light + ice	no light +ice	CDC trap
Cx. tarsalis - F	Coachella	3.33d	1.91e	4.19b	4.62ab	5.33a
	Kern	1.19c	0.85c	1.76b	1.92b	2.50a
Cx. tarsalis - M	Coachella	3.70a	1.12b	3.54a	1.28b	0.80b
	Kern	1.56a	0.51bc	1.33a	0.89b	0.42c
Cx. p. quinquefasciatus - F	Kern	0.93d	1.37cd	1.53c	2.80b	5.05a
Cx. erythrothorax - F	Coachella	0.59ab	0b	0.91a	0.52ab	1.41a
Ae. vexans - F	Coachella	0.19c	0c	1.08b	1.07b	2.11a
Ps. columbiae - F	Coachella	0.29ab	0b	0.75a	0.59a	0.67a
Cs. inornata - F	Coachella	0.78ab	0b	0.97a	0.40b	0.67ab

Data transformed by ln[y+1] and tested by a 2-way ANOVA with traps and sites as main and weeks as replicates. Means within rows followed by the same letter were not significantly different (P>0.05) when tested by a least significant range test.

with the above NJ light trap configuration study, CDC traps always collected more females of all species than NJ light traps [standard operation], except for *An. freeborni* that was attracted equally well by both dry ice and light. In contrast, *Cx. tarsalis* and *An. freeborni* males always were collected more abundantly in NJ than CDC traps.

Catch in paired NJ and CDC traps was correlated over time and space for 12 of 14 groups of females, but only 2 of 7 groups of males. However, the magnitude of the difference in catch between trap types varied significantly among the 8 trap sites within each district, precluding the calculation of a universal ratio to convert counts between trap types.

Counts of *Cx. tarsalis* females in CDC traps then were regressed as function of counts in NJ light traps within each of the four districts. Regressions were done using both untransformed [linear fit] and ln [y+1] – ln [x+1] transformed [curvilinear or power function fit] data. The relationship between trap counts was linear in the Coachella Valley and Kern MVCDs and curvilinear in the San Joaquin County and Sac-Yolo MVCDs, indicating that the relationship between trap counts differed markedly among districts.

DISCUSSION

The comparative features of CDC and NJ light traps for sampling *Cx. tarsalis* mosquitoes is summarized in Table 2. In agreement with previous studies (e.g., Milby et al. 1978; Reisen et al. 1999), CDC traps operated without light and baited with dry ice always collected as many or more female mosquitoes than NJ light traps, even when these larger traps were configured with the light off and baited with dry ice. Differences in light trap sensitivity most likely will be increased by on-going urbanization in most districts. Therefore, we recommend that NJ light traps should be replaced or augmented with systematically operated CDC traps to provide a more sensitive method of sampling females of most mosquito species. No sampling system is perfect. Data on males of species such as *Cx. tarsalis* and *An. freeborni* would be lost using CDC traps, and these data could be useful in locating poorly controlled or newly created larval habitats. In addition, because CDC traps probably would be operated once every week or 2 weeks, inclement weather or other aberrant factors on the night of operation would have a greater impact on CDC trap than NJ light trap data, because NJ light traps are operated continuously and the samples collected weekly.

Cost of operating dry ice-baited CDC traps **per night** is somewhat greater than operating NJ light traps, because of battery and dry ice costs and labor and transportation for deployment. However, if driving distances are comparable and processing time is considered, the overall cost for operating CDC traps is comparable to NJ light traps (R.Takahashi, unpublished), and the enhanced sensitivity of CDC traps results in a better measure of population size. Processing time for NJ light traps must include the tedious job of sorting mosquitoes from the myriad of other insects. Because specimens in NJ light traps can be up to 1-week-old when collected, these specimens can be hard to identify because they frequently are broken and badly rubbed. Mosquitoes collected by CDC traps are alive and suitable for use in bioassays or arbovirus surveillance.

Comparing current catch size to historical data is useful in determining if mosquito populations are significantly high or low and is one of the primary factors used to forecast the relative risk of encephalitis virus transmission (Eldridge1987). Switching trapping methods from NJ light traps to CDC traps will interrupt the historical continuum of data collected by most districts and thereby compromise the interpretation of catch size. However, increasing urbanization and security lighting already has confounded this data continuum by progressively decreasing NJ light trap sensitivity (Reeves and Milby 1989; Wegbreit and Reisen 2000). The use of historical data trends after switching trap methods will require regional statistical analyses to relate catch in traps operated concurrently at varying habitats over time within each district. Our attempts to provide universal conversion ratios were thwarted by marked differences among mosquito catch over space and time.

Table 2. Comparative features of CDC and NJ light traps for sampling *Culex tarsalis*.

	Feature	CDC	NJ light
1	Sensitivity - females	high	Low
2	Sensitivity - males	none	moderate
3	Impact of weather	high	Low
4	Processing time	fast	Slow
5	Pools for virus surveillance	yes	No
6	Cost of operation	high	Low
7	Historical data	few	long term

Regression analyses also failed to provide a universal conversion function. Although both traps measured similar trends in female abundance as indicated by correlation analyses, the functional relationship between trap types varied markedly among the 4 districts studied; i.e., the relationship was linear in 2 districts and curvilinear in 2 districts.

Transition in sampling methodology from NJ light to CDC traps will enhance population measurement and provide improved data for making control decisions and projecting the risk of encephalitis transmission. However, additional comparisons and statistical analyses at the local level will be necessary to adjust historical NJ light trap data to detect anomalies in abundance measured by CDC traps.

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Role of California Birds in the Amplification of Encephalitis Viruses

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ABSTRACT: Birds collected in Coachella and San Joaquin Valleys were infected experimentally by subcutaneous inoculation with 100 or 1,000 plaque forming units of sympatric strains of western equine encephalomyelitis (WEE) or St. Louis encephalitis (SLE) virus, respectively. Overall, 104 and 86 birds comprising 17 and 11 species were infected experimentally, of which 12 and 3 produced a peak viremia sufficient to infect susceptible *Culex tarsalis* mosquitoes, respectively. The small number of bird species that were competent hosts for SLE was unexpected and indicated the probable important role of immature birds in virus amplification.

Although wild birds collectively are well recognized as maintenance and amplifying hosts for mosquito-borne encephalitis viruses in California (Reeves and Hammon 1962; Reeves 1990), the role of individual bird species in virus maintenance and amplification is less well understood, especially in wetland habitats. During the past 5 years we have been studying infection rates to western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses in wild bird populations (Reisen et al. 2000c) and have identified three epidemiological species groupings within our study areas: 1) birds repeatedly antibody positive and abundant, 2) birds occasionally antibody positive and varying in abundance, and 3) birds never found to be infected, but regionally or seasonally abundant (Table 1). Groups 1 and 2 consisted mostly of resident species, whereas group 3 contained mostly migrants and winter residents. In this research we assumed that the frequency of field infection could be ascertained by seroprevalence rates. However, not all infected birds produce a peripheral viremia of sufficient titer to infect vector mosquitoes, and therefore it was necessary to assess host competence by experimental infection with recent sympatric strains of virus. The relative importance of different bird species in virus amplification then may be determined by the frequency of infections that produce a viremia of sufficient titer to infect susceptible *Culex tarsalis* Coquillett females. Our studies extend previous research (Hammon et al. 1951; Hardy and Reeves 1990b) by documenting the response of additional species of wild birds to recent sympatric strains of WEE and SLE from the Coachella Valley and Kern County.

During the past 10 years, WEE has been detected intermittently in the Central Valley including Kern County, whereas SLE has not been detected north of the Tehachapi Mountains since 1992. In contrast, both WEE and SLE have been active consistently in southeastern California, including the Coachella Valley. Recently isolated virus strains were found to differ genetically from historical strains used in previous in host competence studies (Kramer et al. 1997; Kramer and Fallah 1999). The goal of the current on-going research was to determine the host competence of representative bird species from each of the three epidemiological groups. Because of the patterns of enzootic activity in recent years, our study emphasized WEE in Kern County birds and WEE and SLE in Coachella Valley birds.

MATERIALS AND METHODS

Wild birds were collected by mist net or grain baited traps in Coachella Valley and Kern County, and transported to our aviaries in Bakersfield. Birds were held for 2 weeks to adjust to captivity, observed for general health and prebled to determine prior WEE or SLE infection. Five to 6 previously uninfected adult birds (hatching or after hatching year) of each species then were infected by subcutaneous inoculation in the cervical region with 0.1 ml of virus diluent containing either 100 or 1,000 plaque forming units (PFU) of WEE or SLE. Previous research demonstrated that syringe inoculation produced a viremia response comparable to infectious mosquito bites and that *Cx. tarsalis* infected with WEE or SLE expectorated approximately these quantities of virus after extrinsic incubation (Reisen et al. 2000a). Birds from Coachella Valley were infected with either the Coa592 strain of WEE or the Coa608 strain of SLE, whereas birds from Kern County were infected with the Kern5547 strain of WEE or the Kern217 strain of SLE. These strains were isolated within the past 10 years from *Cx. tarsalis*, were at Vero cell passage 1 or 2, and previously elicited a strong viremia response in House Finches (Reisen et al. 2000b). Birds were bled by jugular puncture, and 0.1 ml of whole blood diluted in 0.4 cc of virus

Table 1. Epidemiological groupings of wild birds sampled for encephalitis virus infection in California.

Group	Bird Species: Prevalence	Bird Species: Abundance	Species Tested in Current Study
1	1 to 10%	>100	House Finch, House Sparrow, Mourning Dove, Common Ground Dove, Gambel's quail, California quail
2	<1%	<or>100	Brown-headed Cowbird, Song Sparrow, American Robin, Red-winged Blackbird
3	0%	<or>100	White-crowned Sparrow, Lincoln Sparrow, Lark Sparrow, Brewer's Sparrow, Yellow-headed Blackbird, Western Scrub Jay, Yellow Warbler

Prevalence, seropositivity rate for either WEE or SLE
Abundance, number collected and tested for virus

diluent [BABSB, bovine albumin borate buffered saline with antibiotics, pH 9]. Samples were frozen immediately on dry ice, stored at –80 °C, and then tested for infection by plaque assay on Vero cells.

RESULTS

Overall, 104 and 86 birds comprising 17 and 11 species were infected experimentally with WEE and SLE, respectively (Table 2). Of these, 96 birds were from the Coachella Valley and 94 were from Kern County. Most birds infected with WEE produced a detectable viremia, and 12 of 17 produced $>10^2$ PFU per 0.1 ml for at least 1 day post infection, a titer considered to be infectious for susceptible *Cx. tarsalis* (Hardy and Reeves 1990a). Viremias for all species peaked on days 1-2 post infection, decreased on day 3, and approached 0 by day 4. House Sparrows, Red-winged Blackbirds and White-crowned Sparrows were most susceptible, producing peak mean viremias $>10^6$ PFU/0.1 ml. In contrast to previous studies using virus strains isolated during the early 1950s (Hardy and Reeves 1990b), few birds in the current study succumbed to infection. To determine if this apparent change in virulence was due to methodology or decreased virulence by recent virus strains, we infected White-crowned Sparrows from Kern County with the BFS1703 strain of WEE, isolated from Bakersfield in 1953; 5 of 6 birds succumbed within 5 days of infection.

Only 3 of 11 species of birds experimentally infected with SLE produced a viremia of sufficient titer infect *Cx. tarsalis*. These species represented each of the 3 epidemiological groups and included House Finches, Song Sparrows and White-crowned Sparrows (Table 2). Viremias peaked on days 2 and 3 post infection, but frequently persisted through day 4. Species such as House Sparrows and Mourning Doves, considered to be important amplifying hosts elsewhere in North America (McLean and Bowen 1980), produced low titered fleeting viremias.

DISCUSSION

Most adult birds infected experimentally with WEE produced a detectable viremia, and in 12 of 17 species tested this was sufficient to infect susceptible *Cx. tarsalis* using our cutoff viremia of 10^2 PFU per 0.1 ml. However, *Cx. tarsalis* vector competence for WEE changes markedly over time (Hardy et al. 1990; Reisen et al. 1996), with populations most susceptible during late winter and spring and least susceptible during summer and early fall. Therefore, practically all the bird species evaluated except for the refractory Brown-headed Cowbirds probably would be able to infect *Cx. tarsalis* early in the season, whereas only those producing a viremia $>10^5$ PFU per 0.1 ml would be important during summer when most enzootic transmission is detected.

In contrast to WEE, few adult birds produced a detectable viremia after infection with SLE, and only 3 of 11 species were considered to be able to infect *Cx. tarsalis*. This was unexpected based on the literature (McLean and Bowen, 1980), but supported previous studies in California (Hardy and Reeves 1990b) and was consistent with the idea that SLE strains from the west tend to be less virulent or viremogenic than strains from the midwest or east (Monath et al. 1980).

The viremia response was not related to the frequency of field infection. White-crowned Sparrows, for example, are a

Table 2. Species of birds that were competent hosts for WEE or SLE

Group	Virus	Comp/total[1]	Species (ranked in order of viremia)[2]
1	WEE	5/6	House Sparrow> House Finch = Mourning Dove = Common Ground Dove> California Quail> *Gambel's quail*
	SLE	1/6	House Finch> *Gambel's quail> California quail= Mourning Dove, House Sparrow= common ground dove*
2	WEE	3/4	Red-winged Blackbird> Song Sparrow> American Robin>> *Brown-headed Cowbird*
	SLE	1/2	Song Sparrow>> *Brown-headed Cowbird*
3	WEE	4/7	White-crowned Sparrow>> Lark Sparrow = Lincoln's Sparrow = Yellow Warbler> *Brewer's Sparrow>* Western *Scrub Jay = Yellow-headed Blackbird*
	SLE	1/3	White-crowned Sparrow>> *Lark Sparrow = Lincoln's Sparrow*

[1] Number of competent species/total tested in each group for each virus. Competent species produced a viremia $>10^2$ PFU/0.1 ml for >= 1 day post infection.

[2] Incompetent hosts in italics.

common winter resident in California and rarely or never have been found infected naturally with either WEE or SLE (Milby and Reeves 1990; Reisen et al. 2000c), but produced elevated viremias after infection with WEE or SLE. Failure to find this species antibody positive in nature must relate to its roosting in low desert brush or other areas not frequented by host-seeking *Cx. tarsalis* (Lothrop et al. 2001), because this species usually arrives at our study areas in Coachella Valley before *Cx. tarsalis* enters diapause (Reisen et al. 1995) and when SLE still is being transmitted actively in wetlands managed for migratory waterfowl. In contrast, birds in epidemiological group 1 such as the House Finch are year round resident species, frequently are fed upon by *Cx. tarsalis* (Tempelis et al. 1976), and produce an elevated viremia response to infection with both WEE and SLE.

Differences in the viremia response of adult birds to WEE and SLE infection may impact the seasonality and rate of virus amplification and dissemination. WEE should be amplified rapidly, especially during spring, because a wide variety of bird species produced an elevated viremia in response to infection and *Cx. tarsalis* are most susceptible to infection at this time. In contrast, few species produced an elevated viremia response to SLE, indicating that transmission may be focal in both time and space. Nestlings of several bird species produce an elevated viremia after infection with SLE (McLean and Bowen 1980) and therefore amplification transmission could be widespread during the nesting season and then decline as these cohorts age. Multi-broooded species such as House Sparrows and Mourning Doves, although not producing an elevated viremia as adults, could be very important in amplification by making nestlings available for infection throughout the summer season.

This report summarizes our on-going research on avian host competence. Future plans include experimentally infecting additional bird species, including blackbirds, ducks, coots and herons, and measuring the viremia response by nestlings of representative species, especially those in epidemiological group 1 found to be infected frequently in nature.

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Mosquito Pesticide Tolerance Surveillance in California

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ABSTRACT: Bottle adulticide bio-assays were conducted on numerous California populations of *Cx. pipiens quinquefasciatus/pipiens* and *Culex tarsalis*. The pyrethroids, resmethrin, permethrin and pyrethrum rapidly knocked down the majority of individuals of all populations of both *Culex* species. However, approximately 5% of individuals from every population of *Cx. tarsalis* assayed except from Corona took longer than susceptible standard colony individuals to get knocked down with pyrethrum. Low level tolerance to resmethrin and permethrin was apparent in approximately 10% of *Cx. tarsalis* originating from within the city limits of Fresno. Varying degrees of tolerance to di-methyl and di-ethyl organophosphates (malathion, fenthion, temephos, chlorpyrifos and naled) was found in California populations of both *Cx. tarsalis* and *Cx. p. quinquefasciatus/pipiens*.

INTRODUCTION

Pyrethroids and to a much lesser extent organophosphates (OP) are used as adulticides to control medically important and pest mosquitoes species in California. As early as the 1970s, species of *Aedes* (now *Ochlerotatus)* and *Culex* mosquitoes began to show evidence of tolerance to organophosphates. This OP resistance led to more reliance on the use of pyrethroids such as, pyrethrum, permethrin and resmethrin with and without the synergist piperonyl butoxide. Coordinated surveys to determine susceptibility levels of field-collected mosquitoes to OPs were conducted from 1963 until the late 1980s by the Environmental Management branch of the California Department of Health Services (Thompson 1990). Since 1990 and after the more extensive use of pyrethroids, no surveys to determine susceptible levels of field populations of mosquitoes to OPs and pyrethroids have been done. There is evidence of high levels of resistance to pyrethroids in mosquitoes from other parts of the World such as malaria vectors (Vulule et al. 1999, Hargreaves et al. 2000) and members of the *Cx.pipiens* complex (Amin and Hemingway 1989, Rodriguez et al. 1993, Pasteur et al. 1995, Ben Cheikh et al. 1998, Bissett et al. 1991, Chandre et al. 1998). Synergist and genomic studies on field and pyrethroid resistant strains of *Cx. pipiens* complex members, have so far revealed evidence of carboxylesterase and P450-dependent oxidase mediated insecticide metabolism and insensitive target site resistance (*kdr*) (Bissett et al. 1997, Ben Cheikh et al. 1998, Chandre et al. 1998, Kasai et al. 2000). Given that resistance to pyrethroids in mosquitoes exists, the possibility of California mosquitoes developing resistance to pyrethroids is likely and should be evaluated.

To fill this void of information on current levels of susceptibility and tolerance to adulticides, a time mortality and time required to knock down, insecticide bottle based bioassay survey was conducted on California populations of *Culex tarsalis* Coquillett and *Culex pipiens quinquefasciatus/pipiens* Say in the summer of 2000. The time-mortality bottle bioassay, based on the principal that mortality is proportional to insecticide uptake and its success in reaching the target site, provides an effective method to evaluate adulticide susceptibility (Brogdon and McAllister 1998).

Additionally, non-specific esterase zymogram patterns, using the surrogate substrates *a* and *b* naphthyl acetate were performed on the populations of *Cx. p. quinquefasciatus/ pipiens* that were tested for adulticide susceptibility. This was done to ascertain if esterase phenotype patterns correlated with insecticide tolerance profiles and hence, could be used as a monitoring tool based on gene frequency to predict tolerance ratios of *Cx. p. quinquefasciatus/pipiens* populations.

METHODS

All collections of mosquitoes except for *Cx. p. quinquefasciatus/pipiens* from Fresno and Corona were collected as host seeking females in CO_2 baited traps. The females were bloodfed and all adult bioassays were conducted on male and female 3-4 day old offspring reared from the wild females. Bioassays were not performed directly on the wild mosquitoes but rather on their offspring to ensure that the mosquitoes were uniformly reared and testing was done on mosquitoes that were of the same age. *Cx. p. quinquefasciatus/ pipiens* were collected as larvae from Fresno and Corona and the bioassays were conducted on the emerged adults.

All bioassays were conducted on adults in glass bottles except for assays of temephos that were conducted, on 4th stage larvae reared from wild females, or in the case of collections from Fresno and Corona, directly on the larvae obtained from the field.

For adult bioassays, the interior surfaces of 250ml Wheaton bottles were treated precisely according to the methods described by Brogdon and McAllister (1998) with technical grade solutions (diluted in acetone) purchased from Chem Service (West Chester, PA). All bioassays were conducted on the same day the bottles were coated with insecticide. Before testing field material, discriminate test concentration of insecticide was empirically determined as that concentration that persistently resulted in 100% mortality in the case of the OPs or knock down with pyrethroids in standard susceptible colonies of *Cx. tarsalis* (BSF colony) and *Cx. p. quinquefasciatus (*Cq1 colony) within an hour in a near linear manner. Each time assays were conducted on wild material (4 replicates of 25 mosquitoes), treated bottles were set aside for testing on susceptible colony material to ensure consistency. As controls, wild mosquitoes were placed in bottles treated with acetone only. Mortality (with OPs) and knock down time (pyrethroids) were recorded every 15 minutes for three hours, or sooner, if all mosquitoes had died or had been knocked down before the three hour period. Our criterion for knock down was that mosquitoes could not right themselves or fly when the bottle was rotated. Time-mortality and time- knock down data was plotted on a probability scale on the Y axis using SigmaPlot software (Jandel Corporation, San Rafael, CA).

For assays on temephos, batches of 20 larvae were placed in 99.99 ml of tap water in waxed cups to which 10 ml of appropriate temephos concentrations diluted in acetone was added. Four replicates per concentration of temephos (0.05, 0.01,0.005, 0.001, 0.0005 and 0.0001 ppm) and control (acetone only) were used for each bioassay. Each cup was provided with 5 mg of larval diet and they were incubated at 28°C for 24 h. Larval mortality was recorded 24 h after exposure to temephos.

Phenotypes of non-specific esterase were determined by poly-acrylamide gel electrophoresis (PAGE). All mosquitoes (offspring from wild females, or adults emerged from field collected larvae) were ground as adults in 30 ml of a 10% sucrose, 0.5% Triton X-100, bromophenol blue Tris-citrate (pH 7.1) solution. This homogenate was then electrophoresed through a 6% tris-boric acid-EDTA gel (pH 8.9) in a Hoefer SE600 series electrophoresis system for approximately six hours. The gel was then stained according to the recipe provided by Steiner and Joslyn (1979). The resulting bands were characterized based on color and mobility, as α-esterases preferentially bind α-naphthyl acetate giving a black band and β-esterases preferentially bind β-naphthyl acetate giving red bands. Material from the susceptible Cq1 colony was run alongside field material in each gel for comparison.

RESULTS

Time-mortality and time-knock down responses of field samples of *Cx. tarsalis* and *Cx. p. quinquefasciatus/pipiens* have been summarized in Tables 1 and 2. Time mortality and time-knock down ratios represent the time it took for 50% and 90% of the test population to die or get knocked down divided by the time it took for 50% and 90% of the susceptible colony material to die or get knocked down. Values used in time-mortality and time-knock down calculations were determined graphically. In some instances time-mortality$_{90}$ ratios could not be calculated as the graph reached a plateau before the 90% mortality level in the test mosquito samples. Because temephos was conducted on larvae the data is summarized as lethal dose ratios. Ratio values higher than 2 (*i.e.* require twice as long as the susceptible colony material to die or get knocked down) is considered representative of significant resistance.

Cx. p. quinquefasciatus/pipiens from Fresno was the only population that showed significant tolerance to malathion. The Fresno *Cx. p. quinquefasciatus/pipiens* were also tolerant to all the other organophosphates except for naled. Significant tolerance to naled was apparent from Redding where about 20% of the mosquitoes took more than two hours to die. Naled TMR_{90} was slightly above 2 in the other populations (Centerville and Corona) tested due to about 14% of the individuals showing naled tolerance in each population. All four populations of *Cx. p. quinquefasciatus/pipiens* that were assayed for tolerance to fenthion showed low level tolerance with Fresno being the highest.

An alternative way of representing the comparative time-mortality and time knockdown data is to present it as relative resistance frequencies. Relative resistance frequency is the percentage of test population individuals surviving beyond the period it took for all the susceptible mosquitoes to die or get knocked down. The relative resistance frequencies (tables 3 and 4) however, does not inform one of how long it took for the test mosquitoes to die whereas the resistance ratio data does (Tables 1 and 2). Brogdon and McAllister (1998) reported that the bottle bioassay correlated well with single-insect esterase or oxidase biochemical microplate assays. As a modification to this approach, we attempted to find correlation's between relative resistance frequencies and genetic data of non-specifc esterase PAGE isozyme patterns. We compared the data in two ways, one by comparing

Table 1. Summary of organophosphate bottle bioassay test results expressed as fifty and ninety percent time-mortality resistance ratios for various California populations of *Cx. tarsalis* and *Cx. p. quinquefasciatus/pipiens*.

		Culex tarsalis		*Culex pipiens* complex	
Organophosphate	Location	TMR^1_{50}	TMR^2_{90}	TMR^1_{50}	TMR^2_{90}
Malathion	Redding			1.1	1.25
	Fresno	3.4	>3.16 (55)[4]	3.17	>2.8 (88)[4]
	Centerville –Kings River	0.68	0.79	1.1	1.25
	Reedley			1	1.15
	Corona			1.33	1.63
	Contra Costa	2.8	>4.74 (70)[4]		
	Sacramento	1	2.82		
	Los Banos Duck Club	5.56	>4.74 (70)[4]		
	Coachella	1.76	1.32		
Naled	Redding			1.79	4.6
	Fresno	2.8	4.5	2	1.8
	Centerville – Kings River	1.3	3	1	2.6
	Corona			1.57	2.2
	Contra Costa	2.2	5.06		
	Sacramento	6.15	8.82		
	Los Banos Duck Club	6.5	6.88		
	Coachella	4.6	6.59		
Chlorpyrifos	Redding			1.27	1.84
	Fresno	3.87	4.5	4.55	>3.24 (70)[4]
	Centerville – Kings River	1.53	2.15	1.27	1.62
	Corona			1.95	2.03
	Contra Costa	3.53	4.3		
	Sacramento	1.07	1.5		
	Los Banos Duck Club	2.8	3.35		
	Coachella	2.33	2.75		
Fenthion	Redding			1.4	1.71
	Fresno			2.8	4.94
	Corona			1.52	1.71
	Contra Costa	0.89	0.84		
	Sacramento	0.71	1		
	Los Banos Duck Club	1.21	1.13		
	Coachella	1.43	1.38		
Temephos[3]	Redding			2	8.33
	Fresno	2	1.67	10	11.67
	Reedley			5	8.33
	Corona			2	3.33
	Sacramento	1	1		
	Los Banos Duck Club	1	1		
	Centerville – Kings River	2	1.33		
	Coachella	1	1		

[1] TMR_{50} (Time mortality ratio)= time necessary for fifty-percent morality in the wild, test population divided by the time necessary for fifty-percent mortality in the control, susceptible colony.

[2] TMR_{90} (Time mortality ratio) is the time necessary for ninety-percent mortality in the wild, test population divided by the time necessary for ninety-percent mortality in the control, susceptible colony.

[3] Temephos ratios are given as a LD (lethal dose) ratio, and represent the temephos concentrations that resulted in fifty percent and ninety percent mortality at 24 hours in the wild, test population divided by the concentration that resulted in fifty or ninety percent morality at 24 hours in the control, susceptible colony.

[4] The TM_{90} value was not available due to a mortality plateau that extended throughout the test period. The number in parenthesis represents the percent mortality at which the plateau occurred.

Table 2. Summary of pyrethroid bottle bioassay test results expressed as fifty and ninety percent time-knockdown resistance ratios for various California populations of *Cx. tarsalis* and *Cx. p. quinquefasciatus/pipiens*.

		Culex tarsalis		*Culex pipiens complex*	
Pyrethroid	Location	TKR^1_{50}	TKR^2_{90}	TKR^1_{50}	TKR^2_{90}
Resmethrin	Redding			1.41	1.78
	Centerville – Kings River	1.77	2.22	1	1.08
	Fresno	2.69	2.59	1.25	1.57
	Corona			1	0.79
	Contra Costa	2.15	1.78		
	Sacramento	1.08	1.48		
	Los Banos Duck Club	1.15	1.48		
	Coachella	1.54	1.85		
Pyrethrum	Redding			1	1.04
	Fresno	2.85	2.59	1	1.46
	Centerville –Kings River	1.31	1.7	1	1.04
	Corona			1	1.17
	Contra Costa	2.23	2.19		
	Sacramento	1.15	1.11		
	Los Banos Duck Club	1.92	2.37		
	Coachella	3.85	3.04		
Permethrin	Redding			1.33	1.33
	Fresno	1.33	1.34	0.87	0.85
	Centerville – Kings River	0.95	0.94	1.33	1
	Reedley			0.87	0.93
	Corona			1	1.11
	Contra Costa	0.62	0.66		
	Sacramento	0.67	1.03		
	Los Banos Duck Club	0.9	0.88		
	Coachella	0.86	0.78		

[1]TKR_{50} (Time-knockdown ratio) is the time necessary for fifty-percent mortality in the wild, test population divided by the time necessary for fifty-percent mortality in the control, susceptible colony.

[2]TKR_{90} (Time knock down ratio) is the time necessary for ninety-percent mortality in the wild, test population divided by time necessary for ninety percent mortality in the control, susceptible colony.

electrophoretic esterase patterns of single adults to bottle bioassay resistance frequencies and the other by comparing the frequency of individuals showing more intense staining (reflection of elevated esterase levels) than the susceptible mosquitoes to the bottle bioassay relative resistance frequencies. In all instances, no staining or very slight staining of esterases was observed in the susceptible Cq1 individuals. The test mosquitoes on the other hand showed considerable variability in esterase allele profiles and in intensity of staining (Figure 1). Intense staining, interpreted as presence of highly active esterases, was observed in many individuals from all *Cx. p. quinquefasciatus/pipiens* populations examined. Based on comparisons with published data (Raymond et al. 1987) the active esterases most likely represent the co-amplified α2 and β2 esterase and the β1 esterase genotypes.

Interestingly, the α2 and β2 esterases which was first detected south of the Tahachipi mountain range in 1984 and found to replace esterase β1 (Raymond et al. 1987) has spread northwards and is now present in low frequency in Redding (Table 5). Unfortunately, no consistent associations between bioassay relative frequencies and esterase patterns could be identified. This was unexpected as selection of *Cx. p. quinquefasciatus* colonies originating from California that are pure for elevated α2 and β2 esterases and the β1 esterase did show resistance to chlorpyrifos, temephos and to a lesser extent malathion (Wirth et al. 1990).

Table 3. Relative organophosphate and pyrethroid resistance frequencies of several populations of California *Cx. p. quinquefasciatus/pipiens* based on bottle bioassays.

Chemical	*Cx. pipiens* complex populations				
	Fresno	Centerville – Kings River	Corona	Redding	Reedley Ranch
Malathion 100ug,* 60min@	65.24	10.48	20.22	10.27	5.52
Chlorpyrifos 50ug, 60min	85.3	13.08	25.28	18.79	na
Naled 5ug, 45min	15.72	15.18	12.89	38.49	na
Fenthion 200ug, 45min	60	na	29.43	25.64	na
Permethrin 30ug, 45min	0	1.25	0	6.23	0
Resmethrin 5ug, 60min	16.58	2.78	0	11.07	na
Pyrethrum 20ug, 45min	3.51	3.17	1.14	0	na

* Test concentration
@ Length of time for 100% mortality and knockdown of susceptible mosquitoes

Wild *Cx. tarsalis* from most locations were tolerant to naled, chlorpyrifos, and malathion, but were susceptible to fenthion and temephos (Tables 2 and 4). Sacramento *Cx. tarsalis* were especially tolerant to naled (RR_{50} = 6.15 and RR_{90} = 8.82), however they did not show resistance ratios greater than 2 in all other organophosphates tested except for malathion RR_{90} = 2.82. While OPs have been used to a much lesser extent than they were 10 years ago to control *Cx. tarsalis*, the mosquitoes still appear to remain OP tolerant. This could be due to the continued exposure to OPs, as non-target organisms, used for agricultural purposes or that the OP resistance genes do not confer any cost to the mosquito and hence, are not be selected against in the absence of OPs. Most *Cx. tarsalis* populations showed low level knock down tolerance to pyrethrum and resmethrin. This low level pyrethroid tolerance is cause for concern as pyrethroids are the only registered adulticides left for mosquito control in California. With continued and increased public health and agricultural use of pyrethroids, selection for higher levels of resistance will be inevitable. Delaying development of high levels of pyrethroid resistance should be attempted by using as little pyrethroids as possible and by stressing the use of larvicides.

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Table 4. Relative organophosphate and pyrethroid resistance frequencies of several populations of California *Cx. tarsalis* based on bottle bioassays.

Chemical	*Cx. tarsalis* populations					
	Coachella	Contra Costa	Los Banos Duck Club	Fresno	Centerville - Kings River	Sacramento
Malathion* 200ug, 45min@	13.24	57.99	72.26	84.1	3.12	25.17
Chlorpyrifos 200ug, 30min	64.24	85.02	75	89.67	21.57	10.32
Naled 10ug, 30min	66.87	34.05	73.19	38.12	15.83	88.25
Fenthion 200ug, 60min	11.48	0	2.94	na	na	2.31
Permethrin 20ug, 45min	0	0	0	7.64	2.44	1.92
Resmethrin 5ug, 45min	13.03	19.77	6.92	23.6	12.4	5.65
Pyrethrum 10ug, 60min	22.52	9.4	12.62	14.62	8.33	4.12

* Test concentration

@ Length of time for 100% mortality and knockdown of susceptible mosquitoes

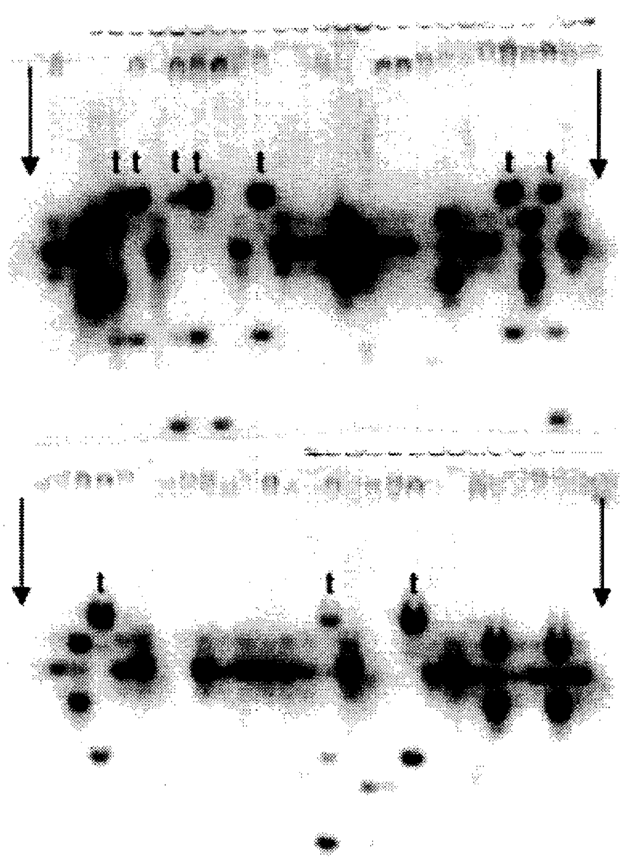

Figure 1. PAGE non-specific esterase patterns of single adult *Cx. p. quinquefasciatus/pipiens* and *Cx. tarsalis* mosquitoes collected from Centerville (California) stained with a and b naphthyl acetate. Lanes marked with t are *Cx. tarsalis*, those unmarked are *Cx. p. quinquefasciatus/pipiens* and lanes with an arrow are adults from the susceptible *Cx. p. quinquefasciatus* colony.

Table 5. Relative frequencies of non-specific esterase alleles observed in various *Cx. quinquefasciatus/pipiens* populations across California using PAGE electrophoresis.

Population	Phenotype (Percentage)			
	α2β2	β1	α2β1β2	None or other
Suscep. CQ1	0	0	0	100
Redding	3.9	32.7	1.9	61.5
Fresno	7.8	7.8	75.4	9
Centerville	3.4	75.9	20.7	0
Reedley	14.3	53	10.2	22.5
Chino	20.8	31.3	6.2	41.7

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A Program to Eradicate Red Imported Fire Ants From Orange County[1]

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ABSTRACT: The Orange County Fire Ant Authority (OCFAA) was created as a special department of the Orange County Vector Control District via contractual agreement between the District's Board of Trustees, State of California, and the County of Orange. The Authority is intended to systematically eliminate and eventually eradicate the Red Imported Fire Ant from Orange County. The eradication effort, which includes cooperative efforts of governmental, professional, commercial (i.e. agricultural pest control industry), and County residents, incorporates state-of-the-art technologies. This paper briefly summarizes the design and operation of the Authority.

The decision to attempt the eradication of the Red Imported Fire Ant (RIFA), *Solenopsis invicta,* from Orange County was made when the California Department of Food and Agriculture (CDFA) determined that the threat from this exotic pest made eradication a goal worth the expense. After placing an agricultural quarantine on the entire County, CDFA developed a RIFA action plan that included the element of local government entities developing a plan to meet the needs and concerns of their communities. In Orange County, the responsibility to develop a local RIFA eradication program was sub-contracted to the Orange County Vector Control District (OCVCD) via a contract with the County of Orange Board of Supervisors and County Agricultural Commissioner. Dr. Robert Sjogren, Orange County Vector Control District Manager, designed the community based eradication program and implemented it following the signing of the sub-contract, with an 18-month budget of $5.9M, on January 20, 2000. Eradication efforts began almost immediately with the creation of the Orange County Fire Ant Authority (OCFAA), the RIFA eradication division of OCVCD. The initial RIFA locations were released by CDFA to the Fire Ant Authority, and treatment protocol began in the northern part of the County bordering Los Angeles on February 1, 2000.

In anticipation of reaching a contractual agreement with CDFA and the County of Orange, the OCVCD Board of Trustees approved numerous program-enhancing policies and resolutions in the months preceding the program's start date. Included in these policies were an emergency-purchasing authorization given to the program manager and the creation of a Trustee Fire Ant Committee to expedite program needs and make recommendations to the entire Board. In addition, the Board made it clear via a resolution that once the OCVCD accepted contract responsibility, it would maintain the local authority to operate the eradication program within the budgeted funds, without State administrative interference.

The title, "Orange County Fire Ant Authority," was selected to give the citizens of Orange County a name they could equate to other existing quasi-governmental entities, such as Transportation Authority and Fire Authority. Since the finding of RIFA in an urban county covering 940 square miles is largely citizen-based, the creation of a moniker that would easily be remembered, by both residents and members of the media, was essential.

Another important aspect of the early stages of the program was the creation of the "Advisory Committee for the Orange County Red Imported Fire Ant Program." This committee is chaired by the County Agricultural Commissioner and includes representatives from CDFA, OCVCD, University of California, and the local nursery industry (the largest agricultural industry in Orange County). This committee meets monthly to discuss RIFA control issues, survey/sampling protocol, exclusion issues, and any other applicable issues.

As job announcements and interviews for the 21 "limited term" OCFAA positions were ongoing, the infrastructure for the program was acquired, with the most important being facility location(s), information system network, and data collection hardware/software. With the heaviest RIFA infestations occurring in the extreme northern and southern parts of the County (an area separated by 20 miles of congested highways) the decision was made to open two facilities in each of these areas to minimize "drive-time." The County was broken up into five treatment zones, with the size of the zones

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[1]This article was not submitted for review by the Editor. Author will assume responsibility for contents.

being determined by infestation levels, and four of the zones being assigned one zone coordinator and three technicians. The remaining zone, located in the low-infested central part of the county, was assigned to one zone coordinator who would operate out of the OCVCD Garden Grove headquarters and utilize OCVCD technicians on an as needed basis.

The ability to create an accurate "paperless" data collection method, as well as a high-speed intranet/internet computer network, required a custom application that would also be capable of hosting the OCFAA website. In addition, without a dedicated computer system technician on the payroll, the system would have to be a "stand-alone" system requiring minimum maintenance. A local computer networking specialist company that utilized Gateway™ hardware was hired to develop the program's custom installation and set up a high capacity server that enabled data storage and file sharing. A "T-1" high capacity internet line was also installed to maximize transfer of data between OCFAA offices and CDFA.

With the computer network operational, a search began for a hand-held data collection device that would utilize GPS and be compatible with an ArcView™ GIS system. After field-testing certain models, a Trimble GeoExplorer 3® system was purchased because of its capability to customize attribute information and interface with the program's GIS system that utilizes County Assessor parcel data. When returning from the field, the technicians place the units into a "cradle," and that day's data (mound location, treatment, acreage, etc.) is automatically downloaded to a customized Access® database. This entire process takes about one minute. The data can be used to create reports, treatment maps, and numerous customized tracking tools.

Other items unique to the OCFAA are specialized treatment vehicles and highly modified golf carts that utilize both a Herd® spreader and a mist duster calibrated for use with Distance™ ant bait. This equipment allows for entire neighborhoods and parks to be treated in only one day.

Budgetary constraints, coupled with a 940 square-mile-area of responsibility, made surveying the entire county by OCFAA staff an impossibility. Instead, it was decided to utilize the citizens of Orange County to be the primary "RIFA-finders." This public outreach effort has utilized effective marketing techniques and has maximized both electronic and print media. CDFA's RIFA hotline was modified to include an Orange County specific menu to assist citizens in reporting suspected RIFA locations, as well as answer commonly asked questions. CDFA hotline personnel enter the caller's information on an internet-based, password-protected database, which is made available in "real time" to OCFAA staff. As many as 200 calls-per-day were being received in the late summer months, with OCFAA maintaining a response time of under 48 hours. When technicians responded to these calls, adjacent parcels were surveyed and informational doorhangers were distributed.

A large part of the program's public outreach campaign includes a citizen-based "pest test," which is comprised of asking citizens to place potato chips in their yards and mail any ants on the chips to the Authority for identification by OCFAA. Aside from involving citizens in a proactive manner, the infamous "potato chip test" has attracted an incredible amount of media attention. The chip test has been duplicated by members of the media and has been seen in over 5 million households in Southern California. Instructions on how to safely conduct the test is explained in detail on the OCFAA website, in brochures, and the RIFA hotline.

Another important aspect of public outreach is the OCFAA staff attendance at the hundreds of community events occurring throughout the year.

An easily recognizable EZ-UP® display featuring the OCFAA logo and RIFA hotline number (888-4-FIREANT) is becoming a fixture every weekend at community events. A public awareness survey conducted by CDFA discovered that only a land-use issue exceeds awareness of RIFA by the citizens of Orange County.

Soon after the discovery of RIFA in Orange County, CDFA convened a Scientific Advisory Panel (SAP), which includes experts on RIFA from Federal agencies and academia. On their first visit to Orange County in 1998, "... members expressed reservations about the possibility of eradicating RIFA from California. Based on what they saw in the May 2000 visit, they now believe that eradication of this pest from the state is potentially possible if the level of enthusiasm and commitment that they saw can be maintained along with the monetary support of the state government." (CDFA, Plant Health & Pest Prevention Services, Report to the Legislature, June 2000).

Black Fly Control Program at Greater Los Angeles County Vector Control District

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ABSTRACT: The Greater Los Angeles County Vector Control District has carried out a black fly control program in the Los Angeles River since 1994. The program is based on surveillance of larval black fly populations, and application of *Bacillus thuringiensis israelensis* or *Bti* (Vectobac 12AS®) at a rate of 18-24 ppm/minute. The threshold for larvacidal application is > 5 larvae/in^2, on 50% or more of the substrates sampled. This paper outlines information on the surveillance and operational aspects of the District's black fly control program.

INTRODUCTION

The Los Angeles River flows approximately 50 miles from the western portion of the San Fernando Valley, east to the City of Burbank, and then south through the Los Angeles Basin, before emptying into the Pacific Ocean at Long Beach Harbor (Fig. 1). Inflows to the River include natural runoff from foothill streams, suburban and urban street runoff, and secondarily treated wastewater discharge from Tillman, Glendale, and Burbank water reclamation plants located along the river's course. These diverse inflows combine to create and sustain a year-round flow of the river, resulting in a riparian corridor that supports a variety of vegetation types and a diverse composite of invertebrate and vertebrate fauna. Among the several nuisance pest species that develop within this system, the black fly, *Simulium vittatum* Zetterstedt, is the most pestiferous.

Other black fly species found in the District include *Simulium argus* Williston, *Simulium aureum* Fries, *Simulium piperi* Dyar and Shannon, *Simulium tescorum* Stone and Boreham, and *Simulium virgatum* Coquillett. Immature populations of these species are generally found in small-stream sources in outlying areas of the District, and with the exception of *S. tescorum* and *S. virgatum*, have not yet been determined as pestiferous species. Therefore, the major focus of the District's black fly control program is controlling populations of *S. vittatum* produced in the Los Angeles River.

BACKGROUND

Efforts to control black flies within District boundaries date back to 1969 when *S. tescorum* populations at the Hansen Dam spillway and Big Tujunga wash were treated with temephos (Abate 4E®). Application of temephos to these habitats provided effective control at the of rate of 0.5 ppm/hr. 100% kill was achieved within 24 hours, and reinfestation occurred 15 to 60 days later (Pelsue et al. 1970).

The current control program began in August of 1994. In June of that year, Kevin Regan, Tree Surgeon Supervisor, Forestry Division, City of Los Angeles, collected samples of a small fly that was causing a tremendous nuisance to visitors, golfers, and equestrians in the Griffith Park area. He took these samples to Entomologist Rosser Garrison at the Los Angeles County Agricultural Commissioner's Office, who identified the fly as belonging to the family Simuliidae, likely *Simulium sp.*, and suggested he contact our District to explore the possibility initiating a program to control the nuisance black fly.

A series of meetings occurred between the District manager and Los Angeles County and City officials, to discuss the extent of the black fly problem associated with the river, and the District's role in mitigating the problem. For the 1994-1995 season, it was estimated that five golf courses along the river were losing approximately $34,000 per month in green fees due to *S. vittatum* (M. Shaw, pers com.). This figure does not include reduction in revenues from other concessions such as golf cart rentals, food sales, etc. Equestrian, picnicing and other recreational park activities were being adversely impacted as well. News broadcasts from Dodger Stadium showed players and spectators performing the "black fly wave," a behavior to "chase" bothersome black flies from the face. Since the economic and political impact of the black fly infestation was so significant, these meetings resulted in a contractual agreement between the City and the District. The District agreed to initiate a program to control *S. vittatum* along a 24-mile section of the Los Angeles River corridor. The City Council agreed to reimburse the District for time and materials, plus 15% overhead. In 1995, the City re-evaluated its position, and determined that the Los Angeles River was not under its immediate jurisdiction, and therefore the City had no authority

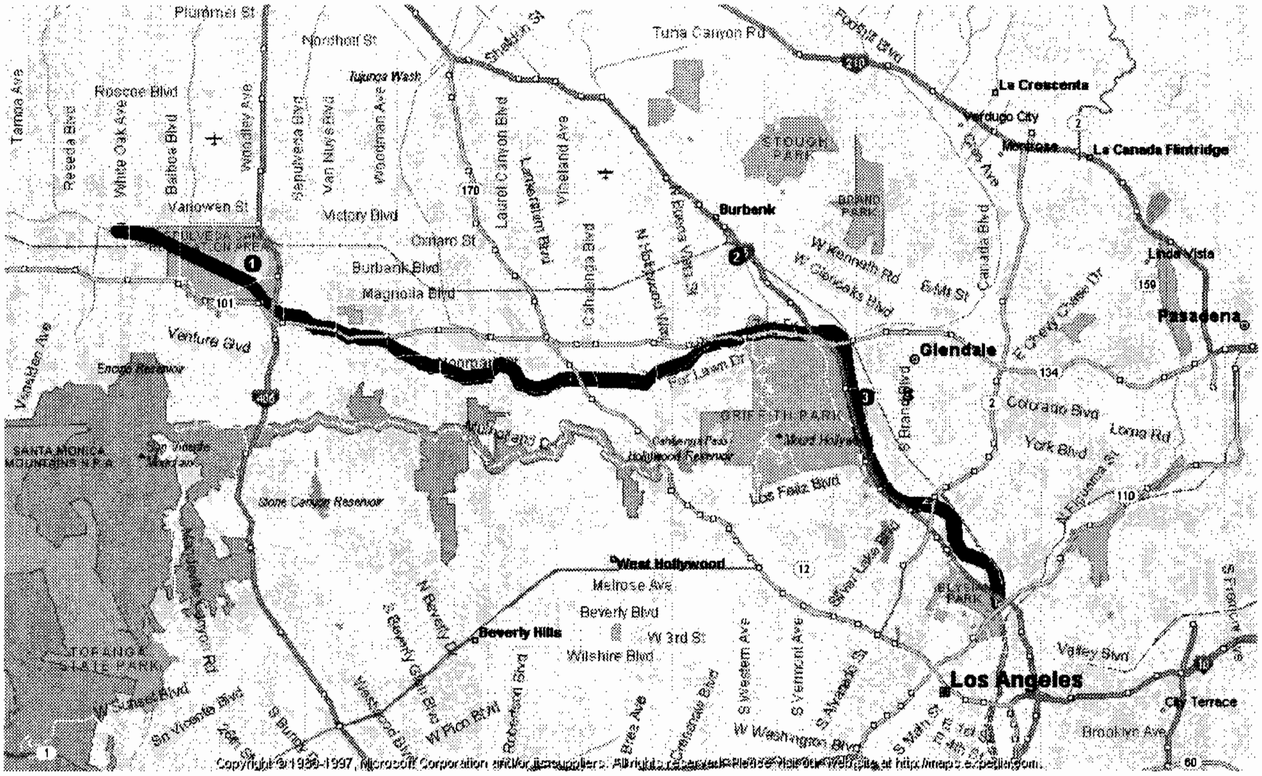

Figure 1. A general view of the Los Angeles River and surrounding communities (map not to scale). The thick black line indicates the portion of the Los Angeles River that is treated to control black flies. Locations of sewage treatment plants are also shown. 1) Tillman Water Treatment Plant; 2) Burbank Water Treatment Plant; 3) Glendale Water Treatment Plant.

to reimburse the District for the control program. In order to continue this much needed service, and to maintain the control program for the benefit of businesses, residents, and visitors along the river corridor, the District created a special benefit assessment zone along a 26-mile long section of the river in 1997. An annual assessment of $0.15 was levied on all properties located within 2 miles of the Los Angeles River and the Arroyo Seco, a small adjoining tributary. This assessment was to remain in place until three successive years of below nuisance levels of black fly activity indicated no need for District intervention (Hazelrigg, 1996, unpublished data).

SURVEILLANCE METHODS

Appropriate sampling methods for black fly larvae have been given much attention in relation to studies of black fly distribution, population studies, and ecology (Calbo, 1987). Black fly larvae are generally found in relatively clean, fast flowing waters. Often, they exhibit a contagious distribution; that is, larvae are often found in concentrated groups on various objects such as rocks, trailing vegetation, leaves, and branches. Adjacent substrates may be devoid of larvae, likely due to variation in water velocity and other flow characteristics. This presents major problems when designing a sampling protocol. Sampling for black fly larvae at equal intervals along a transect is difficult and time consuming, and because of the contagious nature of larval distribution, can lead to misleading estimates of larval density (McCreadie and Colbo, 1991). The use of artificial substrates for sampling immature black flies has inherent problems as well (Walsh et al, 1981), including disturbance or washing away of the sampling device, or entrapment of the sampling device with algae or debris floating downstream.

The choice of sampling method depends in part upon the amount of resources in time and material that can be allocated towards the sampling effort, and the ultimate purpose for data collection. Larval sampling, for District purposes, is to determine when application of control agents for black flies is necessary. A basic sampling strategy was adopted similar to that described by McCreadie and Colbo (1991) that provided adequate data for control purposes, with minimal resources.

Early in the program, ten sites along a 24-mile section of the river were selected as sampling stations. Site selection was based on the presence of favorable larval habitat and the relative safety the site afforded for personnel conducting the sampling.

During the peak season (March through October), eight to ten sites were sampled at approximately 10-day intervals. At each site, a swath of the river was examined for substrates likely to harbor black fly larvae. For each object colonized, larval density was estimated on-site, and categorized into one of three groups: low density (<5 larvae/in^2), moderate density (5-20 larvae/in^2), or high density (>20 larvae/in^2). Frequently, only a few objects may be colonized at any given site, but these objects may have high larval densities. For purposes of reporting and graphical representation, overall larval density for all objects colonized at a site was estimated, and the percentage of objects colonized was recorded. Figure 2 shows one such graph for a site south of Fletcher Blvd. When 50% or more of sampled substrates have larval densities of at least 5 larvae/in^2, treatment was recommended to the District's operations staff. Frequently, only certain sections of the river required treatment.

CONTROL METHODS

One of two methods is generally used for black fly control: 1) restriction or reduction of water flow, or 2) the use of a currently registered control agent. Due to the multiple inflows into the Los Angeles River, and the fact that the main inflows to the river are from wastewater treatment plants, flow restriction or drastic reduction is not a practical solution. Instead, applying the biorational material *Bacillus thuringiensis israelensis* (Vectobac 12AS®) for control is both practical and efficacious. *Bti* was chosen based on its strong host-specificity and potency against black fly larvae (Molly, 1990).

In 1994 and the early part of 1995, the dosage rate necessary for treatment was based on surface area of the river. "Duplex," a 12.5:1 mixture of *Bti* (Vectobac® 12AS) and methoprene (Altosid Liquid Larvicide®), was applied at a

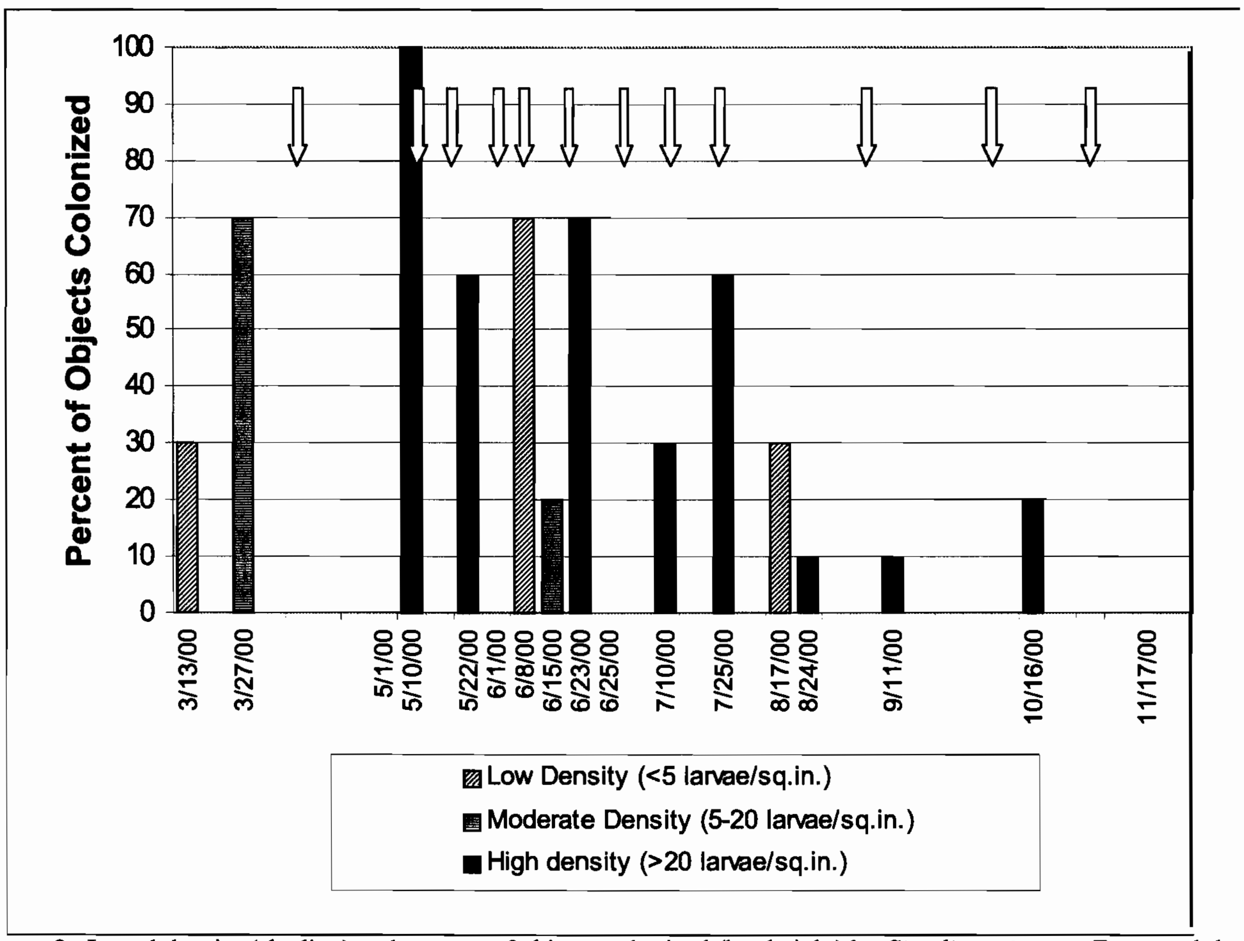

Figure 2. Larval density (shading) and percent of objects colonized (bar height) by *Simulium vittatum* Zetterstedt larvae in the Los Angeles River at a site south of Fletcher Blvd. for the year 2000. Arrows indicate treatment dates.

dosage rate of 189 ml per hectare (16 ounces/acre). Diluted material was injected into the river for approximately 12 minutes at about 160 m (0.1 mi) intervals. This method was partially successful, although it had several drawbacks. First, the applicators had to make several trips out of the river during the course of treatment to refill their tanks with the "Duplex" mixture. This required locating suitable facilities, such as parks, golf courses or fire stations in the area where additional material could be mixed and diluted with water. Dragging hoses from the truck-mounted sprayers to the edge of the river and moving in and out of the river bottom to refill the tanks were burdensome and costly in terms of man-hours. Secondly, larval mortality was inconsistent. Some areas could not be reached with traditional truck-mounted spray equipment. Width of the river and water velocity varied widely among different treatment sites, which affected contact time and the final concentration of the material in the water. Undeen, et. al (1984) recommended calculating dosage rates of *Bti* based on stream width, but they suggested their method be used for small stream sources of not more that 5 m (16.4 ft) wide. Several treatment locations in the Los Angeles River exceeded 70 m (200 ft) in width. For these reasons, in June of 1997, the application method was changed from determining dosage rate based on surface area, to a method based on stream discharge, using a dosage rate of 18-24 ppm.

For stream discharge calculations, the width of the river immediately above each application site is measured with an open reel linear fiberglass tape measure (Lufkin model #HY17100CME). Five to ten depth measurements are used to calculate an average depth. Average flow rate at each site is measured with a digital water velocity meter (Global Water Flow Probe, model FP101). Alternatively, flow rate can be estimated by measuring the time it takes a float to travel a given distance downstream. Several timed-float measurements are taken at a site and averaged. If the float method is used, the calculated flow rate is multiplied by a factor of 0.8 in order to compensate for the effect of the boundary layer in flowing water. Water at the surface flows faster than water in contact with the substrate due to friction and other forces. Multiplying by the factor of 0.8 roughly compensates for this and brings the calculation more in line with the actual flow rate. The required calculations are shown in Table 1.

In terms of planning the logistics for treatment, the 24-mile length of river to be treated was divided up into 5 sections, based primarily on the physical characteristics of the river. These characteristics, such as the number of inflows, "improved" (concrete-lined) versus "unimproved" (soft-bottomed) sections, and the number of access points, in turn determine the type of equipment, vehicle, and manpower required. Three primarily soft-bottomed sections are each treated by teams of two vector control specialists. Each section has approximately ten stops at approximately 0.8 km (0.5 mi.) intervals. Undiluted material is injected from hand-held sprayers at dosage rates of 18-24 ppm. The two remaining sections, Arroyo Seco, from South Pasadena to the Los Angeles River, and south of the Sepulveda Dam to Buena Vista Street, are concrete-lined. Two individual vector control technicians using truck-mounted spray equipment, treat these two sections. On days when treatment is scheduled, the teams work simultaneously, and the 24-mile section of the river is treated in less than four hours. Often, based on the results of larval surveillance, only certain sections of the river require treatment. In the year 2000, for example, no treatment was required for the Arroyo Seco, and only a few applications were required south of the Sepulveda Dam to Buena Vista Street.

DISCUSSION

After six years of sampling and analyzing black fly population data, it is possible to make some general statements about the occurrence of *S. vittatum* in the Los Angeles River. After the winter rains subside, usually in March, *S. vittatum* populations begin to increase rapidly. Adults become most pestiferous during the warm weather from spring through the mid-summer. The river is treated most frequently from early spring to early summer when larval populations are highest. There is a noticeable drop in larval densities from mid to late summer, presumably as result of the District's treatment program. Perhaps, by eliminating a large percentage of larvae early in the season, fewer larvae are able to transform into adults. A reduced adult population consequently results in a smaller larval population as long as control is maintained. Due to low numbers of larvae, treatment is curtailed or suspended until late September or early October, when larval numbers again begin to increase. Generally, a few late-season treatments are conducted, but not as frequently as early in the year. By the time the first rains arrive in late October or November, treatment becomes unnecessary and ceases. In the absence of significant late fall and winter rainfall, populations of immature black flies build up rapidly, and under these conditions, numerous egg masses, and heavy larval and pupal densities can occur.

Evidence strongly suggests that *S. vittatum* does not undergo winter diapause in the Los Angeles River. This hypothesis is supported by the presence of empty pupal cases, collections of pupae from which adults shortly emerge, and the presence of a variety of larval instars during the late fall and winter months. However, despite these heavy densities of larvae and maturing pupae during the late fall and winter, adult populations do not seem to increase to nuisance levels. Similar larval and pupal densities during the warm months would be expected to result in high numbers of nuisance adult black flies in adjacent areas. This may be due, in part, to a change in the behavior of the adult flies in the cooler months.

Table 1. A method used to calculate correct dosage at a treatment site. One needs to determine the desired concentration of material to use (follow label directions), and calculate the volume of water discharged.

Determining Dosage Rate in PPM

Stream Discharge is determined by multiplying the cross sectional area of the stream times the velocity of the water in feet per second.

A. Calculate the Cross Sectional Area (ft^2)

1. Measure the width (in ft) of the river a short distance above the application point.
2. Determine average depth (in ft) across the width of the stream by taking at least 5-10 depth measurements and averaging them.

Cross sectional area (ft^2) = Width (ft) x Average Depth (ft).

B. Calculate the Average Rate of Flow (ft/sec)

1. Measure the average velocity of the flow a short distance above the application point using one of two methods:

   a. By measuring with a flow meter at several points along the width.

   b. By timing how long it takes a float to travel a pre-measured distance. Several readings should be taken. If this method is used, the average flow rate arrived at should be multiplied by 0.8 (see text).

Stream Discharge (ft^3/sec) = Cross sectional area (ft^2) x Average Rate of Flow (ft/sec)

Stream Discharge in ft^3/min = Stream Discharge in ft^3/sec x 60 sec/min

Stream Discharge in gal/min = Stream Discharge in ft^3/min x 7.48 gal/ft^3

Gallons of Vectobac required for a 1 ppm concentration = (Gal/min)/1,000,000

Dosage = Gallons of Vectobac required for a 1 ppm concentration x Desired concentration

Adapted from: VectoBac® Technical Bulletin Aqueous Formulation. Abbott Laboratories. AG-4308/R3. March 1996.

During winters with substantial rainfall, populations of immature black flies in the river are significantly reduced due to uprooting of vegetation, tumbling of cobbles, and the scouring action of sand and silt during these storms. The severe black fly infestation in 1994 was preceded by a lower than average rainfall (as measured at the Los Angeles Civic Center). That year, from July 1993 through June 1994, only 8.14 inches of rainfall occurred as opposed to the normal 15.09 inches. Substantial rainfall disrupts the developmental cycle and significantly changes habitat conditions experienced by immature black flies. Presumably, as a result of a lack of substantial rainfall during the winter of 1993, the black fly population increased unimpeded, adults becoming abundant by spring and summer of 1994. Interestingly, amount of rainfall during the 1988-1990 seasons was similar to that for the 1993-1994 season, however, unlike the 1993-1994 season, an increase in the black fly population failed to occur. The sudden appearance of a severe black fly infestation in and around the Los Angeles River in the summer of 1994 is still not understood. One explanation could be increased volume of discharge from sewage treatment plants that may not have occurred in prior years.

CONCLUSION

The success of GLACVCD's black fly control program, based on larval surveillance and application of Vectobac 12AS® when densities of immatures warrant its use, has greatly reduced the number of service requests, and has been acknowledged by golf course operators and members of the Los Angeles City Council. One of our objectives is to implement a routine post-treatment analysis so that we can better document our program effectiveness. A key component to the success of the District's program is close cooperation between the technical and operational staff, with exchange of information and suggestions for program improvement flowing in both directions between the staff.

Acknowledgements

We would like to thank the District's operational staff for their cooperation, hard work, dedication, and suggestions on ways to improve the program. In particular, we would like to thank Wesley Collins and Howard Mariotta, Vector Control Specialists for the District, both of whom provided valuable input related to seasonal larval occurrence. Vector Ecologists Jacqueline Spoehel (GLACVCD) and Robert F. Cummings (Orange County Vector Control District) were responsible for developing the framework of the initial sampling protocol in 1994. Saeed Tabatabaeepour (GLACVCD) provided valuable assistance with larval sampling in the early years of the program. Special thanks is due to Dorothy Cantrall, formerly of Abbott Laboratories, who provided valuable assistance during the re-evaluation of our treatment program in 1997.

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-Development of a Public Relations Strategy - Creating a Meaningful and Measurable Communications Plan

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ABSTRACT: A five-year Public Relations Strategy was developed based on an in-house survey and a telephone survey conducted by a local research firm. The result of the surveys prompted the redirection of the Districts' communication efforts and illustrates the importance of understanding public perception, knowledge, and awareness of our District. The purpose of the five-year strategy is to give meaningful direction to the District's communication efforts with our public and to ensure that we are adhering to the guidelines set forth in our mission statement. The detailed strategy outlines creative, public relations, and advertising tactics, as well as evaluation methods.

Communication. All businesses try to capture its potential and harness its direction. The Public Relations Strategy is an attempt to steer communication in a chosen direction, to reap specific rewards. With a fresh approach, agencies can evaluate their traditional methods of communication and education to determine if their energy and resources are utilized in the most efficient manner. Traditional communication doesn't necessarily mean successful communication.

The Contra Costa Mosquito & Vector Control District completed our Public Relations Strategy in early 2000. The following information illustrates the process of developing the strategy, the type of information our agency gained, and how that information led to the development of our message, and ultimately, our campaign.

The goal of our strategy is to help us connect with our community. We wanted to know if our residents were satisfied with our services. What do they know about us? What do they *not* know about us? How do they perceive us? Learning this information enabled us to determine what messages needed to be delivered, how they should be delivered, in what medium, and to whom.

Our strategic development process is divided into five factors: Research, Situational Analysis, Strategy Development, Strategy Implementation, and Evaluation. Each factor is dependent upon the preceding factor and the entire process operates in a continuous cycle.

The key element in any strategy is research. We need to understand who our audience is and how to reach them. Are they local farmers who get their daily news from their local newspaper, or are they educated professionals used to communicating by means of the internet? What, how, and where we place our message is dependent on knowing this information. One of the research elements we used was our weekly public survey, mailed to customers who received our services, giving them the opportunity to rate our performance. We ask questions such as: was our response time reasonable? Was the field employee helpful and courteous? Was the pest problem handled? In our case, our customer service was rated at the highest levels in all categories. Customer service is not an area in which we need to spend extra effort, time, and money. It's a strength, not a weakness. Knowing where in your organization you *don't* need to concentrate your communication efforts can be as useful as learning where you *do* need to concentrate efforts.

The other form of research we utilized was a telephone survey. It is conducted once every five years by professional statisticians. Their task was somewhat daunting in that they phoned more than 9,000 residents in order to obtain 400 valid responses to our survey. The sampling tolerance for all of our respondents was ±5 percentage points at the 95% confidence level. For example, in this case our chances are 95 out of 100 that our survey result does not vary by more than ±5% from the result that would have been obtained if interviews had been conducted with all Contra Costa County residents at the time of this survey.

The survey helped us to understand the people in our community, their demographics, and their awareness and perception about our District. For example, we learned that the majority of our respondents are between the ages of 41-60, are college graduates, own their own home, etc. We learned that they are most likely to get their news from the newspaper and that they are least likely to get it from fairs or events. Not once was there a mention that they received information from our District having provided a presentation to their child's class in school.

The Situational Analysis is simply a document that illustrates our situation at *this* moment in time. It is a combination of the two types of research mentioned previously and also statistical information about our District, such as the number of vehicles we maintain, the number of people we employ, total square miles in our county, etc. From this completed document we are able to determine our Strengths, Weaknesses, Opportunities and Threats (concerns), also known as a SWOT Analysis.

Understanding and identifying these qualities allows us to quantify and qualify information (as well as provide statistical information with which to measure our successes on later). For example, we learned of our weaknesses. We learned that 58% of our residents don't know about our services and wouldn't know who to contact for the services we offer. We learned that 63% of our residents are unaware of any of our mosquito control measures. Seventy-two percent of respondents to our survey think of mosquitoes as simply annoyances and not as health threats. Considering that Contra Costa Mosquito & Vector Control District is a public health agency, this information is crucial to our message development. Once we have determined what our strengths, weaknesses, opportunities, and concerns are we can develop our actual strategy. This report contains a summary of each component. Specific tactics for our strategy are listed in the strategy itself and are not part of this paper.

Our Public Relations Strategy consists of the following components:

- Objectives
- Problems/Opportunity Statement
- Creative Strategies
- Advertising Strategies
- Public Relations Strategies
- Evaluation Methods
- Budget

Our research and SWOT Analysis enabled us to develop three objectives. For *our* District, we determined that we need to: 1) Build Awareness. We are a vital health agency, but 58% of our residents don't know that. 2) Improve Recognition. We are still known as simply mosquito control and not for other vectors for which we provide services for. It's our responsibility to inform all of our residents about all of our programs and services. 3) Educate our residents of vector health threats. Seventy-two percent of our respondents think of some vectors as simply annoyances.

Our Problem Statement sums up our situation: The majority of Contra Costa County residents do not know about us, and those who do usually associate us only with mosquito control. Also, they do not understand the significance of mosquitoes and other vectors as health threats. Our Opportunity Statement is: *We strive to become a household name.*

Our creative and advertising strategies will utilize history to illustrate the importance of our agency in the community. We want to show that without us, residents' quality of life would suffer, as illustrated in the past. We will use real headlines from the past to get our message across. For example, before our agency was formed, schools were shut down and real estate sales suffered during peak mosquito seasons. Our advertisements will combine the actual headlines from these times and then read *"We don't let history repeat itself. It's as simple as that."* Or, the same headline visual with the addition of *"Our quality of life just isn't the same anymore. That's a good thing."* Our message of *"We're a public health agency. It's as simple as that!"* will be used throughout our campaign and enable us to meet all of our objectives through repetition and recognition. Newspapers are the vehicle of choice for delivering our messages because our research shows they are the most likely way in which our residents get their information, though other mediums will be utilized as well.

For our public relations strategies, our top priority is to communicate with the media and develop a professional relationship with them. They are our link to the public and again, newspapers are the number one way in which our residents get their information. This medium also allows us to reach out to new audiences, which strengthens our commitment to invoke public confidence by informing our residents of our services and value within the community.

Evaluation is a critical component to any strategy. By evaluating the differences in surveys, an organization can measure the success of their strategy. Efforts become measurable. Also, an evaluation is needed to determine new objectives based on the new information. For example, more people will have become aware of our organization due to our communication efforts and so our statistics for awareness will be different in the future. Only from the new information can we develop appropriate new objectives.

The last component of our strategy is the budget. Contra Costa Mosquito & Vector Control District developed a five-year budget projection to anticipate costs as well as to use as a guide for tactic timing. Every component or tactic in the strategy has a cost associated with it, and is listed under the year in which it will be implemented. Each year in the budget also supports a cost of living allowance. Unusual or substantial costs associated with a one-time or special event is listed outside the five-year budget.

The Public Relations Strategy has allowed the Contra Costa Mosquito & Vector Control District to understand our residents, their knowledge and perception of our District, as well as their knowledge of the vectors in our county. We can now strive to connect with them, enhance their knowledge of us, and clearly illustrate the importance of our organization in the community. After all, we're a public health agency protecting public health for almost 75 years. *It's as simple as that!*

Utility of *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* in Rotational Larviciding Programs for Control of *Ochlerotatus nigromaculis* and *Ochlerotatus melanimon* in California

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ABSTRACT: The recent discovery of reduced methoprene susceptibility in a population of *Ochlerotatus nigromaculis* near Fresno, CA has spurred interest in materials for rotational larviciding of pasture and refuge mosquitoes. This paper reviews both published studies and recent unpublished field trials designed to test the efficacy of *Bacillus thuringiensis israelensis (Bti)* and *Bacillus sphaericus* against *Oc. nigromaculis* and *Oc. melanimon.* Various formulations of VectoBac® (*Bti*) and VectoLex® (*B. sphaericus*) were tested in recent field trials. In most cases, the formulations were found to be highly effective. Recommendations are made for rotational use of *Bti* and *B. sphaericus* in mosquito control programs.

INTRODUCTION

Mosquitoes associated with flood irrigation are a significant problem in the Central Valley of California. Flooding of pastures, duck clubs and waterfowl refuges provides larval habitat for *Ochlerotatus nigromaculis* (Ludlow) and *Ochlerotatus melanimon* (Dyar). These mosquitoes are aggressive biters and are important from an economic and public health perspective (McClelland 1980).

Suppression of these species in the larval stage is an essential component of successful control. Source reduction options are limited, because irrigation is needed for agricultural production and wildlife management in the Central Valley. Success of adulticiding is confounded by dispersal of broods after emergence (McClelland 1980). Consequently, larvicides have been a major component of irrigation mosquito control efforts.

Methoprene is an insect growth regulator (IGR) that has been used for many years to control both *Oc. nigromaculis* and *Oc. melanimon* in these habitats. Following the development of resistance to organochlorine and organophosphate insecticides in *Oc. nigromaculis* during the 1950's and 1960's, methoprene was identified as a very effective agent for pasture mosquito control (Schaefer and Dupras 1973). It has since been used, almost exclusively, for pasture mosquito control in certain areas of the state (Cornell et. al. 2000).

In 1999, reduced susceptibility to methoprene was discovered in *Oc. nigromaculis* in pastures near Fresno. (Cornell et al. 2000) Mosquito and vector control districts have subsequently expressed an interest in identifying effective materials for use in rotation with methoprene in order to protect the viability of this important IGR for the future of mosquito control (Brown 1999), (Farley 1999), (Inman 1999), (Mulligan 1999).

Published Literature

A review of the literature shows that *Bacillus thuringiensis israelensis (Bti)* has excellent potential for control of floodwater mosquitoes. An application of 0.5 lbs per acre of *Bti* powder to flooded pastures resulted in 95% control of *Oc. nigromaculis* and *Oc. melanimon* (Garcia et al. 1980). Bactimos® WP, a wettable powder containing *Bti,* provided 96% and 100% control of fourth instar *Oc. melanimon* and *Oc. dorsalis* in Wyoming pastures at concentrations of 0.3 and 0.9 ppm respectively (Jones and Lloyd 1985) Laboratory assays with fourth instar *Oc. nigromaculis* showed LC_{90} values ranging from 0.2 to 0.45 ppm (Mulla et al. 1990).

The literature also indicates that *B. sphaericus* strain 2362 has excellent potential for control of *Oc. nigromaculis*, and good potential for control of Oc. *melanimon* in warm waters.

Early work with *B. sphaericus* strain 1593 was not encouraging. A wettable powder of this strain provided only 5% control of *Oc. nigromaculis* and *Oc. melanimon* at 1.1 to 3.4 kg/ha (Mulligan et al. 1978). However, subsequent studies with strain 2362 were much more encouraging. A technical powder containing *B. sphaericus* strain 2362 was tested against *Oc. melanimon* in the Owens Valley of California. In warm water, 90% control of stages L2-L3 was achieved at 0.11 kg/ha and 94% control of stages L3-L4 was achieved at 0.56 kg/ha. Lower efficacy was seen in cool water with only 4-31% control being achieved against L3-L4 at 0.28 kg/ha (Mulla et al. 1985). Further testing against *Oc. nigromaculis* in Kings County, CA showed 100% control of stages L2-L3 with a technical powder containing *B. sphaericus* strain 2362, and 98% control of stages L2-L3 with VectoLex® CG (a granular *B. sphaericus* formulation) at 5 lbs/acre. (Mulla et al. 1988) Both of these tests were in warm water.

FIELD TESTS

Encouraged by the published literature relative to efficacy of *Bti* and *B. sphaericus* for pasture mosquito control and by the expressed interest from several districts, Valent BioSciences Corporation entered into a cooperative program with several Mosquito and Vector Control Districts (MVCD's) to explore the utility of its VectoBac® *(Bti)* and VectoLex® *(B. sphaericus)* products for control of flood water mosquitoes. Cooperative field tests were carried out in 1999 and 2000 with Butte County MVCD, Consolidated MAD, Merced County MAD and Sac-Yolo MVCD in California. Tests also were carried out in the Baker Valley VCD and the Jackson County VCD in Oregon.

Nine trials with VectoBac® products were successfully completed. Formulations tested include VectoBac® TP (5000 ITU *Bti* technical powder), VectoBac® CG (200 ITU 10/14 mesh *Bti* corncob granule), VectoBac® G (200 ITU 5/8 mesh *Bti* corncob granule) and VectoBac® 12AS (1200 ITU *Bti* aqueous suspension). Both ground and aerial applications were tested. Formulations were tested both pre-flood and post-flood. Adjuvants were tested with VectoBac® 12AS to assess improvement in deposition of the material.

Eight trials with VectoLex® products also were successfully completed. Formulations tested include VectoLex® CG (50 *Bs* ITU 10/14 mesh corncob granules) and VectoLex® WDG (650 *Bs* ITU water dispersible granule). Both ground and aerial applications were tested. Formulations were tested both pre-flood and post-flood using both ground and aerial applications.

MATERIALS AND METHODS

Trials consisted of unreplicated treatment plots simultaneously compared to untreated control (UTC) plots. In some cases, two rates or two materials were tested in separate plots in one trial. Applications were made using the standard operational methods of the respective districts. Ground applications of granular materials were made both with all terrain vehicle (ATV) mounted Herd® Seeders and hand operated seeders. Ground application of liquids were made with ATV mounted sprayers as well as hand sprayers. Most aerial applications were made with fixed wing aircraft. Aerial application of granules generally was accomplished with Transland® spreaders. Aerial liquid applications were made using both rotary atomizers and boom/nozzle systems. In most cases application equipment was calibrated in static tests, and rates were verified by comparing quantities used to the size of the areas treated. No efforts were made to quantify deposition or swath characteristics of the applications in the test plots.

Sampling was done both pre-treatment and post-treatment (24-72 hours) using 350ml mosquito dippers. For pre-flood studies, initial sampling was done at the time of flooding. Generally, single transects perpendicular to the treatment swaths were sampled. However, in some cases, multiple transects were dipped to address concerns about uneven distribution of larvae in the breeding site. In studies where larval populations were dense and homogeneous, as few as 20 dips were taken per plot. In other cases, more than 100 dips were taken per plot. Percent control was calculated using Mulla's formula (Mulla 1971) to compensate for changes in the UTC populations.

RESULTS

VectoBac® G and VectoBac® CG post-flood testing included a total of eight individual plots. Percent control greater than or equal to 90% was achieved in seven of the eight plots. These results are summarized in Table 1.

VectoBac® TP on sand was tested against *Oc. nigromaculis* in one trial in the Consolidated MAD. The sand mixture was prepared by following the directions on the VectoBac® TP label, and applied at 7.6 lbs per acre using an ATV mounted Herd Seeder. This application resulted in 100% control at 72 hours post-treatment. The use of VectoBac® TP sand for pasture mosquito control has since been adopted by the Consolidated MAD and others in the San Joaquin Valley. Sand has been used as a carrier for methoprene in these districts for several years. Consequently, the operational change to VectoBac® TP was simple. The districts report consistently good results with the VectoBac® TP sand.

Table 1. Summary of VectoBac® G and VectoBac® CG Post flood tests.

Species & Location	Researcher & Year	Material & Application	Rate & Timing	Percent Control
Oc. nigromaculis Consolidated	Mulligan 1999	VectoBac® CG Ground/ATV	6.8 lb/ac L3-L4	90% 48 hours
Oc. nigromaculis Consolidated	Mulligan 1999	VectoBac® CG Ground /ATV	10.7lb/ac L2-L4	97% 48 hours
Oc. melanimon Butte County	Bearden 2000	VectoBac® G Aerial Transland®	5 lb/ac L1-L4	98% 48 hours
Oc. melanimon Butte County	Bearden 2000	VectoBac® CG Aerial Transland®	5 lb/ac L1-L4	93% 48 hours
Oc. nigromaculis Merced MAD	Inman 2000	VectoBac® G Aerial Transland®	5 lb/ac NR	100% 48 hours
Oc. nigromaculis Merced MAD	Inman 2000	VectoBac® CG Aerial Transland®	5 lb/ac NR	100% 48 hours
Oc. nigromaculis Sac-Yolo MVCD	Yoshimura 2000	VectoBac® G Aerial Transland®	5 lb/ac L2-L4	44% 48 hours
Oc. nigromaculis Sac-Yolo MVCD	Yoshimura 2000	VectoBac® G Aerial Transland®	10 lb/ac L2-L4	92% 48 hours

VectoBac® G and VectoBac® CG were also tested in pre-flood applications. Two tests were carried out in the Merced MAD, and one in the Sac-Yolo MVCD. The materials were applied at 10 lbs per acre in all three studies. Results were mixed with nearly complete control achieved in the Merced test and no control achieved in the Sac-Yolo test. These results are summarized in Table 2.

Table 2. Summary of VectoBac® G and VectoBac® CG; pre-flood tests.

Species & Location	Researcher & Year	Material & Application	Rate & Timing	Percent Control
Oc. nigromaculis Merced MAD	Inman 2000	VectoBac® G Aerial Transland®	10 lb/ac 4 days pre-flood	98% 48 hours
Oc. nigromaculis Merced MAD	Inman 2000	VectoBac® CG Aerial Transland®	10 lb/ac 4 days pre-flood	100% 48 hours
Oc. nigromaculis Sac-Yolo MVCD	Yoshimura 2000	VectoBac® G Aerial Transland®	10 lb/ac 5 days pre-flood	0% 48 hours

VectoBac® 12AS was tested against *Oc. nigromaculis* in a total of seven plots. Dilute aerial and ground applications (1-3 gallons total volume per acre) did not perform well, while applications with little or no dilution resulted in very good control. The addition of Sta-Put® adjuvant (a penetration and drift control agent) to ULV aerial applications resulted in 100% control. These results are summarized in Table 3.

VectoLex® CG was tested against *Oc. nigromaculis* in six post flood tests. Percent control of this species was very high in every test. One late season test was implemented against *Oc. melanimon* that did not result in successful control. Water temperatures were very cool during this test. Results of the tests are summarized in Table 4.

Table 3. Summary of VectoBac® 12AS Tests.

Species & Location	**Researcher & Year**	**Material & Application**	**Rate & Timing**	**Percent Control**
Oc. nigromaculis Baker, OR	Janosek 1999	VectoBac® 12AS Aerial HV boom/CP nozzle	24 oz/ac 3 GPA L1-L3	17% 24 hours
Oc. nigromaculis Baker, OR	Bissell 1999	VectoBac® 12AS Ground HV Chappin Sprayer	24 oz/ac 3 GPA L1-L3	37% 18 hours
Oc. nigromaculis Baker, OR	Bissell 1999	VectoBac® 12AS Ground HV Chappin Sprayer	18 oz/ac 3 GPA L1-L3	65% 18 hours
Oc. nigromaculis & *Oc. melanimon* Baker, OR	DeChant 2000	VectoBac® 12AS Ground LV Chappin Sprayer	19 oz/ac 0.8 GPA L1-L3	98% 24 hours
Oc. nigromaculis & *Oc. melanimon* Baker, OR	DeChant 2000	VectoBac® 12AS Plus Sta-Put Ground LV Chappin Sprayer	19 oz/ac 0.8 GPA L1-L3	100% 24 hours
Oc. nigromaculis Merced MAD	Inman 2000	VectoBac® 12AS Aerial ULV Beecomist	24 oz/ac undiluted NR	84% 48 hours
Oc. nigromaculis Merced MAD	Inman 2000	VectoBac® 12AS Plus Sta-Put Aerial ULV Beecomist	24 oz/ac undiluted NR	100% 48 hours

Table 4. Summary of VectoLex® CG post flood trials.

Species & Location	Researcher & Year	Material & Application	Rate & Timing	Percent Control
Oc. nigromaculis Baker, OR	Bissell 2000	VectoLex® CG Ground/hand crank seeder	8.33 lb/ac L2-L3	97% 48 hours
Oc. nigromaculis Baker, OR	Bissell 2000	VectoLex® CG Ground/hand crank seeder	10 lb/ac L2-L3	100% 48 hours
Oc. nigromaculis Consolidated MAD	Mulligan 2000	VectoLex® CG Ground/ATV	8 lb/ac L1-L2	100% 48 hours
Oc. nigromaculis Consolidated MAD	Mulligan 2000	VectoLex® CG Ground /ATV	6 lb/ac L1-L2	100% 48 hours
Oc. nigromaculis Merced MAD	Inman 2000	VectoLex® CG Aerial Transland®	10 lb/ac NR	100% 24 hours (20% @ 48 hrs)
Oc. nigromaculis Merced MAD	Inman 2000	VectoLex® CG Aerial Transland®	10 lb/ac NR	98% 24 hours (100% @ 48 hrs)
Oc. melanimon Sac-Yolo MVCD	Yoshimura 2000	VectoLex® CG Aerial Transland®	10 lb/ac L1-L3 (cool water)	0% 72 hours (low larval numbers)

VectoLex® WDG was tested against in two ground and three aerial post flood tests. Percent control of *Oc. nigromaculis* was very high in ground tests. Lower levels of control were seen in the aerial tests. Results of the tests are summarized in Table 5.

Table 5. Summary of VectoLex® WDG post flood trials.

Species & Location	Researcher & Year	Material & Application	Rate & Timing	Percent Control
Oc. nigromaculis Jackson County, OR	Clover 1999	VectoLex® WDG Ground/ATV	0.5 lb/ac L1-L4	97% 48 hours
Oc. nigromaculis Jackson County, OR	Clover 1999	VectoLex® WDG Ground/ATV	1.0 lb/ac L1-L4	98% 48 hours
Oc. nigromaculis Merced MAD	Inman 2000	VectoLex® WDG Aerial Transland®	0.75 lb/ac NR	37% 24 hours (0% @ 48 hrs)
Oc. nigromaculis Merced MAD	Inman 2000	VectoLex® WDG Aerial Transland®	0.75 lb/ac NR	93% 24 hours (70% @ 48 hrs)
Oc. melanimon Sac-Yolo MVCD	Yoshimura 2000	VectoLex® WDG Aerial Boom/CP nozzle	0.5 lb/ac L1-L3 (cool water)	46% 72 hours

Three pre-flood tests were carried out with VectoLex® CG against *Oc. nigromaculis*. Results of these tests were mixed with control ranging from zero to 100%. These results are summarized in Table 6.

Table 6. Summary of VectoLex® CG Pre-flood tests.

Species & Location	Researcher & Year	Material & Application	Rate & Timing	Percent Control
Oc. nigromaculis Merced MAD	Inman 2000	VectoLex® CG Aerial Transland®	10 lb/ac 1 days pre-flood	82% 96 hours
Oc. nigromaculis Merced MAD	Inman 2000	VectoLex® CG Aerial Transland®	10 lb/ac 4 days pre-flood	100% @ 24 hours 83% @ 48 hours
Oc. nigromaculis Sac-Yolo MVCD	Yoshimura 2000	VectoLex® CG Aerial Transland®	10 lb/ac 5 days pre-flood	0% @ 96 hours (dead larvae noted)

DISCUSSION

Bti and *B. sphaericus* are effective for the control of *Oc. nigromaculis* and *Oc. melanimon.* These materials can be integrated into larviciding programs for the control of these species in California.

Post-flood *Bti* corncob and sand granular applications are generally the most reliable. However, *B. sphaericus* is also highly effective for control of *Oc. nigromaculis,* and is likely to be useful against *Oc. melanimon* in warm waters. *B. sphaericus* may not be as useful against *Oc. melanimon* in late season applications to cool waters.

Low and ultra-low volume applications of liquid *Bti* in both ground and aerial tests produced very high levels of control. Efficacy of ULV aerial applications appeared to be enhanced by the addition of Sta-Put® brand adjuvant. High volume liquid *Bti* applications were not found to be sufficiently effective in pasture tests in Baker, Oregon. Specific reasons for this have not been identified. Conversely, some districts in California and Nevada report routine operational success with high volume liquid *Bti* against pasture mosquitoes (Wargo 1999), (Lucchesi 2000). Further study in this area is warranted.

Variability was also seen in aqueous applications of VectoLex® WDG, with ground applications showing a high level of control and variability seen in aerial applications. Again, the specific reasons for this variability have not been identified. Future tests should include more complete assessment of spray droplet deposition to the plots.

Pre-flood tests with granular *Bti* and *B. sphaericus* indicate that there is potential for pre-flood larviciding with these materials. However, additional testing will be needed to determine the conditions under which success can be achieved.

There are several factors that can affect the success of biological larvicide applications. This is particularly true of aerial applications. Mosquito abatement districts will need to test materials and application methods under local conditions. It will also be important to characterize swaths produced by the specific aircraft and/or spray system in use, and use methods that optimize deposition of spray material into the breeding site. Complete and even coverage of the breeding site, as well as penetration of vegetative canopy are essential to success. Finally, since biologicals are more effective against early instar larvae than late instar larvae, application timing will be important. Treatments can be effective as late as the early fourth instar, but efforts should be made to treat earlier stages if possible.

Specific recommendations for rotational frequency are not the purpose of this paper. However, the following general concepts may be useful. Since biologicals tend to be more effective on early instars, their use may be less practical during the hottest part of the season when larval development is extremely rapid. Use of *Bti* during the cooler times early and late in the season and use of methoprene during the warmest months is a logical scenario. Since cross-resistance between *Bti* and *B. sphaericus* is unlikely, *B. sphaericus* can provide an additional mode of action in rotational programs. Use of this agent early in the fall could be valuable during refuge and duck club flooding that results in both *Culex* and *Ochlerotatus* production.

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Use of Albino Mosquitofish (*Gambusia affinis*) in Mosquito Control

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ABSTRACT: For ten years the Contra Costa Mosquito and Vector Control District has raised albino mosquitofish (*Gambusia affinis*). Their yellowish-white appearance and therefore high visibility and vulnerability to predation seems to preclude them from stocking in most mosquito breeding sources. However, their use in ornamental ponds and water gardens has shown that they are an efficient mosquito larval predator as well as a valuable public relations tool. Albino mosquitofish have also been used as a "marked" fish in estimating population sizes of standard mosquitofish in research ponds and keeping track of unauthorized stocking by residents. The importance of these fish to mosquito control agencies as well as the research community is just beginning to be realized and future use will only be limited by the creativity of mosquito control personnel.

INTRODUCTION

Over the decades, mosquito control agencies have battled mosquitoes, as well as the elements and sometimes our own machinery in order to control diseases. Through trial and error and ingenuity mosquito control personnel have been able to control mosquitoes in efficient and in some cases unique ways. Some of these methods can be used universally while others are specific to that district that develops them. Drawing on this history of ingenuity and problem solving the Contra Costa Mosquito & Vector Control District has cultured albino mosquitofish to be used in their integrated pest management program.

In August of 1990 approximately 300 pregnant mosquitofish were isolated in breeding trays in order to obtain new born fry. From this group of females one yellowish-white fry was produced. Upon closer examination its pink eyes identified it to be an albino mosquitofish. This genetic anomaly generated some interest and a ten gallon tank was dedicated to rearing this unique fish. From 1991 through 1995 our albino mosquitofish population ranged from 20 to 300 fish. In April of 1996 we dedicated a few large tanks to the production of albinos. Starting with only 15 fish we produced approximately 10,000 albino mosquitofish in six months. Production has since been 36,768 in 1997, 42,128 in 1998, 35,245 in 1999 and 35,547 in 2000.

DISCUSSION

On the surface it seems counterproductive to produce yellowish white fish to be used to control mosquito larvae. Their lighter color makes them more visible in most environments than their gray counterpart. The high visibility of these fish would make them an easy target for predatory fish as well as fish-eating birds. High predation by these natural predators would reduce populations to inefficient or even non-existent levels. Although the color of the albino mosquitofish will preclude use in more "natural" environments, the popularity of these fish with ornamental pond and water garden enthusiasts, as well as their efficiency in these environments make the albino mosquitofish a valuable tool in public relations as well as in mosquito larval control. In addition, their distinct color will also provide a means of keeping track of non-authorized stockings by residents. With changing environmental attitudes and laws these fish may be an important tool in the ever changing climate of mosquito control.

PUBLIC RELATIONS

The value of albino mosquitofish in terms of public relations is threefold. The first is the unique and attractive appearance of albino mosquitofish. These fish can easily be seen swimming around the pond providing enjoyment to the owner. We have received many positive comments by pond owners using albino mosquitofish and continue to have people request these fish when the "self-serve tank" is empty. Most people appreciate the fact that albino mosquitofish are an attractive as well as a functional pond fish. The second is the educational value. By adding a few albino mosquitofish in with the standard wild-type mosquitofish during class presentations, children and adults are captivated by the stark contrast in color. While explaining the differences we can include a genetics lesson describing the inheritance of the recessive gene. The third benefit is to provide the public with a "selection" of mosquitofish to choose from. The general public see choices in a positive manner. The end result is to provide the public with a positive view of the District and its operation.

TRACK UNAUTHORIZED STOCKING

The California Department of Fish and Game regulations allow mosquito and vector control agencies to use mosquitofish for mosquito control. They require that we keep a record of where, when and the number of fish we have stocked. As part of our stocking protocol we evaluate a site for the presence of threatened or endangered species and suitability for stocking mosquitofish. Although mosquitofish coexist in many habitats with other species, due to the perceived threat to threatened and endangered species we choose not to stock some sites. Because we provide mosquitofish to Contra Costa County residents for use on their private property we are also required to keep track of these fish. Residents are notified before they are given mosquitofish that they can only stock their own property. While this program works on the honor system there has been some concern that a well intended resident may release mosquitofish into a site that may contain threatened or endangered species. Until now there was no way to track this type of occurrence. By stocking our public "self-serve" tank with a mix of both albino and standard mosquitofish we are providing marked fish. If albino mosquitofish show up in a creek or pond we know that a member of the public stocked this site. By looking at our records we may be able to find out who stocked these fish. This method may not be foolproof but it will help us detect non-authorized stocking and may help us answer the concerns of various environmental groups.

MARK-RELEASE-RECAPTURE STUDIES

Albino mosquitofish have been used to estimate population sizes of mosquitofish in small research ponds (Lawler et al. 1999). Ordinarily, a mark-release-recapture study is very labor intensive because it requires marking individuals in such a way that they can be identified later. Handling of hundreds or thousands of animals twice during a study is both stressful and time consuming. The albino mosquitofish do not have to be fin clipped, dyed, or marked in any manner. This saves a significant amount of time and labor and eliminates stress, mortality and behavioral differences caused by the marking process. While Lawler et al. (1999) found that using albino mosquitofish was useful in estimating mosquitofish population sizes, this study was of short duration. Because of the differential survival between wild-type mosquitofish and albino mosquitofish, their usefulness in large bodies of water or studies of long duration is questionable.

AQUARIUM TRADE SALES

Because albino mosquitofish are unique, attractive and useful fish, pond owners and fish enthusists are requesting these fish. We have marketed albino mosquitofish to nurseries with aquatic plants, national pet supply stores and an independent aquarium/pet store outside our county. The nurseries were not interested in selling fish and the national pet supply stores purchased fish from large wholesale fish dealers. The independent aquarium pet store was very interested and easy to work with. We currently sell albino mosquitofish for $0.35 each with a 1,000 minimum order (delivered) when we have fish available. Although the amount of revenue generated by the sale of albino mosquitofish is minimal, it does indicate that there is potential for increased sales in the future. By providing revenue through the sales of albino mosquitofish we hope to help offset costs of the mosquitofish production program.

CONCLUSION

While albino mosquitofish cannot be used in areas where predators are not limited, they still have an important role in mosquito control. Their attractive appearance, as well as their functionality, make them a nice addition to the pond or water garden. The importance of these fish to mosquito control agencies as well as the research community is just beginning to be realized. Future use will only be limited by the creativity and determination of those individuals raising this unique strain of mosquitofish.

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Caltrans Stormwater Retrofit Pilot Study - Local Vector Management Aspects

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ABSTRACT: The State of California Department of Transportation (Caltrans) is currently conducting a study to assess the efficacy of treating stormwater runoff, by retrofitting Caltrans right-of-ways using Best Management Practice (BMP) technology. In southern California a total of 39 BMP study structures of eight designs have been constructed. All BMP sites were surveyed on a weekly basis since May 1999, and most of them were producing mosquitoes either intermittently or on a regular basis during this period.

Among the sites monitored by Greater Los Angeles County Vector Control District (GLACVCD), the Multi Chambered Treatment Train designs produce the most mosquitoes. *Culex quinquefasciatus* was the most abundant mosquito species; *Cx. tarsalis*, *Cx. stigmatosoma Culisetas incidens*, *Cs. inornata* and *Anopheles hermsi* were also collected.

INTRODUCTION

As a result of litigation between the State of California, Department of Transportation (Caltrans) and the National Resources Defense Council (NRDC), Caltrans is currently carrying out a Best Management practice (BMP) Retrofit Pilot Program in southern California to reduce contamination in stormwater runoff. A total of 36 BMP study structures consisting of eight designs, have been constructed. Since some of these stormwater devices require constant presence of standing water to function, potential vector control problems were foreseen, and mosquito larval surveys were included as an important component of the post-construction monitoring program. Thirteen of these BMP structures are located in San Diego County and are being monitored for vector activity by the Vector Surveillance and Control Division, County of San Diego Department of Environmental Health Services. The remaining 23 BMP structures are located within Los Angeles County. Surveillance and control of mosquito larvae in L.A. County is conducted by San Gabriel Valley Mosquito and Vector Control District (6 BMP), L.A. County West Vector Control District (1 BMP) and Greater Los Angeles County Vector Control District (GLACVCD) (16 BMP). This paper discusses the results of the first 1½ years of larval surveillance, emphasizing data collected within GLACVCD boundaries.

MATERIAL AND METHODS

Between May 1999 and December 2000, GLACVCD conducted weekly vector monitoring and control services for 16 operational stormwater BMP structures (Fig. 1), representing 8 different technologies. Each site was sampled for mosquito larvae when water was present at the time of inspection. At sites where the water was easily accessible, samples were obtained with a standard dipper.

A modified dipper, the "Madon-Kluh dipper" (Fig. 2), was utilized when stagnant water was encountered in deep cement basins. The latter sampling device consists of a standard sized dipping bowl attached by nylon lines at three points. A weight is welded to the outside of the bowl on one side to ensure penetration of the water surface. The bowl is either dragged along, or submersed to obtain a sufficient amount of water, constituting the standard dip sample. Mosquito larvae were taken to the laboratory and identified to species (Bohart and Washino, 1978).

RESULTS

During the surveillance period each BMP site was inspected 85 times. *Culex quinquefasciatus*, *Cx. stigmatosoma*, *Cx. tarsalis*, *Culiseta incidens*, *Cs. inornata* and *Anopheles hermsi* were found in San Diego and Los Angeles Counties at various BMP sites. *Culiseta inornata* and *Anopheles hermsi* were present only in San Diego County. *Cs. inornata* was found in all the different systems and *An. hermsi* occurred in samples taken from the Wet Basin, the Media Filters and the Extended Detention Basin (unpublished data, County of San Diego).

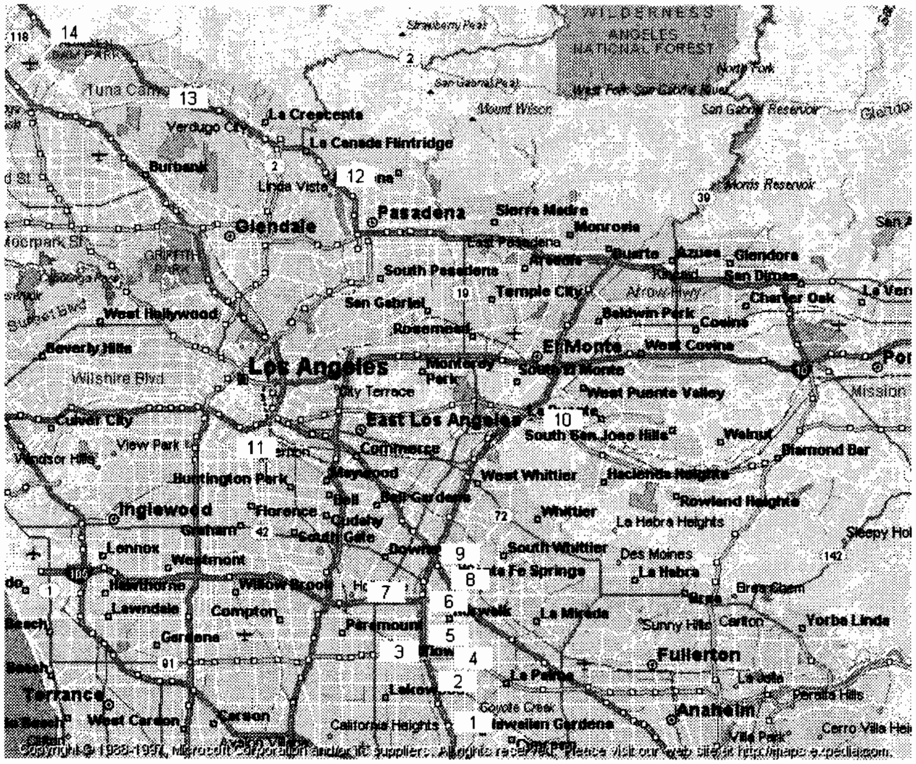


Figure 1. Location of BMP sites

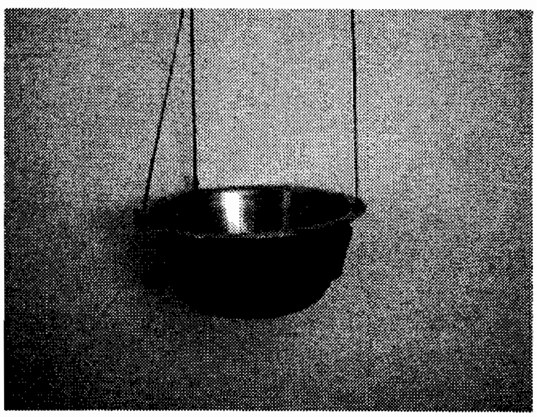
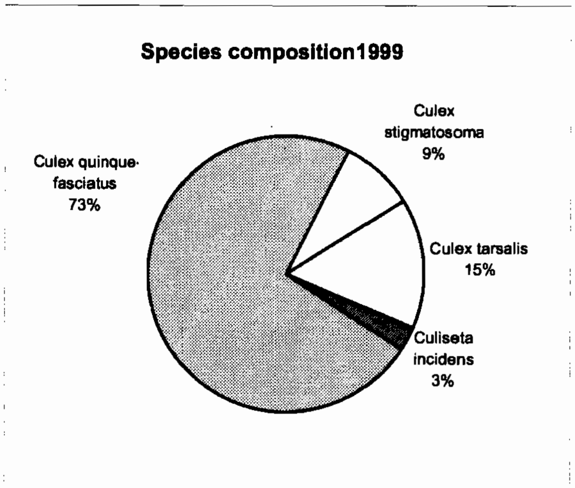


Figure 2. "Madon-Kluh" dipper

Figure 3 shows a comparison of species composition in Los Angeles County in 1999 and 2000. During both the first and second year of the study, *Cx. quinquefasciatus* was the most prevalent mosquito species (>72%). In 1999, however, *Cx. tarsalis* was still common, representing 15% of the population, with *Cx. stigmatosoma* and *Cs. incidens* representing 9% and 3% respectively. As the BMP structures began to accumulate increasing amounts of debris and organic matter in 2000, *Cx. quinquefasciatus* larvae became predominant, comprising 96% of the mosquito population sampled and the species most likely to occur in sources heavily charged with organic matter (Fig. 3).

The primary purpose of the BMP pilot study is to evaluate the effectiveness of each of the eight designed technologies in removing stormwater runoff pollutants and costs associated with site maintenance. This study was also to assess the potential of each of these structures to produce mosquitoes. At this point in the project, it is already very clear that some of the designs have been identified as producing significantly more mosquitoes than others. For example, larvae occurred 66% of the time during weekly site inspections (sampling) at the Multi Chambered Treatment Trains (MCTT) sites and 52% of the time at Continuous Deflection Systems (CDS) sites



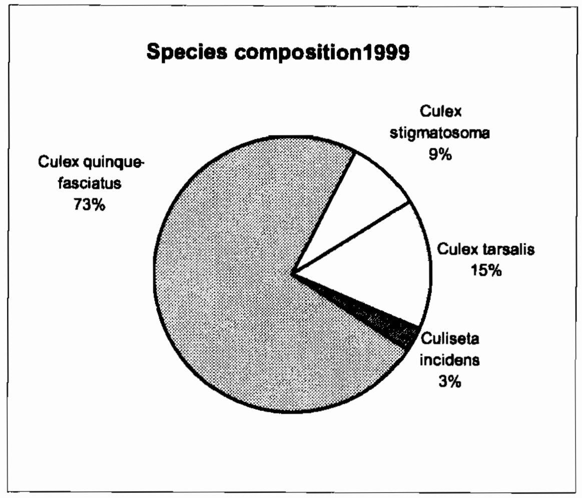


Figure 3. Mosquito species composition at BMP sites during 1999 and 2000.

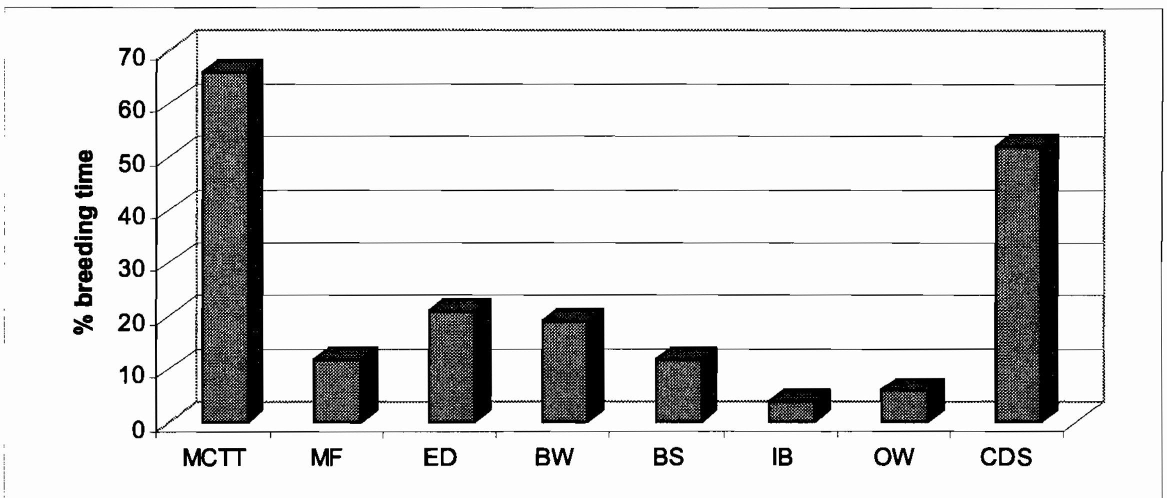


Figure 4. Percent of weekly site inspections with larval occurrence in Los Angeles County BMP sites May 1999 through December 2000.
MCTT=Multi Chambered Treatment Train, MF=Media Filter,
ED=Extended Detention Basin, BW=Bio-filtration Swale, BS=Bio-filtration Strip,
IB=Infiltration Basin, OW=Oil-Water Filter, CDS=Continuous Deflection Systems

(Fig. 4). Both designs require standing water, which presumably explains the high rate of occurrence associated with these sites. These designs would become extremely problematic if implemented in the field as part of a local or statewide, expanded BMP program.

Mosquito production at some sites was not directly related to the actual BMP structure. At the Oil Water Separator (OW) for example, breeding did only occur in a little puddle created by the outlet water sampling equipment, which would not be part of the device in normal field deployment.

These data clearly demonstrate that selecting the design with the shortest possible water retention time is an important step towards mosquito prevention. Additional findings, however, suggest that site location is also important.

A comparison of mosquito abundance at bio-filtration swales at four ecologically different sites showed that factors in the vicinity of a site or structure can significantly influence its mosquito productivity. Although the bio-filtration swales

(Fig. 5) are structurally identical, breeding activity was nearly four times higher at the freeway site I-5 (south) / I-605. The reason for the difference in productivity at this site was a sprinkler system that constantly replenished water in two concrete lined portions of the structure referred to as energy dissipaters, thus preventing the site from drying.

Energy dissipaters are structures near the inlet of the bio-filtration swale designed to reduce the force of water flow before it reaches the salt grass filtration area and thus protecting the grass from uprooting. They originally consisted of a square and a triangular basin filled with loose rocks. Since water would accumulate in these rock-filled shallow basins after each rain, mosquito larvae would usually be present before eventual evaporation. In cooperation with the California Department of Health Services (CDHS), Vector Borne Disease Section (VBDS), GLACVCD suggested that these basins be grouted with cement. The rocks were still placed into the concrete to break the water force, but water could no longer stagnate. Figure 6 illustrates mosquito production at a bio-filtration swale during the early months of the project. After the dissipaters were grouted, mosquito breeding no longer occurred.

Mosquito breeding was also prevented by covering the

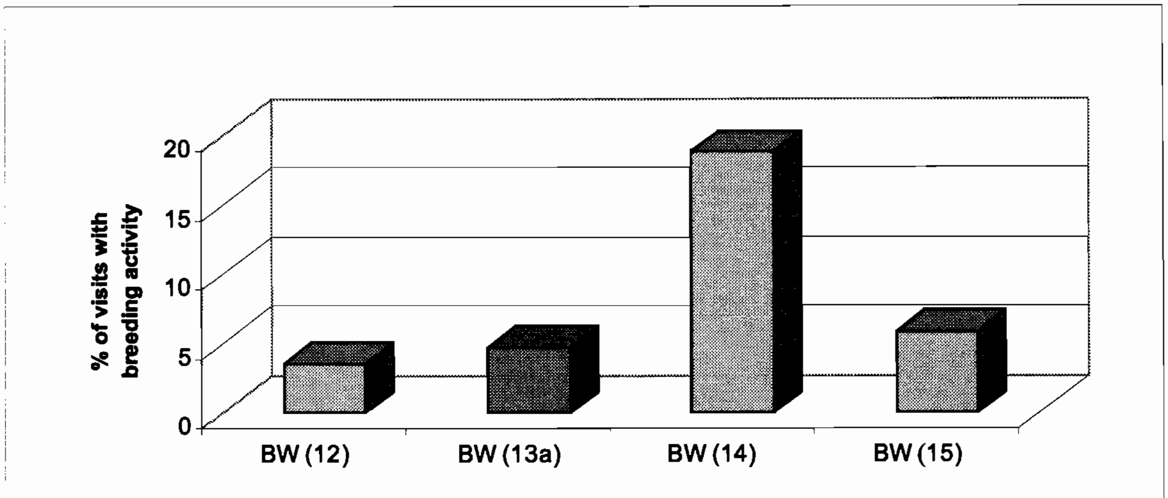


Figure 5. Comparison of percent of weekly inspections with breeding activity at different bio-filtration swales: BW (12) = Cerritos Maintenance Station, BW (13a) = I 605 (north)/I 91, BW (14) = I 5 (south)/I 605, BW (15) = I 605 (north: Carson/Del Amo).

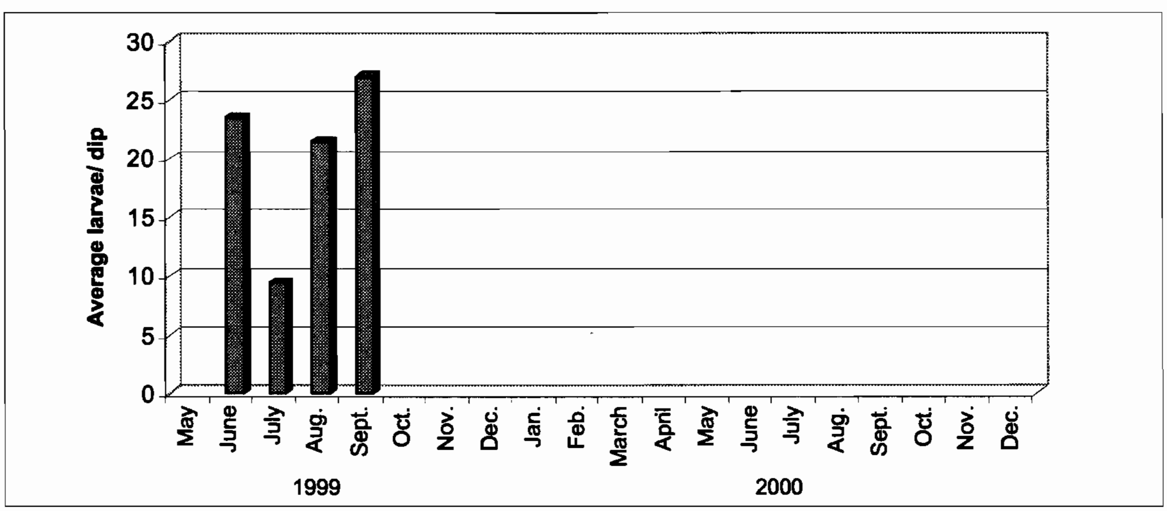


Figure 6. Mosquito breeding at freeway site I-5 (south)/I-605 before grouting of energy dissipaters.

outlet sumps with mosquito netting. The sumps had constantly held small amounts of water that could not be removed completely and consequently produced mosquitoes until the installation of the screening.

Continuous Deflection Systems (CDS) are designed to remove solid debris from non-point stormwater runoff. In order to function properly, they require the constant presence of standing water and, therefore, continuously produced mosquito larvae. In November 2000, attempts were made to "mosquito-proof" the CDS unit by caulking openings, fitting the lid tightly, and replacing the netting of the outlet pipe with a finer mesh. During the following month, larval numbers decreased steadily and further breeding has not occurred since.

CONCLUSION

In conclusion, many of the above-mentioned BMP structures have been shown to produce mosquitoes at some point during 1½ years of surveillance. Factors contributing to mosquito production are as follows:

- poorly drained sumps and spreading trenches
- settling basins that retain water in excess of 72 hours because of clogging or faulty design
- high water tables at infiltration basins
- rock-filled energy dissipaters
- excess vegetation at wet basins

Modifications to the design and constant maintenance have helped to minimize mosquito problems in many sites, but have not completely prevented them. Should hundreds of similar devices be built across California, collectively they may present a significant mosquito-breeding problem. If continued maintenance of the BMP structures is not sustained at the same high level as during this study (a CDHS survey of similar BMP structures in other states shows it usually is not) these non-point stormwater treatment sites may pose a potential threat to public health.

Taking into consideration the enormity of future projects planned by Caltrans, we highly recommend that mosquito and vector control agencies in California (including the anticipated support of the Mosquito & Vector Control Association of California), initiate legislative change that would demand review and permit authorization of such devices by state and local public health agencies before their clearance for field-implementation.

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Comparison of Larvicidal Application Methods in the Underground Storm Drain Systems in Urban Los Angeles

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ABSTRACT: The purpose of this project was to evaluate and compare the effectiveness of aerosolized applications of VectoLex® WDG (*Bacillus sphaericus*) for larval mosquito control in underground storm drain systems (USDS). Six sections of USDS located in the Los Angeles Basin were selected. At three locations, VectoLex® applications were made via ULV cold fogger, using the Curtis Dyna-Fog® Sewer Attachment Kit (adapted to a LECO® Machine). Two drain systems were treated with the "LAvector/USDS Larvicide Applicator." One of the USDS was used as an "control" (untreated) site. Adult trapping as well as bioassays demonstrated that VectoLex® WDG is an effective larval mosquito control agent for the underground storm drain environment. Both application systems achieved adequate control. The "LAvector / USDS Larvicide Applicator," however, was easier and safer to use and treatment was less susceptible to air currents caused by temperature gradients between above and below ground.

INTRODUCTION

The underground storm drain systems (USDS) in urban Los Angeles is a complex network of drains, catch basins and manhole chambers. These systems catch runoff from rainstorms as well as from irrigation and residential runoff. Within the City of Los Angeles alone, there are 1,500 miles of storm drains, 6,700 miles of street drains and 34,000 catch basins (Moore, 2000.). Los Angeles County contains 2,650 miles of county-maintained storm drains and about 60,000 catch basins (Ariki, 2000.). These numbers do not include various street drain systems in all the other 87 incorporated cities in Los Angeles County.

The USDS is historically known to be capable of producing large numbers of mosquitoes. Interest in mosquito productivity of the USDS increased in the early 1980s, following outbreaks of St. Louis encephalitis (SLE) in the southeastern United States (80 human SLE cases in Houston in 1980). *Culex pipiens quinquefasciatus* Say was determined to be the primary vector of SLE in the Houston epidemic of 1964 (Pigford 1964). Since this *Cx. quinquefasciatus* is also predominant in the USDS in southern California (Dhillon and Mulla 1982, 1983 & 1984, UC MCR Annual Reports), there may be a potential for a similar occurrence. In 1984 there were 26 confirmed cases of SLE in the Los Angeles Basin. During the following years, data were collected on the ecology of urban underground mosquitoes and the development of integrated vector management strategies (Dhillon and Mulla 1982, 1983 & 1984, UC MCR Annual Reports). In 1999, Greater Los Angeles County Vector Control District (GLACVCD), using Encephalitis Virus Surveillance (EVS)/CO_2-baited traps, confirmed that USDS still produce significant numbers of mosquitoes (up to 18,000 adults/trap-night at a single site). The trapping results demonstrated clearly that the district should:

1. seriously consider implementing a routine mosquito surveillance and control program in the USDS.
2. evaluate the efficacy of currently registered larvicidal agents in the USDS, and
3. determine the most efficient larvicidal application method.

MATERIAL AND METHODS

Six segments of the USDS historically known to produce mosquitoes (located within a 5 square mile area in the L.A. Basin), were selected as trial sites (Fig. 1). Five systems were treated with a *Bacillus sphaericus* (VectoLex®) suspension (1 lb WDG/1.33 gal of water) at an application rate of two gallons per acre. The application was made on September 26, 2000, at three locations with a ULV cold fogger, using the Curtis Dyna-Fog® Sewer Attachment Kit adapted to a LECO®

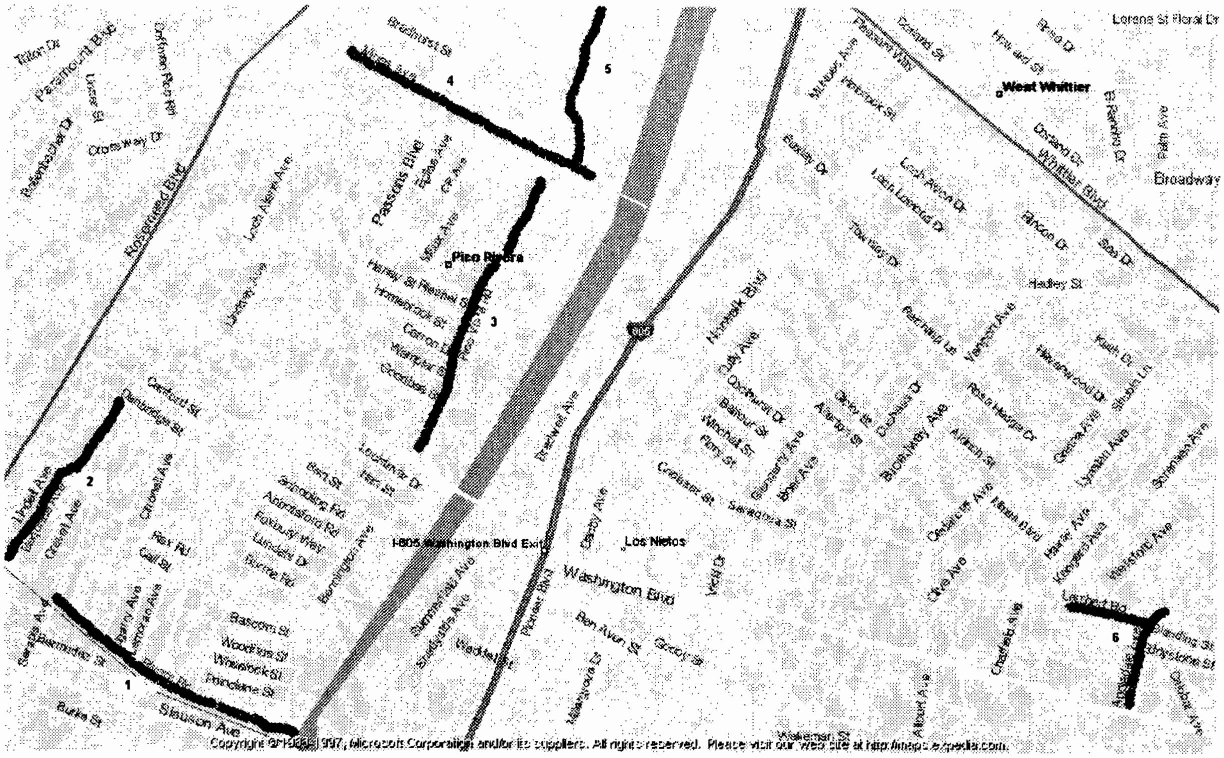

Figure 1. Location of trial sites 1. Rivera Rd 2. Bequette Avenue 3. Pico Vista Rd (S) 4. Mines Ave 5. Pico Vista Rd (N) 6. Lambert Ave

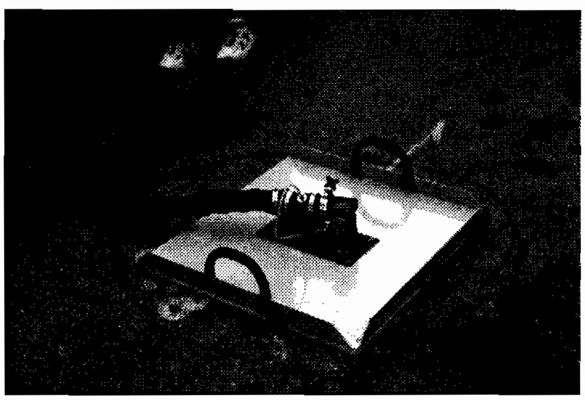

Figure 2. Curtis Dyna-Fog[a] Sewer Attachment Kit

Figure 3. "LAvector/USDS Larvicide Applicator"

Machine (Fig. 2). This method of delivery was first used in similar trials by the Harris County Health Department, Houston, TX (Harris County 1999, unpubl. data). We (GLACVCD) treated two drainage systems on September 27, 2000, with a compressed air sprayer, the "LAvector/USDS Larvicide Applicator." The sprayer consists of an Amflo Hydro-blast cleaning tool and siphon, usually used for high-pressure cleaning of car engines (Fig. 3), attached to an air-compressor unit. One drainage system was used as "control" (untreated).

Adult and larval mosquito populations were monitored to assess treatment efficacy. The adult mosquito population was monitored by using EVS/CO_2-baited traps, placed into each system just below the manhole cover, prior to and over several weeks following treatment. All adult mosquitoes were counted and identified to species.

Larval populations were monitored by placing 20 laboratory-reared 3rd and 4th instar *Cx. p. quinquefasciatus* larvae into water buckets [0.39 gal, Ø 0,64 in (1.5 liters, Ø 16 cm)] and lowering the buckets into each accessible manhole prior to treatment. No sooner than 4 hours post-treatment the buckets were removed and returned to the laboratory. Rates of larval mortality were determined at 24- and 48-hour post-treatment. In addition, water samples were taken at every accessible manhole (with exception of the ones at which treatments were performed) 4 hours post-treatment after 10 and 20 days respectively. Water samples were taken with disposable Zip-loc® bags that were weighted with a stiff wire to keep the bag open. A string was attached to the weighted system and the bag was dragged along the water surface to obtain a water sample. Using a new Zip-loc® bag at each site assured no cross-contamination between sample sites. The water samples were transferred into waxed paper cups and twenty 3rd and 4th instar *Cx. quinquefasciatus* larvae were added to each of the samples for bioassays.

RESULTS AND DISCUSSION

The results of adult monitoring at the "control" (untreated) site at Lambert Ave (unincorporated area of L.A. County) throughout the duration of the trial are summarized in Figure 4.

Adult numbers in the pre-treatment sample were relatively low. It had rained in the Los Angeles area September 22/23, just a few days before the scheduled field trial, apparently flushing the mosquito larvae out of the systems. During the next 4 weeks, however, breeding activity gradually increased, and adult counts were high (3379 adult *Cx. p. quinquefasciatus*/ trap night). The relatively high percentage of adult males trapped indicated that breeding sites were actually within the USDS. Since males emerge first, male mosquito numbers were relatively high on October 5 and October 12, while female numbers increased later in the surveillance period.

Water samples taken from the "control" (untreated) system at 5 different manholes (L1–L5) generally showed low larval mortality rates in laboratory assays. All 48 h mortality rates remained below 10%, except for one sample taken on September 26 at manhole L4, which showed a mortality of 45% after 48 hours (Fig. 5). This comparatively high value is presumably due to unknown contamination at that particular sample site on that day. Only one sample could be taken from manhole L1, since the water puddle was too shallow to retrieve further samples.

One of the drainage systems treated with the "LAvector/ USDS Larvicide Applicator," was located at Rivera Road (City of Pico Rivera). This system is 0.6 mile (1 km) long with 5 manholes that allow access for treatment and sampling. Treatment was conducted on September 26, 2000 at manhole R3, the water flowing in the system from manhole R1 to R5 (Fig.6).

Bioassays conducted with water samples taken from the Rivera Road system 4 hours post-treatment showed 100% mortality at 48-hour exposure at two manholes downstream from the treatment point and up to 45% mortality after 48-hour at one manhole up-stream of the treatment site. The 10 day post-treatment mortality rate had increased (90% at 48h) up-stream and decreased (R4=20%, R5=90% at 48h) down-stream. Ten days later there seemed to be no residual effect at the upstream location, while mortality rates downstream were still at 20% and 5% at 48 hours respectively (Fig.6). It was again not possible to retrieve water samples at manhole R1 on 10/5 and 10/16 due to insufficient water.

Adult monitoring results show low pre-treatment numbers, which may be attributed to the rain on September 22/23.

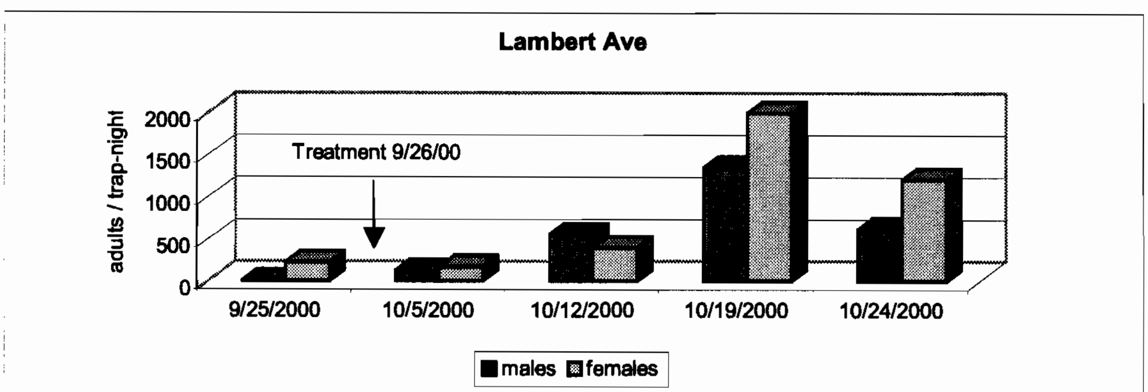

Figure 4. EVS/CO_2-light trap results at "control" (untreated).

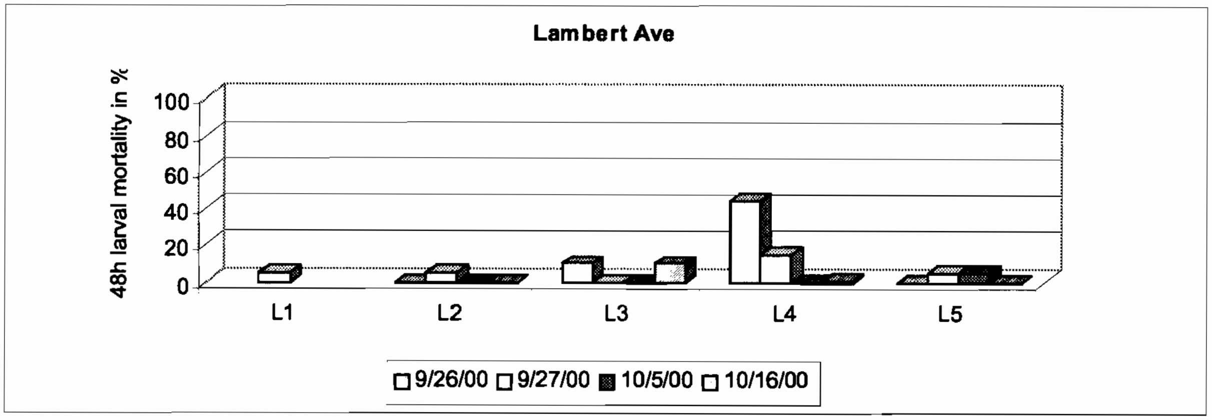

Figure 5. Forty-eight hour larval mortality in water samples taken from "controls" (untreated). L1-L4: accessible manholes.

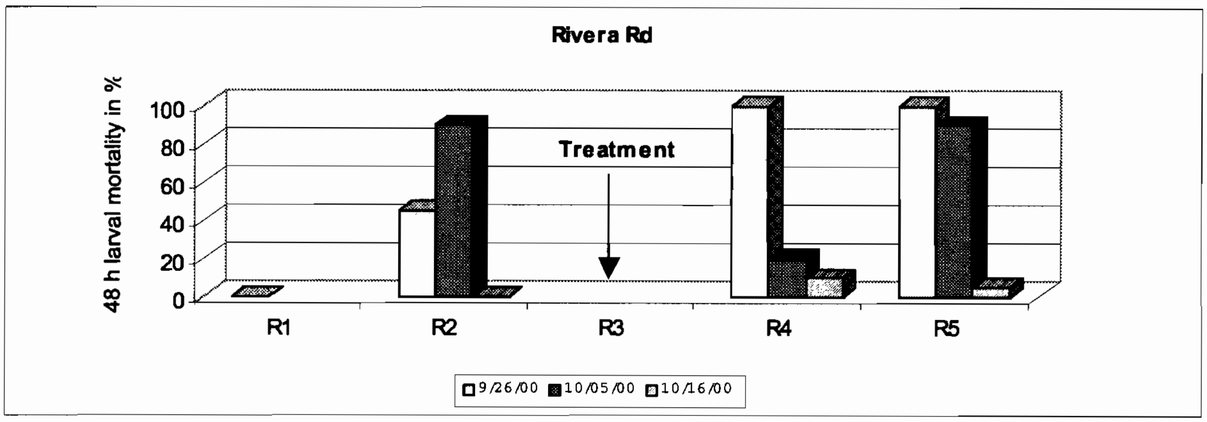

Figure 6. Forty-eight hour larval mortality in water samples, treated with "LAvector/USDS Larvicide Applicator," R1-R5: accessible manholes.

Numbers of female adults increased steadily throughout the observation period, but male numbers remained very low (<50 males/trap-night) in comparison to the number of males trapped at the untreated control site (up to 1350 males/trap-night), thus indicating that the mosquito productivity of the source had been affected as a result of the treatment. Because weather conditions remained favorable for mosquito breeding throughout the remainder of the trapping period, increasing numbers of females could be interpreted as "fly-ins," perhaps attracted to the CO_2 from other areas below- or above-ground (Fig. 7).

The USDS at Pico Vista Road (N, City of Pico Rivera) was treated with VectoLex® using the Curtis Dyna-Fog® Sewer Attachment Kit (adapted to a LECO® Machine). This system is 0.3 mile (0.5 km) long and is accessible through 7 manholes for pre- and post-treatment sampling. Pre-treatment adult numbers were low, as at the other trial sites due to the rain on September 22/23. During the surveillance period, the number of females trapped at this site also steadily increased (apparently "fly-in" females attracted to the CO_2 from other areas either below- or above-ground), while male numbers remained low (<50 males/trap-night), demonstrating once again that the treatment performed on September 26 was effective (Fig. 8).

Larval mortality at 48 h in water samples taken 4 hours after treatment was 100% at one manhole upstream (P2) and also at all of the downstream sample sites (P4 – P7). On October 5, 2000, the 48 h mortality rate was still 100% at P4 and P5, and 70% at P6 (P7 was too shallow to sample). Mortality at P2 (upstream) was 40%. Post-treatment bioassays (10 days later) showed 35% mortality at P2 and mortality rates between 15% and 55% at the downstream locations P4-P7

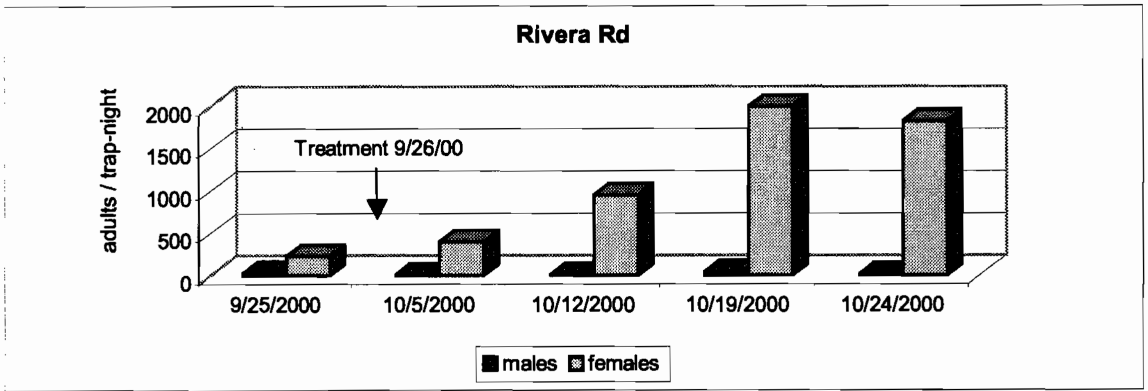

Figure 7. CO_2/EVS trap results, system treated with "LAvector/USDS Larvicide Applicator."

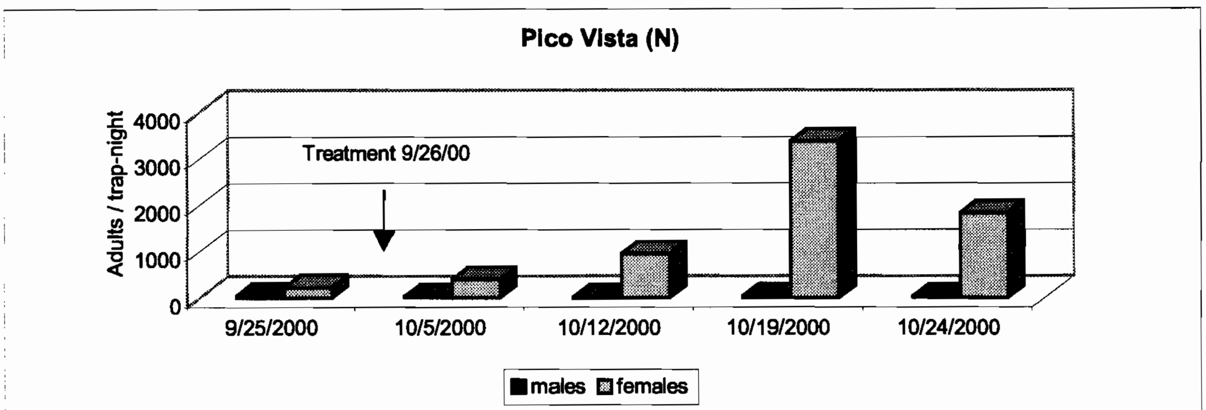

Figure 8. EVS/CO_2-baited light trap results, treated with ULV cold fogger.

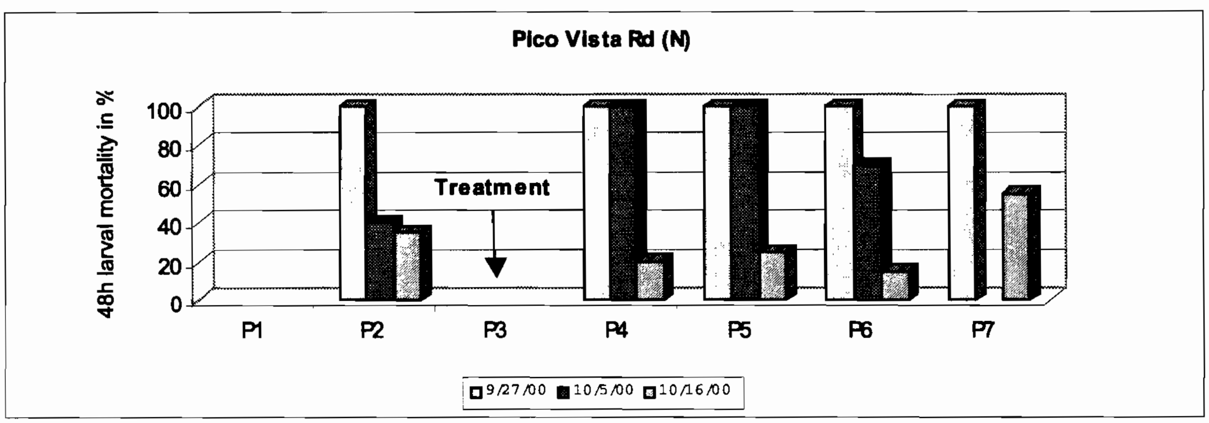

Figure 9. Forty-eight hour larval mortality in water samples, treated with ULV cold fogger. P1-P7: accessible manholes.

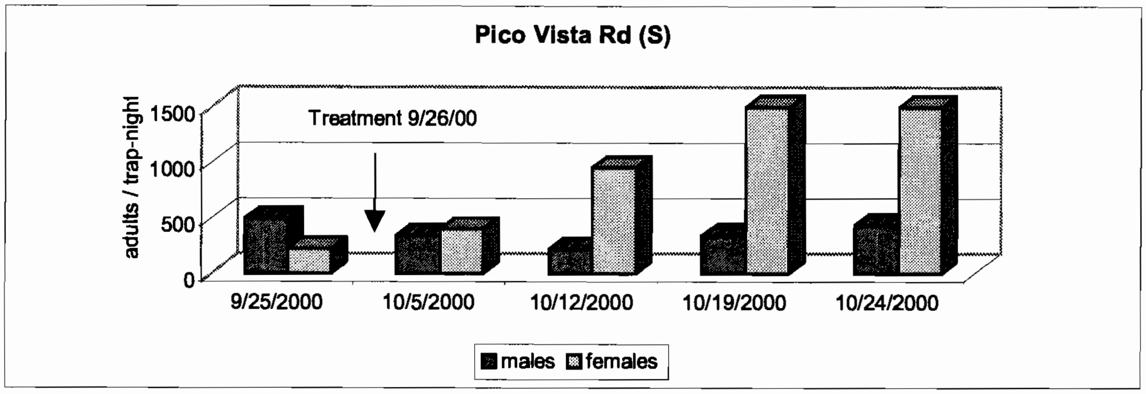

Figure 10. EVS/CO_2-trap results, treated with ULV cold fogger.

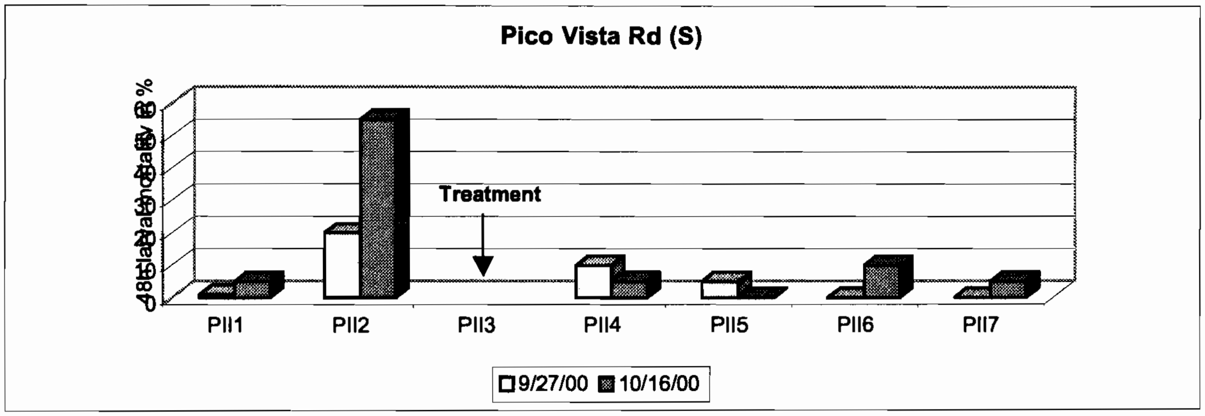

Figure 11. Forty-eight hour larval mortality in water samples, treated with ULV cold fogger. PII 1-PII 7: accessible manhole covers.

(Fig. 9).

At first glance, results appear to be more consistent when using the ULV cold fogger in comparison to the "LAvector/ USDS Larvicide Applicator." However, the Pico Vista (N) site system is only half as long as the Rivera Road site system and has more sampling points, resulting in sampling locations much closer together and therefore with less variation.

One of the disadvantages of the ULV cold fogger is that spray droplet deposition into the USDS appeared sensitive to extreme temperature gradients. In a separate southern section of Pico Vista Rd, temperatures below ground were higher than aboveground, creating an updraft that led to the loss of visible amounts of ULV material through the openings at the catch basins located near the manhole treated. Since temperatures within USDS remain relatively constant, we believe that treatment with this applicator would have to be confined to the later hours of the day, when temperatures are higher aboveground.

Adult monitoring results indicate that this treatment at Pico Vista Road (south) was less effective (Fig. 10). The number of male mosquitoes trapped remained high (>200 per trap-night), indicating that breeding within the system continued, following larvicidal application.

When bioassayed, water samples also showed less efficacy due to the above-mentioned loss of material. Mortality rates after 48 h were generally below 20% in the 4 h post-treatment sample. The highest mortality rate (>50%) was encountered at one manhole upstream from the point of treatment on October 16, 2000 (Fig. 11).

CONCLUSION

The field trial of two different application methods of VectoLex[a] WDG in the USDS demonstrated that VectoLex[a] WDG was an effective larval mosquito control agent for the underground storm drain (USDS) environment. Adult monitoring indicated residual effects for at least 30 days and water samples taken 20 days post-treatment still produced larval mortality rates of up to 55% after 48 h of exposure. Both delivery systems provided satisfactory treatment. Comparisons between these systems indicate that the

ULV Cold Fogger:
- involves a high initial investment
- costs more to operate/maintain
- requires considerable effort and manpower to operate safely (removal of manhole cover), especially in congested urban traffic
- subject to erratic dispersal due to temperature gradient sensitivity

whereas the
"LAvector/USDS Larvicide Applicator:"
- involves a low initial investment
- costs little to operate/maintain
- requires minimum effort to operate (single-person operator) and is safer (does not require removal of manhole covers)
- is less affected by temperature gradients due to larger droplet sizes produced

Overall, treatment with the "LAvector/USDS Larvicide Applicator" proved to be easier and much safer. We are currently modifying the nozzle and trigger mechanism to allow treatment from within the vehicle and to optimize droplet size and drift potential, to achieve control right below the treated manhole as well as within inaccessible parts of the drain between manhole chambers.

Though drastic and lasting reduction of male mosquitoes indicate the effectiveness of the larvicidal application, female mosquito numbers frequently remained high, most likely due to "fly-ins" from other sources in the vicinity. These sources may be within untreated sections of the USDS or even above ground. In earlier "mark and release" studies 10% of the mosquitoes released above ground were recaptured in catch basins and manhole chambers without using CO_2 as an attractant (Dhillon and Mulla 1982, 1983 & 1984, UC MCR Annual Reports), thus demonstrating that adults readily re-enter the USDS. Since CO_2 baited traps were used in this trial it is not surprising that female mosquito numbers remain high even though the immediate source has been removed. Using CO_2 baited traps has thus the advantage of indicating local treatment success (low number of males) while monitoring general mosquito abundance in the vicinity of the treated site. To further enhance overall control efficacy, an additional application of a residual adulticide to the inside surfaces of manhole and catch basin walls, both preferred adult resting places (Dhillon and Mulla 1982, 1983 & 1984, UC MCR Annual Reports) may be useful.

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Preliminary Study on Optimizing Ground ULV Application

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ABSTRACT: Penetration and swath width for ULV ground adulticiding using Pyrocide ® and Pyrenone ®, (Pyrethrins: piperomyl butoxide, 25:5 mixture) were determined by bioassay with sentinel mosquitoes positioned at three vegetation types (desert, vineyards and citrus), and two elevations, canopy side and top. Five tests done during September and October 2000 indicate that, in all habitats, ULV particles followed the contour of vegetation. Good control was achieved at mixed desert <1m height, whereas 1.5 m grape vines and 3-m high citrus trees imposed a barrier and deflected the ULV particles up and over the canopy.

INTRODUCTION

Annual seroconversions of sentinel chickens and infections in mosquitoes for western equine encephalomyelitis (WEE) and St. Louis encephalitis viruses (SLE) trigger the Coachella Mosquito and Vector Control District to apply ground ultra-low volume (ULV) adulticides to suppress populations of infected Culex tarsalis Coquillett and thereby reduce the threat of human infections. Presently, ground ULV routes and timing are determined by proximity to the positive chicken flock, adult abundance measured by CO_2 traps, 24 hour flight pattern measured by a time sequential trap (Meyer et al. 1984), wind direction, and access roads.

Previous research defined host-seeking and temporal abundance of Cx. tarsalis, at microhabitats in the Coachella Valley (Lothrop and Reisen, 1998, 1999). Traps at vegetation >3m height such as citrus, tamarisk and cattails collected the greatest number of host seeking Cx. tarsalis. We designed the current assessment protocols based on this information of adult female host-seeking aggregation. Our ultimate goal is to improve ULV ground adulticide control by focusing on adult congregation sites and/or flight pathways. The current experiments determine adulticide penetration through different vegetation types and dispersal at various elevations.

MATERIALS AND METHODS

Standard size sentinel cages (15 cm diameter) were constructed of PVC with dark fiberglass window screen. Sentinel mosquitoes mortality in these cages was equivalent to standard cardboard cages; however PVC cages were more durable and provided a lighter background surface for counting dead mosquitoes. Each cage contained 20 field collected Cx. tarsalis or laboratory reared Culex quinquefasciatus, Say. At each location, cages were placed downwind 30 min. before and removed 30 min. after application, after which dead adults were counted. Cages were returned to the laboratory, and dead and live mosquitoes recounted the following morning. For all trails, Pyrocide 7396® -mosquito adulticiding concentrate was used at the rate of 5 oz/min., except on October 5, when Pyrenone 25-5 ®, public health insecticide was used. The truck speed was 5 mph and a Beecomist Systems®, Pro-Mist HD 6 CS ULV Sprayer was used in all trials. Multiple experiments were done at 3 habitat types near the town of Mecca in the southern Coachella Valley.

Desert vegetation: Mixed low desert scrub, consisted mostly of Salicornia sp., Artiplex sp., and Tamarisk.

Test 1 - September 18, 2000: Twenty-one cages each stocked with Cx. quinquefasciatus from CVMVCD colony were set in three rows, at a distances of 0, 15, 30, 45, 60, 75 and 90 m downwind from the ULV source. Three controls were set 25 m upwind. At spray time the wind was NW, at 2-5 km/h.

Test 2 – October 6, 2000: Fifteen cages stocked with wild Cx. tarsalis were set in three rows, at 0, 30, 60, 90 and 120 m from the source of ULV. Three controls were set upwind of the spray route. At spray time the wind was NW, at 5.6 km/h.

Vineyard: The purpose of selecting two sites was to test the effects of drift perpendicular to and parallel with rows of grape that were 1.5 m in height.

Test 1 - October 3, 2000: Eighteen cages were set in two rows, at 0, 30, 60, 90 and 120 m downwind from the ULV source, at the elevation of 1 m., and at 30, 60, 90 and 120 m at the elevation of 2 m. Two controls were set 25 m upwind of the spray route. At spray time, the wind was NW, at 2.6 km/h, and spray drifted at 45 ^{0} to the rows.

Test 2 - October 5, 2000: Twenty-eight cages were set in three rows, at 0, 30, 60, 90 and 120 m, at an elevation of 1 m, and at 30,60,90 and 120 m, at the elevation of 2 m. Three controls were set upwind at 30 m. At spray time, the wind was NW, at 4.8 km/h and spray drifted parallel to rows.

Citrus orchard: This young orchard had trees 3 m high with 5 m gaps between trees within rows.

October 20, 2000: Thirty-nine cages were set in three rows, at 0, 15, 23, 30, 45, 60, 75, and 105 m downwind, at the elevation of 1 m. Two extra cages were inserted into the citrus foliage at the distance of 15 m and 45 m downwind. Two cages were elevated 3.5 m above the canopy at 30 m and one at 75 m downwind. Three controls were set upwind of the spray route. At spray time, the wind was NW, at 4.8 km/h, and spray drifted at 45 degrees into the orchard rows.

RESULTS AND DISCUSSION

Two applications over desert habitat indicated that sentinel mortality and therefore particle dispersal was greatest at distances within 60 m and 90 m downwind (Fig. 1). In contrast, the results from two trials at the vineyard habitat indicated high sentinel mortality at 1m height within, 15 m downwind, weak penetration beyond 15 m at 1-m elevation and increased mortality above the canopy, at 2-m elevation at 60-m distance (Fig. 2). Citrus trees formed a barrier for the downwind dispersal of ULV particles. High mortality (97%) of sentinel mosquitoes was recorded only at cages 8 m downwind, with a sharp decrease in mortality at 23 m. Spray particles apparently drifted above the citrus canopy where ≥ 40% mortality was recorded at a distance of 30-m downwind (Fig.3).

Adulticiding from air or ground has been accepted as the management strategy to rapidly eliminate infected female Culex mosquitoes from a designated area. During outbreaks of WEE in Canada and SLE in Texas, the number of new human cases and the virus isolation rates from vector populations declined following ULV adulticiding by air (Mahady et al. 1979). The choice of the product, type of application equipment and habitat where infected females are to be controlled, greatly influences control effectiveness (Reisen et al. 1985, 1985a, 1984).

In California, control agencies use ground ULV spray in situations when they need to control infected Culex mosquitoes, because these species are active at night and therefore exposed to adulticide droplets. In most cases applications target specific areas known to support virus transmission and are done at specific times during the night based on weather conditions. Far less is understood about the distribution of the target mosquito population and the effectiveness of bringing the adulticide particles into critical contact areas.

Our study was based on previous research on the host-seeking behavior of Cx. tarsalis at microhabitats in Coachella Valley (Lothrop and Reisen, 1998, 1999,) and on the

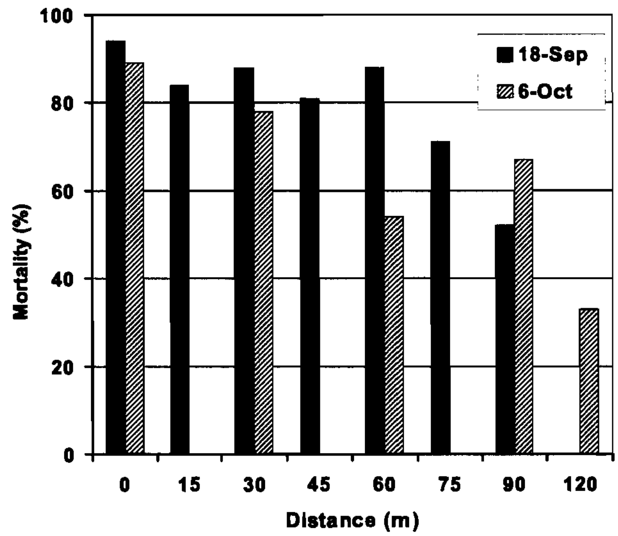

Figure 1. Desert habitat – percent mortality of sentinel mosquitoes at different distances from the ULV source.

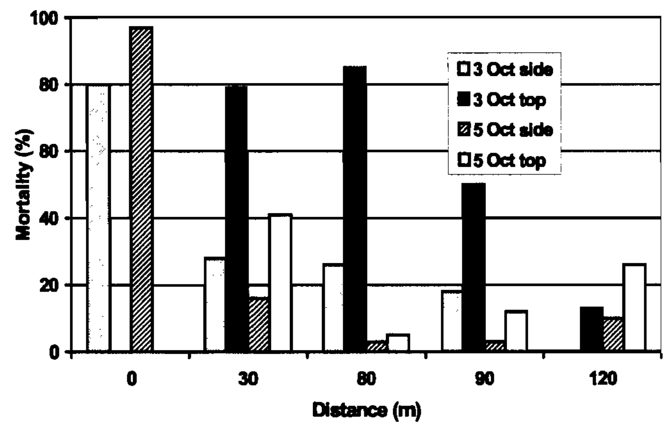

Figure 2. Vineyard - percent mortality of sentinel mosquitoes at different distances from the ULV source.

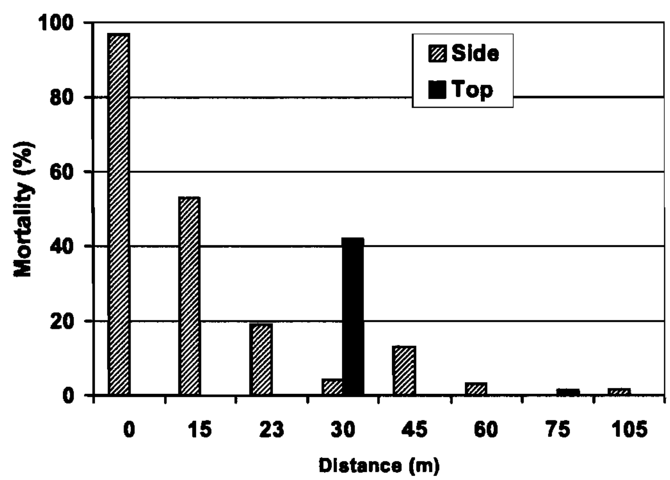

Figure 3. Citrus - percent mortality of sentinel mosquitoes at different distances from the ULV source.

manufacturer's claims of swath width under ideal conditions. Our preliminary results indicated that the spray particles did not penetrate dense vegetation, but rather were carried above the vegetative canopy. In addition, marked shifts in wind direction after sunset frequently compromised applications by driving adulticide particles away from planned downwind cages and into upwind control cages. For example, on October 9 and 10, cages were set downwind from the established spray route; however, at the time of spray, prevailing winds shifted up to 180 ^{0} placing cages upwind. On other occasions, the winds died and ULV particles did not dispersed to cages even at 8 –15 m distance up or downwind. On October 12, during one trail at the citrus orchard the NW wind died during the test, and there was no kill in the cages 45 m downwind. We also noticed that after the hot days, a thermal inversion did not occur, and the spray particles instead laying closer to the ground most likely were pulled upwards by convection currents, away from the target vegetation.

These preliminary results helped us to understand the patterns of ground ULV spray drift and its failure to penetrate elevated and dense vegetation. Future applications must be modified to improve penetration and get the spray to the target mosquito population or to use different protocols that target vegetative ecotones used by Cx. tarsalis.

Acknowledgements

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Field Evaluation of Aqua-Pyrenone and Pyrenone Against *Ochlerotatus nigromaculis* in Merced County

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ABSTRACT: Aqua-Pyrenone EC (3% pyrethrins and 15% PBO) and oil based Pyrenone (5% pyrethrins and 25% PBO) were evaluated as ground-applied ULV treatments at 0.0022 pounds AI/acre against *Ochlerotatus nigromaculis*. At 1-hour post treatment Aqua-Pyrenone achieved greater than 97% knockdown out to 200 ft and 89% knockdown at 300 ft. At 100 ft and 200 ft, Aqua-Pyrenone achieved 99% mortality 12-hours post-treatment and 96% mortality at 300 ft. One hundred percent knockdown and mortality was recorded for Pyrenone at all distances tested.

INTRODUCTION

Aventis Environmental Science is developing water-based formulations that reportedly may replace or augment the use of oil based materials as ultra low volume (ULV) adulticide treatments. Aqua-Permanone, a water based permethrin product, was originally assessed in the late 1980's. The material was reintroduced as Aqua-Reslin and a field evaluation of the material was presented at the 1997 Mosquito and Vector Control Association of California Annual Conference (Inman et al. 1997). The results were encouraging despite marginal weather conditions during the test. The Merced County Mosquito Abatement District (MAD) utilizes pyrethrin as its primary adulticide. Consequently, the District was keenly interested in evaluating the water based pyrethrin product Aqua-Pyrenone.

MATERIALS AND METHODS

Adult mosquitoes were collected the morning of the test with hand held insect vacuums from natural populations of *Ochlerotatus nigromaculis* in a recently irrigated pasture south of the Merced city limits. The mosquitoes were placed in polystyrene ice chests and transported back to the Merced County MAD facilities. They were anesthetized with CO_2 and transferred from collection tubes to screened adulticide test cages. The test cages were stored in the ice chests, covered with moist cotton padding, and held in the laboratory facility of the Merced MAD until treatment. The total number of mosquitoes in each test cage ranged from 9 to 35.

Testing was conducted on October 4, 2000, in a fallow parcel of property containing knee-high vegetation adjacent to the runway of the Merced County MAD. Aqua-Pyrenone was applied as a ULV ground treatment at 6:39 PM and Pyrenone was tested at 7:20 PM. Weather conditions were recorded with a Capricorn II weather station. Surface temperatures ranged from 70.5 to 74.6 degrees with northwest winds 3-4 mph. The weather station confirmed a temperature inversion of 1.5 to 2.0 degrees at 27-30 ft altitude during the applications.

Pyrenone was mixed with Crystal White Oil 90 at a ratio of 1: 0.94. Aqua-Pyrenone was diluted with water at a ratio of 1: 0.69. Two trucks equipped with LECO 800 cold aerosol sprayers were used to apply the materials. During the application, the trucks traveled in a south to north direction at a speed of 10 mph. Droplet size analysis and flow rate calibrations were completed prior to the treatments. Droplets were collected and measured utilizing the AIMS. Droplet sizes were 21.5 microns, mass median diameter (MMD), for Aqua-Pyrenone and 25.5 microns MMD for Pyrenone. Nozzle pressures were 5.0 psi for Aqua-Pyrenone and 6.0 psi for Pyrenone with flow rates of 9.0 fl. ounces per minute for both materials.

Three test plots 100 ft apart were used as replicates. The plot layout (3x3 design) consisted of three rows of metal stakes placed at 100, 200, and 300 feet perpendicular to the spray path of the truck. Caged mosquitoes were placed in the plots just before each treatment. The cages were collected 20 minutes after treatment and transported back to the laboratory. After the initial 1-hour post-treatment observation, the mosquitoes were anesthetized with CO_2 and transferred to clean ½ pint paper cans with screen lids. Raisins were wrapped in water-soaked cotton balls and placed on top of the screen lids. Untreated control cages for each treatment were handled in a similar fashion. Initial knockdown was observed at 1-hour while mortality was recorded at 12-hours post treatment. Data were corrected for control mortality by using Abbott's Formula. Means for each insecticide by distance were analyzed using ANOVA. Treatment means were separated by Sheffe's method of multiple comparisons.

RESULTS AND DISCUSSION

Average 1-hour knockdown and 12-hour mortality for Pyrenone and Aqua-Pyrenone treatments by distance from the spray vehicle are presented in Table I. No knockdown was observed 1-hour post-treatment in the untreated controls for both materials. Untreated control mortality at 12-hours was 7.4% for the Aqua-Pyrenone treatment and 13.6% for Pyrenone. Average 1-hour knockdown for Aqua-Pyrenone was 94.4% while Pyrenone knockdown was 100% at 1-hour post-treatment. Aqua-Pyrenone knockdown by distance ranged from 89% at 300 ft to 97% at 100 and 200 feet. Aqua-Pyrenone knockdown values were slightly lower than the corresponding mortality figures. No recovery was observed in any of the treatments. Mortality was greater than 99% for Aqua-Pyrenone at 100 ft and 200 ft with 96% mortality observed at 300 ft. One hundred percent mortality was recorded for Pyrenone at all three distances. A statistical analysis of the data indicated significant differences in treatment means by distance at 1-hour for Aqua-Pyrenone at 100 and 300 feet, and 200 and 300 feet. Additionally, a significant difference in treatment means between Aqua-Pyrenone and Pyrenone was observed 1-hour post-treatment at the 300 ft distance. Both materials provided impressive knockdown and mortality against *Ochlerotatus nigromaculis* adults.

Acknowledgements

The authors wish to thank Matt Stanich and Anton Cornel of UC Davis for providing a statistical analysis of the data and personnel of the Merced County Mosquito Abatement District for providing the necessary support to complete the evaluation.

Table I. Mean percent knockdown and 12 hr. mortality of caged *Ochlerotatus nigromaculis* treated with Aqua-Pyrenone and Pyrenone.

		Post-Treatment	
Distance	Insecticide	1-Hour	12 Hour
100 ft.	Aqua-Pyrenone	97.5%a	99.1%a
	Pyrenone	100%a	100%a
200 ft.	Aqua-Pyrenone	97.1%a	99.1%a
	Pyrenone	100%a	100%a
300 ft.	Aqua-Pyrenone	89.1%b	96.1%a
	Pyrenone	100%a	100%a
	Untreated (AP)	0.0%	7.4%
	Untreated (P)	0.0%	13.6%

Means by distance and treatment in the same column followed by a different lower case letter are significantly different ($P < 0.05$) by Scheffe's method of multiple comparisons.

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Use of Non-Attractant Traps to Determine Patterns of Mosquito Abundance in Habitats in the Coachella Valley, California[1]

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ABSTRACT: Four non-attractant trap designs including ramp, malaise, CDC-style [without dry ice or light], and suction were compared for their ability to collect *Culex tarsalis* Coquillett in habitats near the Salton Sea. CDC and suction traps collected significantly more females than ramp or malaise traps. In further trials, suction traps proved to be more effective than CDC traps in shoreline tamarisk and citrus. Suction traps then were used to compare *Cx. tarsalis* abundance in differing microhabitats at fish farm, desert scrub, vineyard, and duck club environments. Traps placed at vegetative ecotones collected significantly more mosquitoes than traps placed over low vegetation or within the canopy of elevated vegetation. At inland sites, collections contained mostly unfed females and few engorged and gravid females or males. Our data indicated that unfed females may congregate and disperse at the margins of elevated vegetation, possibly providing a visual target at which to focus adulticide applications.

INTRODUCTION

Previous studies (Lothrop and Reisen, 1998) using CO_2-baited CDC-style traps showed significant differences in the abundance of host-seeking mosquitoes among habitat types. These habitats represented most of the vegetative types found around the northern margin of the Salton Sea within the zone of greatest *Cx. tarsalis* abundance and Saint Louis encephalitis and western equine encephalomyelitis virus transmission. Although CO_2-baited traps were useful in defining differences in the abundance of host-seeking females among habitat types, a finer scale sampling method was necessary to determine differences in abundance within microhabitats. Non-attractant traps fulfill this requirement because they intercept flying mosquitoes within a discrete area. In addition, these traps should collect other portions of the population including blood fed and gravid females and males. Data from the current study are directly applicable to ground ultralow volume adulticide applications, because they indicate which microhabitats have the highest abundance of the target species.

This paper describes comparisons among four non-attractant trap types and evaluates *Cx. tarsalis* abundance in different vegetation types at six sites along the Salton Sea from April through November 2000.

MATERIALS AND METHODS

Four non-attractant trap types (ramp, malaise, CDC-style, and eight-inch suction) were constructed following previous design guidelines (Service 1976, Muirhead-Thomson1991). The Ramp trap was constructed of dark fiberglass window screen with aluminum screen frame, and was four directional, with a square base 1.22 m per side and 0.76 m height. The malaise trap was four paneled, 1.83 m in diameter and had openings 0.91 m high. The CDC-style trap was constructed of 8.9 cm to 8.3 cm (ID) PVC pipe reducer within which was mounted a four-vane 7.6 cm fan driven by a 6-volt Mabuchi motor. Suction traps were constructed of 20.3 cm (ID) class 100 PVC pipe 46 cm long, in which was mounted a 19 cm four blade fan driven by a 12 volt Bueler motor that moved 4.8 cubic meters per minute. Mosquitoes were drawn through an inverted wire screen funnel into a holding cage. This trap was compared running at 12 and 18 volts. Although there was an arithmetical increase in airflow, there was no significant increase in catch size, and we used 12 volts throughout.

Habitat types included shoreline tamarisk thickets, citrus orchards, fish farm ponds and levees, desert scrub, vineyard, and duck club. In all experiments, CO_2-baited CDC-style traps were operated concurrently as controls to measure regional abundance. Initial comparisons with the four trap types were conducted in early April in shoreline tamarisk thickets along

[1] Detailed manuscript has been submitted for publication to the Journal of Medical Entomology.

the Salton Sea. CDC and suction traps then were compared at shoreline tamarisk in mid May and a citrus orchard at the end of May. Subsequently, suction traps were used alone at shoreline tamarisk in early June, at citrus and desert scrub in mid June, at a fish farm and desert scrub in late June, at a vineyard in late October, and at a duck club in early November. In each experiment, 5 to 8 traps were set in 3 to 5 cross-sectional transects in each habitat and were operated for 2-3 consecutive nights. The final experiment at a duck club was an exception with 8 rows of 3 traps. Trap openings were set at different heights according to habitat. In desert scrub and low grass, traps were oriented both up and down to sample possible stratified mosquito movement.

Mosquito counts per trap were transformed by ln[y+1] and compared within experiments using multivariate analyses of variance [MANOVA]. Means were compared by least significant difference posteriori multiple range tests [a = 0.05] and presented as a percentage of the mean total of transects.

RESULTS AND DISCUSSION

Ramp (0.04/trapnight) and malaise (0.88/tn) traps collected few *Cx. tarsalis* females compared to CDC-style (2.88/tn) and suction (5.27/tn) traps. The lack of sensitivity, high cost, and logistical difficulties of transport and setup combined to eliminate these traps from our study. Further experiments comparing CDC-style and suction traps indicated that, although the former were more effective per area of trap opening, suction traps collected more mosquitoes per trap night. For this reason, we selected the suction trap to describe the distribution of *Cx. tarsalis* females among microhabitats.

Suction traps produced similar results within habitats among experiments. Data for each habitat are listed from highest to lowest within habitat as the percentage of sum total abundance for trap positions in the habitat.

Counts of *Cx. tarsalis* females were greatest at shoreline tamarisk (75%) along the ecotone with the Salton Sea, where the beach-vegetative interface was well defined. There were no significant differences in counts among traps set at the inland side (11.5%), under canopy (8%), and over the top of canopy (5.5%) of the tamarisk. The ragged and uneven density of the inland tamarisk/low desert scrub ecotone may have accounted for the lack of significant difference among this position and traps set inside and at the canopy top.

Citrus had significantly greatest abundance at the end of the row (56%) at the desert scrub–orchard interface. Traps set at the canopy side along the row (23%) and at the top (14%) were statistically similar; counts at traps inside the canopy (7%) were lowest.

Traps set in desert scrub had greatest counts at the interface of elevated vegetation at the edge of the habitat (59%). Counts at traps oriented to draw upward at 0.3 meters (7%) and downward at 1.5 m (5%) and 0.75 m (6%) in the desert were similar statistically. Traps set at 0.3 m at the base of elevated vegetation had the lowest counts (1.5%). No vertical stratification was found in mosquito abundance at the elevations sampled. Traps sampled mostly unfed females.

Traps were set along a transect from a fish farm into desert scrub in an attempt to detect spatial changes in the proportion of empty females at inland locations removed from breeding sites. Traps were situated between breeding areas associated with a freshwater marsh adjacent to fish farm ponds and the two previously studied inland sites. Traps were set between ponds, at the edge of a pond adjacent to desert scrub, in tamarisk at the edge of desert scrub, and in desert scrub. Significantly greater numbers of males and gravid females were collected at traps set nearest to breeding sites. The percentage for these two segments of the population was greater in these traps (13% male/9% gravid) compared to others (8% male/6% gravid) in this sample as well as the nearby desert sites (1%/1%). These data supported our assumption that suction traps were not biased toward empty females and that our traps representatively sampled populations present at the sites investigated.

Vineyard habitat showed results similar to citrus, with row end catch greatest (45%) followed by inside canopy (11%), canopy top (1%), canopy side 10 m along row (37%) and an additional sample between rows (6%). Catch size at traps set between rows was statistically similar to size at the top and inside canopy. It should be noted that abundance was quite low at this site and time of year (October) with an average catch <3.5 females per trap night at row end traps (CO_2 trap = 23) compared to over 100 per trap night at row end in citrus (CO_2 trap = 3824).

Duck club habitat was sampled to contrast abundance in tamarisk/arrowweed breaks with adjacent open Bermuda grass. Traps set at the tamarisk ecotone collected more females (75%) and males (92%) than over grass (25% and 8%, respectively) and proportionately more males (77% male/23% female) than over grass (48% male/52% female). These results may have been influenced by attraction to flowers on the tamarisk and arrowweed. It would be useful to test captured mosquitoes for sugar to determine if they are feeding on the flowers.

We concluded that *Cx. tarsalis* host-seeking abundance was greatest at traps set along the ecotone of elevated vegetation and that the sides rather than the top or interior of the canopy was frequented most often. In the Coachella Valley, male *Cx. tarsalis* do not venture far from larval sites and do not frequent the same vegetation as females, except for possible sugar feeding.

Acknowledgements

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Integrating Geographic Information Systems in Mosquito Control in Fresno

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ABSTRACT: The Mosquito Control Research Laboratory at the U.C. Kearney Agricultural Center in Parlier, CA, is adapting Geographic Information Systems (GIS) technology to mosquito research and control. The project design provides two distinct yet cooperative approaches to mosquito control: 1) a GIS-driven approach to mosquito abatement for the Fresno Mosquito and Vector Control District (FMVCD), and 2) a map-based platform to investigate the spatial dynamics of pesticide resistance in mosquitoes.

The goals for the FMVCD are to facilitate daily and weekly planning protocols by consolidating information now held in several paper and digital databases and archives into one GIS interface. Daily activities and data management will be automated, reducing time spent finding and checking mosquito-breeding sites. The district will have rapid retrieval of spatial and temporal archived data initiated by map-related requests such as where and when pesticides have been applied. Service calls will be located on parcel maps and routes designated before the technician leaves the office. Furthermore, which pesticide was used at each specific location and when it was applied enables research to identify the pattern of pesticide use in mosquito abatement. Understanding the geographic pattern will facilitate management procedures that can mitigate the spread of increased levels of resistance.

INTRODUCTION

Geographic Information System (GIS) technology and methodology are valuable tools to deal with some of the challenges facing vector control districts such as resistance to mosquitocides, paucity of development of new mosquitocides, increased public and federal awareness of environmental and non-target toxicological effects of mosquitocides, environmental preservation, and increased costs of vector control. GIS technology essentially provides tools for computer-mediated data management, and as technology advances, GIS can effectively take a leading role by providing a foundation for mosquito and vector research and control. Briefly described, GIS brings together data and their map locations so that patterns can be seen in the data (maps, legends, charts). With this connection made (map to data), field data are stored, retrieved, visualized, and ultimately analyzed within a spatial context. Data can be retrieved by spatial query for report generation, or structured into a spatial model to produce other maps to derive answers and make predictions.

Conceptually, the GIS, as designed for the FMVCD, will provide a spatial structure to plan day to day mosquito control activities such as inspection and chemical application, service call response, and state regulatory reports. Additionally, GIS will support investigation of the growing problem of insecticide resistance in target mosquito populations.

MATERIALS AND METHODS

The GIS for FMVCD is designed to produce map and database products that increase efficiency in day-to-day workflow, and provide a spatial framework for field research in insecticide resistance.

As the GIS is utilized, several functions of mosquito abatement will be facilitated:

a. weekly and daily planning protocols
b. record keeping
c. responding to service calls with address locations
d. locating and identifying mosquito breeding sources
e. submitting regulatory data to state agencies
f. managing technicians' daily activities.

Likewise, field research will be facilitated by the visualization provided by GIS maps that show:

a. location and distribution of mosquito breeding sites
b. spatial and temporal pattern of insecticide use

c. location of evidence of existing insecticide resistance
d. pattern of the spread of resistance
e. predictions of future spread.

The first year has been devoted to understanding the needs of FMVCD – the type of work that is done and how GIS could improve the workflow. Once the "work culture" of the district was understood and the needs of the technicians explained, the type of data (map layers and databases) needed to build the GIS was apparent. To produce the GIS several tasks needed to be preformed: assemble existing base maps, process (clip) these maps to section-sized (one square mile) maps, create a series of map layers of treatment sites, design a database foundation for data acquisition and storage, and eventually provide the map interface to the technician in the field with a hand-held computer. During the first year, the GIS was designed and existing base maps assembled and processed. Activity in the second year will be data (map layer) creation, database design and production of any field hardware. The third year will be testing and adjusting the system.

To provide the type of support outlined above, particular map layers are clearly needed. Working with various agencies in Fresno County and the State of California, the essential base map layers were acquired. The Township, Range and Section map data were acquired from the California Teale Data Center. A county parcel map, street map, and point address file, were provided by the County of Fresno. Swimming pools were mapped onto the parcel layer with information supplied by County Assessor's office. And the Fresno Metropolitan Flood Control District, kindly provided the basin and canal location data. Because the FMVCD works in rural as well as urban settings, rural land use maps are needed. Initially, California State Department of Water Resources (DWR) land use maps were acquired. These will be replaced with Fresno County land use data as they becomes available in April 2001.

The GIS is being assembled and processed using ArcView software developed by Environmental Systems Research Institute (ESRI), Redlands CA. ArcInfo (ESRI) software is used for large data editing tasks. Hardware consists of a Gateway Pentium III 800 MHz Dual Processor and a Xi Athlon 800 MHz computer.

RESULTS AND DISCUSSION

As is usual with maps acquired from several state and county agencies there were problems with mis-registration between the maps because they have been constructed using different projections. For example the Department of Water Resources uses CA 105 projection, while the County of Fresno uses State Plane Zone 4 projection and the Teale Data Center uses Teale Albers projection. To implement a GIS, map layers must coincide in geographic extent as nearly perfect as possible, so the maps needed to be re-projected. Of the three projections, the State Plane Zone 4 was chosen for its relative accuracy to the local area. The Projection! Utility extension in ArcView served to accomplish this task. All the data sets were originally in NAD27 (datum) so the translation to a different projection worked smoothly in ArcView.

The results of the spatial data assemblage accomplished thus far can be viewed in Figures 1 through 4. For several years, the FMVCD has used section, township and range information to locate and reference fieldwork. When this GIS project began technicians used street maps created in Generic CADD software, and printed out to section-scaled maps. The maps were outdated, and showed no parcel detail. To stay within this approach, Teale Public Land Survey System (PLSS) data are used as the base reference to retrieve urban (Fresno County Parcel maps) and rural (DWR land use maps). Figure 1 demonstrates the connection between the Township, Range and Section reference map and the base maps.

A streamlined user interface is being developed for easy access to section maps (Fig. 2). Field technicians can access the day's work by using the Hotlink tool in ArcView to call up the section map and quickly print it. As the map is accessed, so are the databases attached to it which hold pertinent information (i.e. type of pest, insecticide, etc.) necessary for planning their daily activities. Before leaving the facility the technicians will have a good sense of which sites to visit including those that need to be re-inspected, and those that were irrigated three days previously (based on watering days allocations from the irrigation district). Because the database is accessed at the same time as the map, the technician also has information about recent insecticide use for the purpose of insecticide rotations.

Figure 3 demonstrates several aspects of the project. Initially, individual section-scaled parcel maps were printed for the district. During winter 2000-01, technicians used these maps to field check and record the location of sumps, storm drains and gutters. This information is currently being digitized at U.C. Kearney Ag Center's GIS facility using ArcView software. The database for each layer is created in ArcView and some field editing is done in Microsoft Excel. Each week the layers are merged for analysis.

With these layers in place, spatial queries of the databases can be made. One of the most important uses of the GIS will be service call response. As shown in Fig. 3, addresses can be located on the map also calling up the generalized area. Further, treatment sites (sumps, gutters, storm drains) and swimming pools within a specified distance can be located as well. Any relevant information about the sites is instantly retrieved. This function of the GIS is viewed as invaluable to the field technicians because of the time it will save.

One vital piece of the puzzle that is missing is the distribution of above-ground swimming pools. Swimming

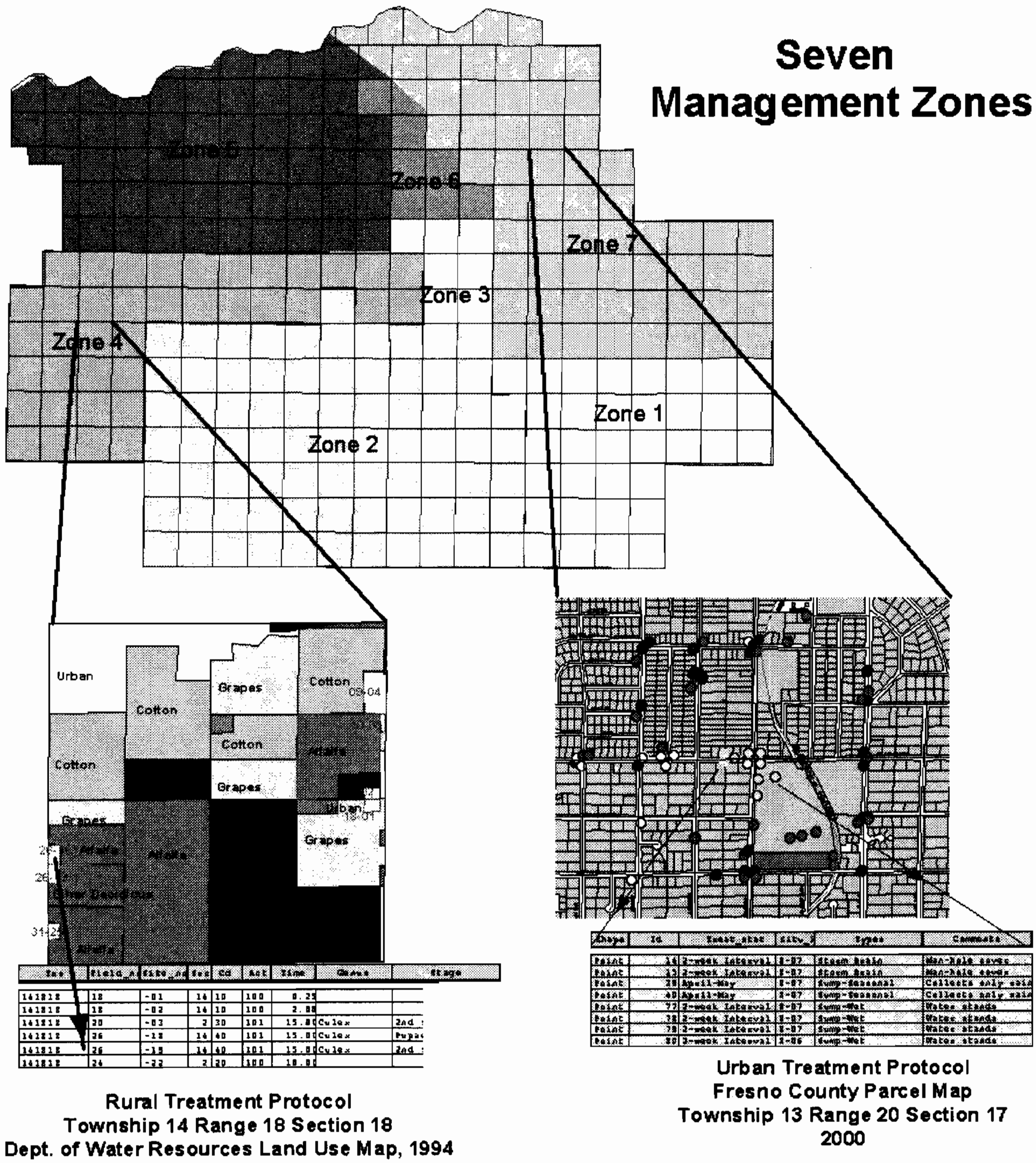

Figure 1. Land use and parcel map retrieval from Township, Range and Section reference layer

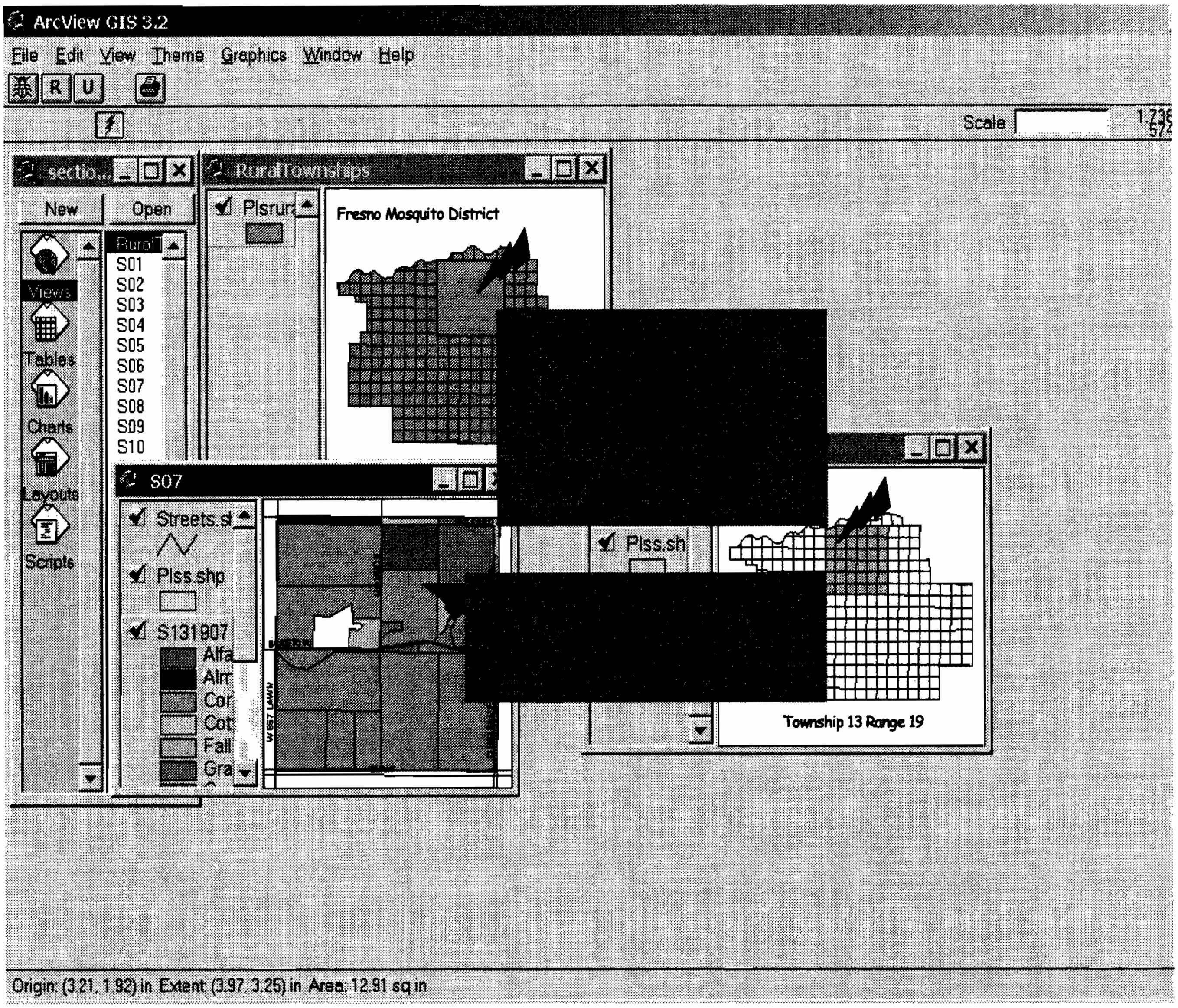

Figure 2. Customized graphical user's interface. Section maps are retrieved and printed by using Hotlink tool.

pools that are neglected, particularly common in houses in escrow, can be a major source of *Culex* mosquitoes. Using county pool permits tied to Assessor Parcel Numbers (parcels map) in-ground pools can be mapped. However, above-ground pools do not require a permit. Locating these pools requires more effort. One method to detect these pools is to use 1-meter aerial photos to detect the pools. The FMVCD purchased August 2000 air photos at 1-meter resolution from AirPhotoUSA, to investigate this method.

Pesticide usage data are available from County Agricultural Commissioners' offices (at the resolution of the field) and from the California Environment Protection Agency (at resolution of township, range, sections). While there is a lag period for data entry, reasonably recent pesticide usage can be visualized spatially by integrating the pesticide databases with maps using GIS as depicted in Fig. 4. Maps can be created that facilitate more rapid visualization of the amount of insecticide usage and can serve as important platforms for insecticide resistance studies and management. As an example, in Fig. 4 a map depicting the overall use of organophosphorus chemicals for 1999 in Fresno County is provided. This map illustrates the extensive and widespread use of organophosphates in Fresno County. The data were queried to depict malathion usage and the hotspots (circled)

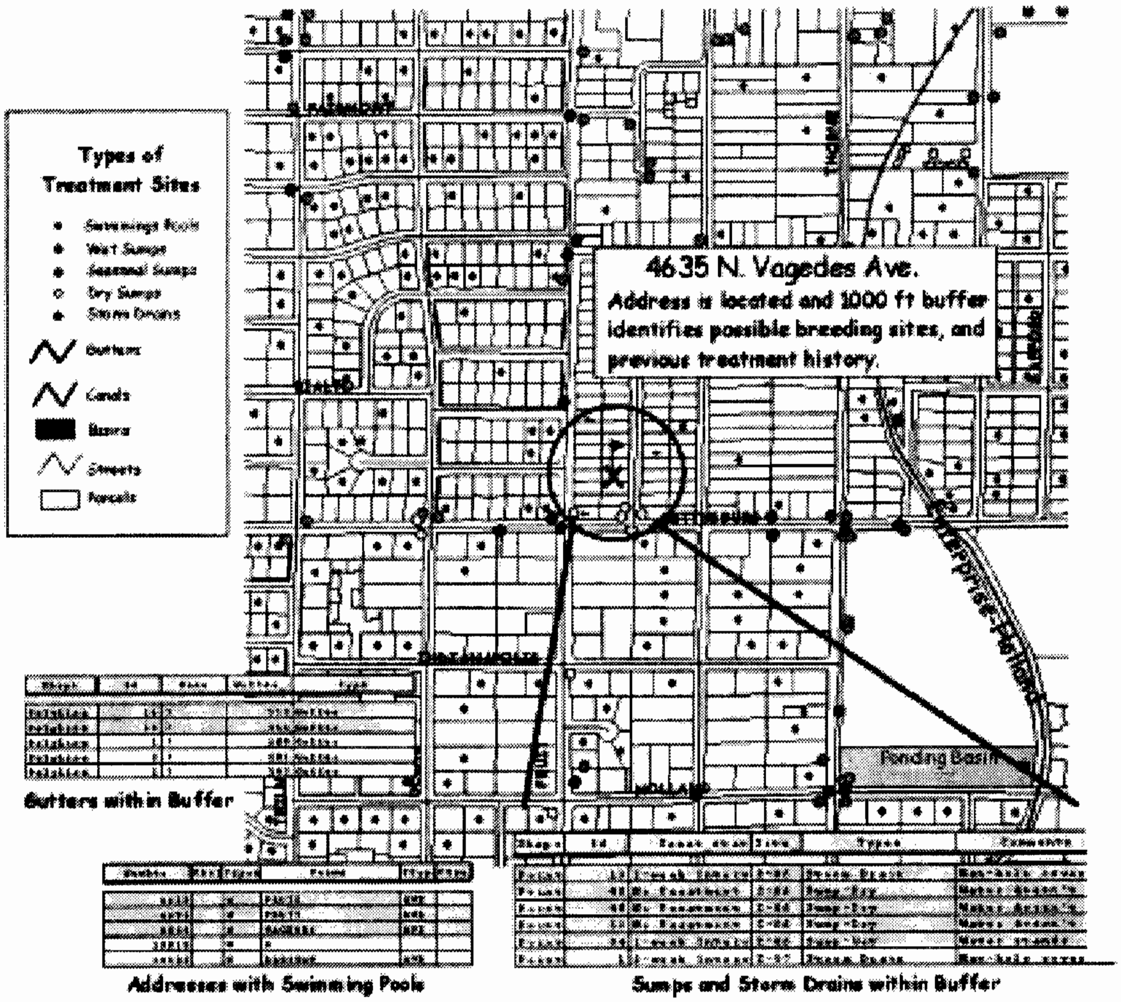

Figure 3. Service response using point address map layer and automatic buffer for database information.

can immediately be recognized. If the likelihood of resistance to malathion were to be investigated in mosquitoes then hotspots would represent the best locations to collect mosquitoes to test. The maps would inform us as to where resistance would likely arise and spread. Using GIS tools, associations between insecticide resistance gene frequencies and insecticide usage can be made. Recognition of overall insecticide usage for agricultural purposes also allows mosquito control operators to make wise choices about which chemicals to use that do not cross-resist with chemicals that are heavily used in that area for mosquito and other pest control.

The concept of using GIS to manage mosquito surveillance and control is not new and many mosquito districts are presently applying GIS technologies (Spradling et al. 1998, Lothrop and Reisen 1999). However the time is ripe for more districts to become familiar with what GIS has to offer as a method of management for mosquito control operations to make it more efficient and cost effective. With the ever-increasing threat of insecticide resistance and introduction of medically important viruses, more sophisticated and directed mosquito control is required. Mosquito districts can ill afford to lag behind especially as the demands made by the general public to live in a safe and pest free environments increase.

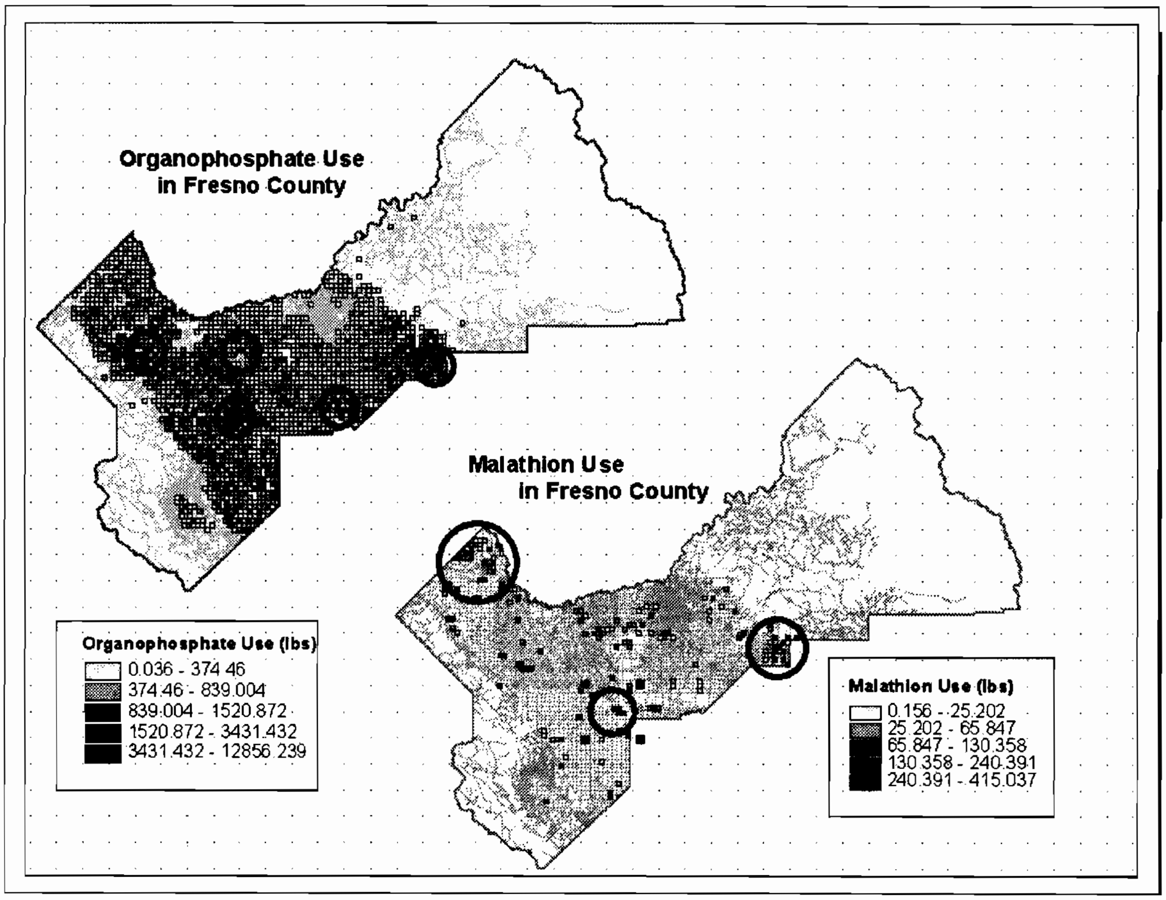

Figure 4. Pesticide use mapped with information from Department of Pesticide Regulation.

Acknowledgements

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Biology of the Endangered San Francisco Garter Snake (*Thamnophis sirtalis tetrataenia*) and its Relationship to Mosquito Control at a Site in San Mateo County, California

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ABSTRACT: The largest remaining population of San Francisco garter snakes (*Thamnophis sirtalis tetrataenia* Cope in Yarrow) occurs on a remnant of undeveloped land owned by the San Francisco International Airport in a highly urbanized section of San Mateo County. This parcel (Mills Field) contains a number of mosquito breeding sites and is one of the primary sources of Western Encephalitis mosquitoes (*Culex tarsalis* Coquillett) in the county. Recently, construction of a light rail line through the center of Mills Field brought the site to the attention of the US Fish and Wildlife Service and the general public. This paper will review the biology of the San Francisco garter snake in relation to mosquito control at Mills Field.

The San Mateo County Mosquito Abatement District (SMCMAD) occupies 163 square miles of urban area just south of San Francisco. The District encompasses most of the land in San Mateo County between the Santa Cruz Mountains and San Francisco Bay. At one time, this area contained vast expanses of tidal saltmarsh, riparian corridors and seasonal marshes. Most of these habitats have disappeared within District boundaries due to urban expansion. Loss of wetlands has contributed significantly to the decline of many species in California and San Mateo County is no exception. At least 6 species of vertebrates once common to wetlands on the Peninsula are now listed by the US Fish and Wildlife Service (USFW) as endangered or threatened: the California clapper rail (*Rallus longirostris obsoletus* Oberholser), California least tern (*Sterna antillarum browni* Mearns), salt marsh harvest mouse (*Reithrodontomys flaviventris* Dixon), red-legged frog (*Rana aurora draytonii* (Baird and Girard)), and southern steelhead trout (*Onchorhynchus mykiss irideus* Behnke). The San Francisco garter snake (*Thamnophis sirtalis tetrataenia* Cope in Yarrow) (SFGS) was declared an endangered species in 1967. It was one of the first species listed for federal protection under the Endangered Species Act of 1973 (US Fish and Wildlife Service 1985). This species is endemic to the San Francisco Peninsula and was once common in fresh water marshes throughout the county (Figure 1) (Fox 1951). Factors contributing to its decline include overcollection, saltwater intrusion, predation and competition by introduced species such as bullfrogs (*Rana catesbeiana* Shaw) (US Fish and Wildlife Service 1985, Wharton 1989).

Habitat loss is the primary factor responsible for the decline of the SFGS. Of the 28 populations surveyed in 1978, only a few were considered viable (Barry 1978). In 1984, 16 potential sites were surveyed for SFGS. Snakes were found at only 8 of these sites (McGinnis 1984). The most vigorous population of SFGS was eliminated in the 1960's when a housing development was built over a site known as Skyline Ponds (Figure 1) (Barry 1978). This site contained a series of sag ponds, small seasonal ponds formed by subsidence along the San Andreas Fault. Skyline Ponds supported a dense population of SFGS and was well known among local reptile fanciers and collectors. Population densities here remained high despite the fact that collectors had removed hundreds of SFGS (Barry 1978, Jennings 1991). A population of SFGS at Laguna Salada in Pacifica disappeared after saltwater intrusion made the site unsuitable for the snakes and their amphibian prey species (McGinnis 1984). Crystal Springs and San Andreas Lakes in the San Francisco Watershed support small populations of SFGS but fluctuating water levels and periodic clearing of vegetation keep their numbers well below carrying capacity (Barry 1978). Small colonies still exist in marshes at Año Nuevo State Park and Pescadero Creek Preserve in the southwest corner of the county (Figure 1), but their numbers are low (<50 snakes) (McGinnis 1984, US Fish and Wildlife Service 1985).

The snakes at Mills Field constitute the largest and healthiest population of SFGS remaining (200 to 500 individuals) (US Fish and Wildlife Service 1985, Wharton 1989). Sandwiched between Highway 101 and the Southern Pacific Railroad, Mills Field is one of the last remaining freshwater wetlands in the District (Figure 2). It has a number of features that make it ideal habitat for SFGS, including the presence of both permanent and seasonal wetlands, availability of multiple prey species and absence of competing species of garter snakes (McGinnis 1984). Mills Field is the remnant of a large marsh that once extended along the shore of San Francisco Bay from Hunters Point to Coyote Point (Goals

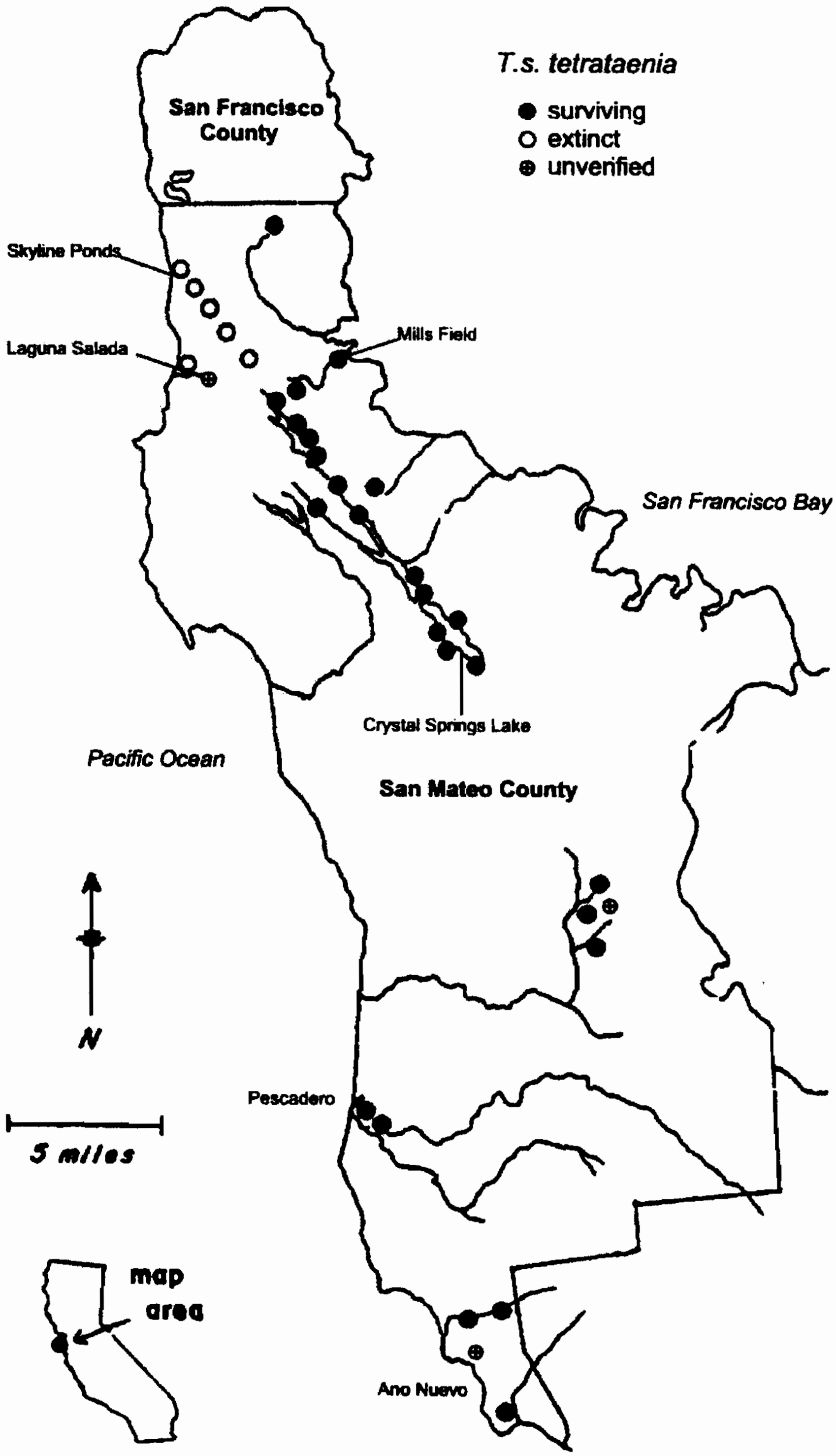

Figure 1. Occurrence of *Thamnophis sirtalis tetrataenia* on the San Francisco Peninsula. Figure adapted from Barry (1978).

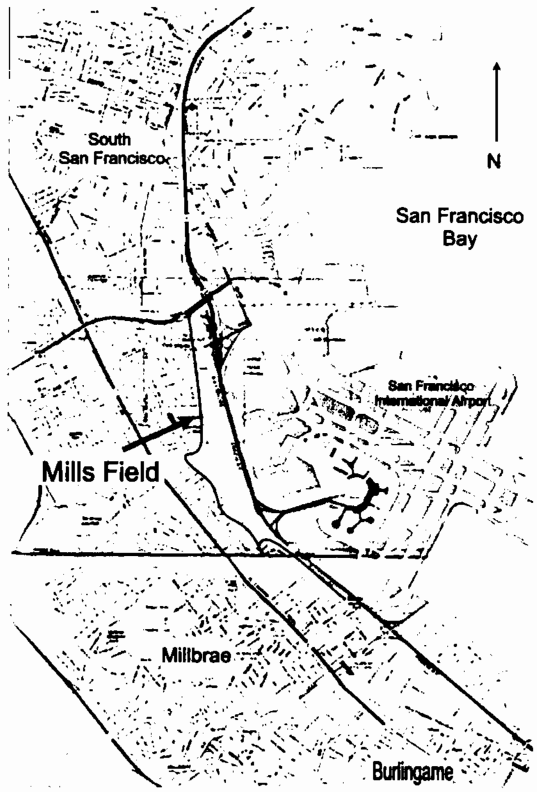

Figure 2. Location of Mills Field in San Mateo County California.

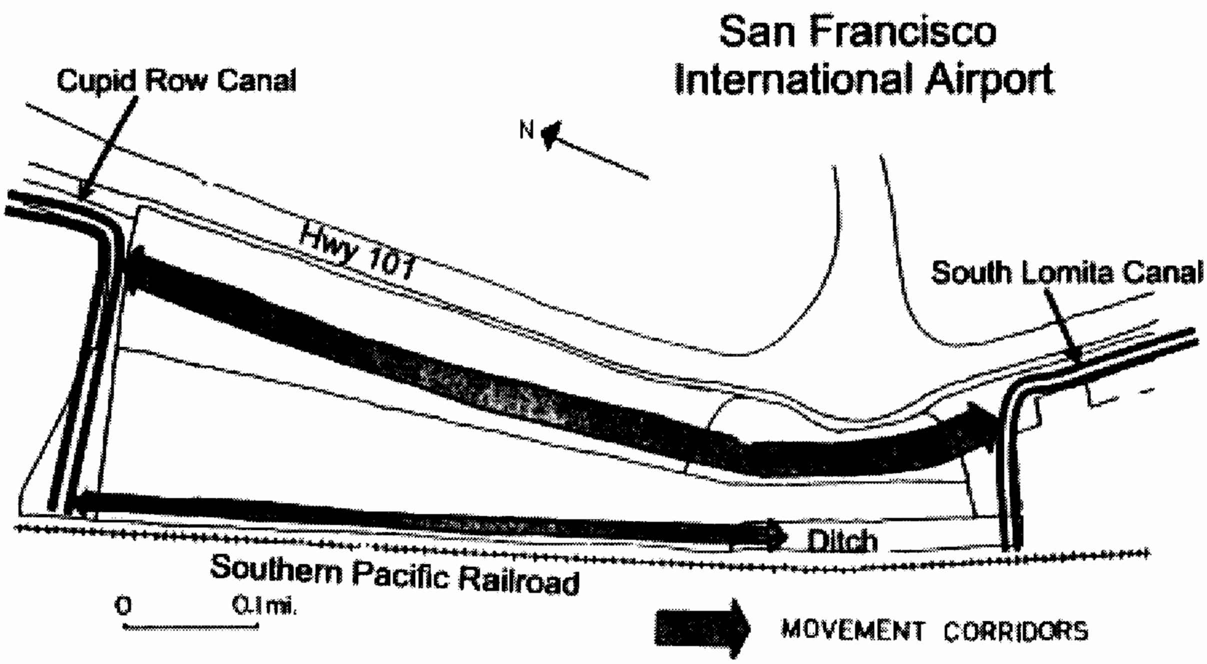

Figure 3. Landscape features of Mills Field and seasonal migration corridors for San Francisco Garter Snakes (*Thamnophis sirtalis tetrataenia*) (from Wharton, 1989).

Project 1999). Much of this land (the Mills Estate) was diked and drained for agriculture during the late 1800's to early 1900's. The San Francisco International Airport now occupies a large portion of this land. Mills Field itself is bordered by 2 canals that were installed for drainage between 1949 and 1956 (Figure 3). These canals empty into San Francisco Bay through tide gates located near the airport. The canals receive runoff from the surrounding suburban stormwater drainage systems and retain water year-round. Red-legged frogs inhabit the canals and are an important food source for SFGS during the summer and fall (Wharton 1989). In winter months, the snakes retreat to upland sites along the banks of the canals to hibernate in rodent burrows (Wharton 1989). Mating occurs in fall or spring and appears concentrated in the first few warm days of March, when the snakes emerge from their hibernacula (US Fish and Wildlife Service 1985).

Later in March, the SFGS migrate to the seasonal marshes covering much of the interior of Mills Field (Wharton 1989). These marshes are breeding grounds for Pacific tree frogs (*Hyla regilla* Baird and Girard), which provide an abundant food resource for gravid and newborn snakes (Fox 1951, Wharton 1989, McGinnis and Larsen 1991). San Francisco garter snakes are livebearers, giving birth to 12 – 24 young in May-June (US Fish and Wildlife Service 1985). Young snakes have difficulty capturing prey in deep water. They are most successful in shallow, clear water that is relatively free of vegetation (McGinnis et al. 1987, McGinnis and Larsen 1991, Larsen et al. 1991, Larsen 1994). The seasonal marshes of Mills Field with their high densities of tree frog adults and tadpoles are ideal hunting grounds for young snakes.

In June or July, the seasonal marshes of Mills Field become dry and Pacific tree frogs begin to estivate (Wharton 1989, McGinnis 1984). San Francisco garter snakes return to the peripheral canals at this time, where they feed on the tadpoles of red-legged frogs (Wharton 1989). The tadpoles of red-legged frogs develop more slowly (3.5 – 7 months to metamorphosis) than those of tree frogs (1.5 - 2.5 months). In most years red-legged frogs cannot complete metamorphosis before the water in the seasonal marshes disappears (US Fish and Wildlife Service 1985, Wharton 1989, Wright and Wright 1995). For this reason, red-legged frogs rarely occur in the interior seasonal marshes of Mills Field.

Small fish serve as a backup food source for the snakes during their residence along the canals (Wharton 1989). Sticklebacks (*Gasterosteus aculeatus* L.) are regularly consumed by SFGS but are somewhat of a hazard to the snakes. There are several reports in the literature of snakes found with their jaws impaled on the spines of sticklebacks (Wharton 1989, Jennings 1991). Mosquitofish (*Gambusia affinis* Baird and Girard) are also present in the canals and are an important part of the snake's diet. The origin of the mosquitofish in Mills Field is uncertain. The District does not currently stock fish in natural sources and hasn't for at least 35 years. These fish may have been released by local residents or stocked during the early days of the District.

San Francisco garter snakes at Mills Field travel between the canals and the seasonal marshes along specific "movement corridors" (Figure 3) (Wharton 1989). One of these is a low area filled with cattails along the eastern border of the property, parallel to Highway 101. The second corridor is a ditch parallel to the railroad tracks along the western boundary of the property that empties into the South Lomita canal (Figure 3). This ditch lies under a grove of eucalyptus trees and holds water throughout most of the summer. This area is a significant mosquito-breeding source and is monitored and treated regularly by District personnel. It is also potentially impacted by construction activities for the light rail line since it is located adjacent to the haul road and material staging area.

The wetlands of Mills Field are also a major breeding source for mosquitoes and have been the focus of SMCMAD control programs for many years. The seasonal marshes in the interior of the field produce large numbers of *Ochlerotatus washinoi* Lanzaro and Eldridge in early spring followed by *Culiseta inornata* Williston, and *Cx. tarsalis* as temperatures rise later in the year. The ditch along the railroad tracks produces mosquitoes throughout the summer. The high organic content of this ditch provides ideal conditions for larvae of *Cx. pipiens* L. and *Cx. stigmatosoma* Dyar. If left unchecked, mosquitoes from Mills Field generate numerous service requests from residents of neighborhoods adjacent to the parcel (Figure 3). These mosquitoes also pose a potential disease threat to the surrounding community. Mills Field is one of the largest sources of *Cx. tarsalis* in the SMCMAD. The district maintains a light trap and a flock of sentinel chickens at a 4H Club just north of the property, to monitor for encephalitis viruses in these mosquitoes.

The public health significance of mosquito breeding in Mills Field is further increased by its proximity to the San Francisco International Airport (Figure 3). In 1979, a 4th instar *Aedes aegypti* Skuse larva was found in a site adjacent to the airport (Jewell and Grodhaus 1984). A company near the airport that imported aircraft tires is believed to have been the source of this introduction (Jewell and Grodhaus 1984, Jewell and Schoeppner 1987). Several months of intensive surveillance failed to turn up any additional larvae or adults, and the tire company is now closed. However, the marshes of Mills Field provide an ideal establishment site, should an exotic vector species be introduced at the airport again.

Mosquito control at Mills Field has focused primarily on the seasonal impounds in the center of the property and the ditch along its western border. These impounds are monitored weekly from the beginning of the rainy season (November through May) until the water dissipates in early to mid- summer. Technicians continue to visit the area on a monthly basis for the remainder of the year. From the early 1970's through 1998, technicians relied primarily on application of larvicidal oils (Flit Larviciding Oil®, Golden Bear 1356® and GB1111®) and treated only the seasonal impounds on the interior. Teknar® (*Bacillus thuringiensis israelensis*) was applied on several occasions during the 1980's. In 1982, the District conducted a trial of Altosid® sand in the seasonal impounds. In 1995, the southern end of the field was treated with Altosid® pellets prior to commencement of the winter rains. The two drainage canals and the cattail marsh bordering Highway 101 have not historically bred mosquitoes, and have never been treated. However, the ditch along the western boundary retains water throughout most of the summer, and is treated with oil or with Bti and Altosid® (duplex) on a regular basis.

Although district technicians have monitored mosquitoes in Mills Field for years, none of the district employees has ever seen a San Francisco garter snake. This may be due to the reclusive nature of the snakes (US Fish and Wildlife Service 1985, McGinnis 1984). Unlike other species of garter snakes, SFGS generally stay within a body length of dense cover (McGinnis 1984). The SMCMAD has been aware of the presence of snakes at this site since the 1980's. However, little was known about their biology, or utilization of habitats at the site.

Mills Field began attracting the attention of the USFW and local residents in 1991 when the Bay Area Rapid Transit District proposed it as part of the route for an extension to San Francisco Airport. Trains would follow a route along the Southern Pacific Railroad's right-of-way and cross the center of Mills Field to reach the airport (Figure 1). The proposal also included the possibility of constructing a station and parking lot in Mills Field. An Environmental Impact Report (EIR) was prepared for the project and the transit district held numerous meetings with the USFW and members of the general public. Results of biological surveys conducted in 1989-90 suggested that populations of SFGS and red-legged frogs in Mills Field had decreased dramatically (McGinnis and Larsen 1991, McGinnis 1992). Only 79 snakes were captured in the area surveyed in 1990, while a survey conducted at the same site in 1983-85 detected 375 individuals (Wharton 1989, McGinnis and Larsen 1991, Larsen 1994). Several factors were thought to have contributed to the decline. Rainfall during the previous 5 years had been far below average and the seasonal marshes had dried out by late April in 1990 (McGinnis and Larsen 1991). In 1983-85, these marshes retained water until late June (McGinnis and Larsen 1991). The shortened season had drastically reduced populations of Pacific tree frogs. Many of the tadpoles in the seasonal marshes were unable to complete metamorphosis before the water disappeared (McGinnis and Larsen 1991, McGinnis 1992, Larsen 1994). Human traffic in Mills Field had increased dramatically (McGinnis and Larsen 1991, McGinnis 1992, Larsen 1994). Census traps placed during the 1990 study were regularly stolen or vandalized, and trespassers had constructed a racetrack for dirt bikes within the field (McGinnis and Larsen 1991). The snakes were also impacted by a malfunction of the tide gate on the northern canal (Larsen 1994). The resulting saltwater intrusion eliminated red-legged frogs and SFGS from this canal (Larsen 1994). To further complicate matters, bullfrogs had invaded the southern canal. Within a year of their introduction, bullfrogs had spread along the entire length of the southern canal and the density of red-legged frogs had declined. SFGS do not eat bullfrogs or their tadpoles; therefore introduction of bullfrogs would also have a significant impact on the snakes (McGinnis 1984). The decline of 2 endangered species at this site was alarming and highlighted the need for protection of this site.

Construction of the light rail system was allowed to proceed, but a number of measures were required to minimize the impact of construction on endangered species in Mills Field. The train station proposed for Mills Field was moved to a site outside the property. All construction areas within Mills Field were surrounded by snake exclusion fences (Figure 4). Extensive trapping was carried out on the interior of these fences. Snakes collected here were placed outside the fenced area or used in a captive breeding program. Most construction activities, staging of materials and movement of heavy equipment was restricted to a corridor along the western edge of the field. Unfortunately, this is also an important movement corridor for the snakes (Figure 3). Construction that crossed the seasonal wetlands in the center of the field was done from raised platforms to avoid compacting the soil (Figure 5). Biological monitors were hired to observe all construction activities and all personnel entering Mills Field were required to receive training on the biology of these snakes. Vehicular traffic within the property was closely monitored. Excessive speed by more than 2 trucks within a single week would cause the entire operation to be shut down for 2 days. The discovery of a dead snake would halt all construction for 1 week and a fine of $30,000 would be imposed.

All of this controversy focused increased attention on the District's own activities in Mills Field. In 1999, biological monitors found a dead snake on the property. Several agencies were questioned but the cause remains unknown. This incident led to stricter regulations for access to the property, and a requirement personnel from all agencies to receive training and certification on the snake's habits. Materials used by the district for mosquito control are currently under review by USFW. The USFW and the property owner (San Francisco Airport) are preparing a management plan for the protection of endangered species on the property. The airport has discussed monitoring district activities in order to detect correlations with changes in snake or frog populations.

CONCLUSION

Mills Field is a significant breeding area for local and introduced mosquitoes of public health importance. It is also one of the last freshwater wetlands left on the Peninsula, inhabited by at least 2 species whose existence is threatened by loss of habitat. The SMCMAD has conducted mosquito control at this site for over 50 years with no impact on snake populations. However, the precarious status of the SFGS at this site has brought all activities there under intense scrutiny. While the presence of endangered species has not affected mosquito control at this site in the past, we expect our activities to be more closely monitored in the future. The district is responsive to the environmentally sensitive nature of the site and has been gathering information on the biology of the snakes. We are working to educate Airport and USFW personnel about the importance of mosquitoes at this site and the nature of our control program there.

Acknowledgements

Assistance was generously provided by personnel from the San Francisco International Airport and The Bay Area Rapid Transit District in collecting background information on the biology of endangered species in Mills Field. Most of the research on San Francisco garter snakes presented in this report was conducted by faculty and students from the California State University at Hayward (Samuel McGinnis, Sheila Larsen, J.C. Wharton and others).

Figure 4. Snake exclusion fencing around construction site at Mills Field.

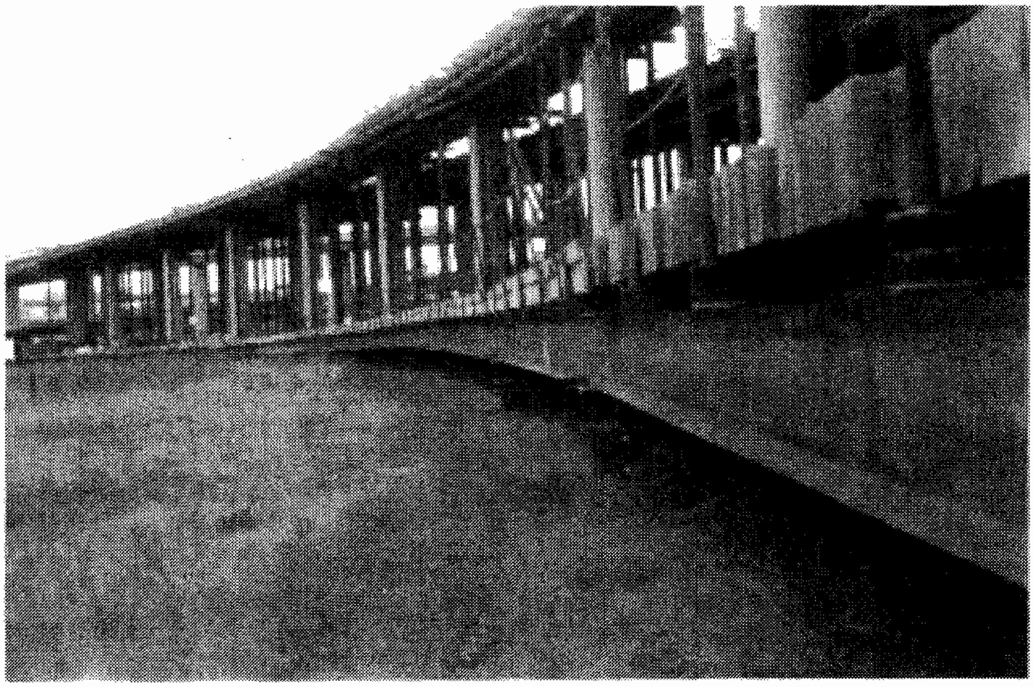

Figure 5. Elevated construction platform crossing seasonal wetlands at Mills Field.

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William C. Reeves New Investigator Award

The William C. Reeves New Investigator Award is given annually by the Mosquito and Vector Control Association of California in honor of the long and productive scientific career of Dr. William C. Reeves, Professor Emeritus, School of Public Health, University of California at Berkeley.

The Award is presented to the outstanding research paper delivered by a new investigator based on quality of the study, the written report, and presentation at the annual conference.

Christopher Barker was the recipient of the 2001 award at the 69th Annual Conference held in Napa, California. The other finalist was Valkyrie P. Kimball.

Previous William C. Reeves New Investigator Award Winners:

2000 - Jason Rasgon
1999 - Parker D. Workman
1998 - Yvonne Ann Offill
1997 - John Gimnig
1996 - None
1995 - Margaret C. Wirth
1994 - Merry L. Holliday-Hanson
1993 - Jeffrey W. Beehler
1992 - Darold P. Batzer
1991 - David R. Mercer
1990 - Gary N. Fritz
1989 - Truls Jensen
1988 - Vicki L. Kramer

Evaluation of Species Diversity on Two Artificially Created Vernal Pools in Sonoma County, California

Valkyrie P. Kimball

Biology Department, Sonoma State University, Rohnert Park, California

ABSTRACT: Vernal pool ecosystems, once commonly found in many landscapes throughout California, are becoming endangered habitats due to increased urban and suburban development. However, little information is available regarding the dynamics of created vernal pools and their contribution to species biodiversity. This study focuses on this issue of species diversity, particularly aquatic invertebrate diversity, and the relative abundance of such animals as they occur in two mitigated wetland properties in Sonoma County. Two study sites located within five miles of each other on the Santa Rosa Plain were compared using physical measurements (pool size, dissolved oxygen, pH, water depth, water temperature, etc.) as well as invertebrate counts and classification. Species diversity in a total of four pools at each site was evaluated using the Shannon Index. Likewise, statistical analysis using a grouped means one-way ANOVA was applied to compare any significant ecological differences between the two sites studied. It was concluded that there were no significant differences in water depth, water temperature, pH, or dissolved oxygen between the Alba Lane and Gobbi Ranch sites (grouped means one-way ANOVA $p>0.05$). When comparing the invertebrates collected, 1½ times more individual invertebrates were noted at the Alba Lane site than the Gobbi Ranch site yet species richness was equal at both sites (n=16). Species diversity was slightly higher at the Gobbi ranch site than the Alba Lane site according to the Shannon indexes (2.01 versus 1.82 respectively).

INTRODUCTION

Vernal pool ecosystems are unique ephemeral habitats for animal and plant communities. Many plants and several animals that inhabit vernal pools in California are listed as threatened or endangered. In Sonoma County two species of animals and thirteen species of plants are listed as threatened (CH2M Hill, 1995). Organisms that are able to reproduce efficiently and quickly in these pools have adapted cyst, egg and seed production enabling them to tolerate the dry summer months (California Biodiversity News, 1996). These adaptations make the survival of these organisms all the more interesting from both an evolutionary and ecological view.

California vernal pool habitat loss has been estimated to range from 50 to 90 percent since Europeans settled in California (California Biodiversity News, 1996). This loss of habitat has increased substantially in recent years as urban and suburban development increased. Seasonal and year-round wetlands are being placed into mitigation banks in an effort to re-create an environment for the plant and animal species that inhabit these unique environments. It is hoped that these mitigation wetlands can mirror the natural conditions that have been lost for both the macro- and microbiological populations (Zedler, 1987).

Biological abundance and richness are important components of biodiversity in a biological system. Healthy ecosystems should have adequate numbers of individuals in each species that will allow for self-perpetuating populations. The questions in this study are (1) whether or not artificial vernal pools simulate natural pools in their aquatic animal species diversity, and (2) do the 2 sites vary in their species composition. It is hoped that this knowledge can be applied toward current issues regarding mosquito and vector control and vernal pool development and management.

Methods

Two sites were chosen for collection and identification of the aquatic invertebrates that inhabit vernal pools. These areas were chosen for the following reasons:

1. created within the same year (1997),
2. developed by the same contractor,
3. exist within a few miles of each other on the Santa Rosa Plain,
4. developed on land that was previously grazed by cattle and considered pasture land,
5. exist at approximately the same elevation,
6. contain about 20 pools of varying sizes,
7. ease of access to both sites.

Sampling procedures:

Four pools of approximately the same size at each site were sampled randomly using an aquatic net. Sampling occurred in the center of the pool as well as along the edges to collect organisms that may inhabit both areas of the pools. A twenty-foot swath at the water's surface was utilized and the net rinsed into a 15 liter bucket with about 1 liter of water from the pool being sampled. Benthic sampling was not done. Each pool was sampled twice within a 10 day period at approximately noon. At that time, GPS location, perimeter measurements, air and water temperature, pool depth, pH and dissolved oxygen measurements were taken at each pool as well as a general description of weather (Tables 1a and 1b). Pools with 150-180 meter perimeters were chosen to be a part of the study.

Once collected, each sample was placed in a plastic 1 liter container and refrigerated for 1-3 days until identification of the invertebrate organisms could be performed. All organisms were individually counted and classified such as ostracod, cladoceran, mayfly, etc. The samples from each site were grouped collectively and the Shannon index (Molles, 1999) was calculated for both sites. Additionally, an analysis of variance was evaluated when comparing significant physical differences between the pools at each study site.

RESULTS

Environmental conditions:

The water level decreased from approximately 5-13 centimeters in all pools during the study period except Alba 3, which remained at a depth of 18 centimeters (Tables 1a and 1b). The Alba pools average loss was 6 centimeters, while the pools at the Gobbi site averaged a loss of 15 centimeters (Tables 1a and 1b). As this occurred, dissolved oxygen averages increased at the Alba Lane site from 8.90 mg/L to 9.60 mg/L. Conversely, the pools at Gobbi Ranch averaged a decrease in dissolved oxygen content from 11.53 mg/L to 10.47 mg/L. Pools #4 at both sites dried up prior to sampling on May 4 and May 5 (Table 1b). The average pH at the Gobbi Ranch became much more acidic, from 7.8 to 4.5 yet the Alba Lane pools average pH remained virtually unchanged (6.7 to 6.6). When comparing physicochemical factors of the pools, there were no significant differences in water depth, water temperature, pH, and dissolved oxygen content between the Alba Lane site and the Gobbi Ranch site (grouped means one-way ANOVA $p>0.05$).

Invertebrates collected:

The most abundant invertebrates collected were copepods (n=742), ostracods (n=514) and cladocerans (n=817) (Tables 2a and 2b). Dipterans, water boatmen and backswimmers were prevalent at the Gobbi Ranch site (total of all three n=202) while snails were prevalent at the Alba Lane site (n=157).

Species diversity:

At the Gobbi Ranch site, 1,093 individual invertebrates were counted and classified into 16 different general orders. The Shannon index calculated for these pools was 2.01 (Table 2a). For the Alba Lane pools, 1,747 individual animals were counted and identified as 16 separate species with a Shannon Index of 1.82 (Table 2b).

Table 1a. April 24 and 25 pool measurements

Pool	*GPS location*	*Pool size (perimeter in m)*	*Air Temperature (C)*	*Weather*	*Water Depth (cm)*	*Water Temperature (C)*	*pH*	*Dissolved oxygen (mg/L)*
Alba 1	N. 38 29.29W. 122 44.44	156	24	Sunny/warm	30	19.1	6.6	8.1
Alba 2	N. 38 29.30W. 122 44.44	183	24	Sunny/warm	30	19.3	6.5	8.3
Alba 3	N. 3829.33W. 122 44.33	153	24	Sunny/warm	18	21.7	6.7	9.2
Alba 4	N. 38 29.30W. 122 44.33	152	24	Sunny/warm	10	27.0	6.9	10.0
Gobbi 1	N. 38 23.13W. 122 44.33	152	20	P. cloudy/warm	24	24.6	7.0	9.5
Gobbi 2	N. 38 23.20W. 122 45.19	158	20	P. cloudy/warm	18	24.3	6.8	11.4
Gobbi 3	N. 38 23.15W. 122 45.26	171	20	P. cloudy/warm	25	26.0	8.0	11.3
Gobbi 4	N. 38 23.18W. 122 45.32	155	20	P. cloudy/warm	8	27.8	9.4	13.9

Table 1b. May 5 and 7 pool measurements

Pool	GPS Location	Pool size (perimeter in m)	Air Temperature (C)	Weather	Water Depth (cm)	Water Temperature (C)	pH	Dissolved oxygen (mg/L)
Alba 1	N. 38 29.29W. 122 44.44	156	17	Overcast/cool	18	18.1	6.3	9.8
Alba 2	N. 38 29.30W. 122 44.44	183	17	Overcast/cool	13	19.7	6.8	9.7
Alba 3	N. 3829.33W. 122 44.33	153	17	Overcast/cool	18	21.7	6.7	9.2
Alba 4	N. 38 29.30W. 122 44.33	152	17	Overcast/cool	N/A*	N/A*	N/A*	N/A*
Gobbi 1	N. 38 23.13W. 122 44.33	152	15	Rainy/cool	17	15.5	3.7	8.1
Gobbi 2	N. 38 23.20W. 122 45.19	158	15	Rainy/cool	5	16.4	6.6	9.0
Gobbi 3	N. 38 23.15W. 122 45.26	171	15	Rainy/cool	13	16.3	4.2	14.3
Gobbi 4	N. 38 23.18W. 122 45.32	155	15	Rainy/cool	N/A*	N/A*	N/A*	N/A*

*N/A refers to pools that had dried up during the study and therefore measurements were not taken.

DISCUSSION

Approximately one and half times more individual invertebrates were collected at the Alba Lane site than at Gobbi Ranch. The species richness was equal at both sites (n=16) yet the species diversity was slightly higher at Gobbi Ranch according to the Shannon indexes. Although the invertebrate species diversity was similar among all pools tested, personal observations point to differences in biodiversity between the two study sites. The Gobbi Ranch vernal pools closely resemble native vernal pools in their plant composition and substrate (Holland and Jain, 1988). The Gobbi Ranch site allows for cattle grazing during the summer months once the pools have dried up. The presence of cattle may have positive effects on the biodiversity of artificial and natural vernal pool ecosystems. The presence of cattle may be two-fold: 1) seasonal grazing may keep non-native grasses in check, thereby allowing native plants to flourish and 2) cattle manure may add organic nutrients vital to the continued existence of the plant and animals species which inhabit the pools (CH2M Hill, 1995). Additionally, the topography at Gobbi Ranch also appears to resemble natural vernal pool systems as the pools are shallower, evaporate sooner and may have less predation by birds, fish and amphibians. Conversely, the Alba Lane pools are subject to flooding from a creek that divides the property and the pools are overall deeper than the Gobbi Ranch pools thus allowing for long periods of water retention during the

Table 2a. Shannon Index for species diversity at Gobbi Ranch site

Species	Species ID	Number of individuals
1	Copepods	323
2	Cladocerans	250
3	Ostracods	171
4	Dipterans	103
5	Water Boatmen	53
6	Back Swimmers	46
7	Amphipods	40
8	Snails	37
9	Damselflies	34
10	Mayflies	8
11	Dragonflies	6
12	Springtails	5
13	Dytiscids	5
14	Water Striders	4
15	Spiders	4
16	Water Beetles	4
	TOTAL	**1,093**
		2.01 diversity

Table 2b. Shannon Index for species diversity at Alba Lane site

Species	Species ID	Number of individuals
1	Cladocerans	567
2	Copepods	419
3	Ostracods	343
4	Snails	157
5	Dipterans	80
6	Water Boatmen	49
7	Damselflies	43
8	Backswimmers	30
9	Water Striders	18
10	Mayflies	18
11	Dytiscids	10
12	Dragonflies	5
13	Leaf Beetles	3
13	Water Beetles	3
14	Water Scorpions	1
15	Water Mites	1
	TOTAL	**1,747**
		1.82 diversity

wet season on into the summer months. Cattails have been noted at the Alba Lane site presumably due to the water depth and the softer substrate.

Another observation of interest is the presence of migratory bird species that visit the pools. The path of migration flyways may affect the biological diversity at the sites as aquatic birds introduce eggs and cysts into the pools from their feet while they feed and rest at the pools. Previous experiences with these sites have shown a difference in bird species that visit the pools. The Alba Lane pools have been noted to have ducks, geese, herons and egrets. At the Todd Road site, only egrets and herons were seen. Additionally, two distinct fish species have been noted to inhabit the Alba Lane site during the first year of operation, presumably due to flooding from a nearby creek.

From a mosquito control aspect, poorly designed vernal pool ecosystems offer many challenges. First would be the disadvantage of providing an excellent habitat for mosquito reproduction, particularly the species *Culex tarsalis* and *Culex stigmatosoma*. Given the fact that many vernal pools provide habitat for migratory birds, the potential transmission of arboviruses such as western equine encephalomyelitis and West Nile increases greatly. The public health importance of these pools would be heightened by the difficulties involved in the treatment of vernal pools that are harboring mosquito populations. Vehicle access is limited because of the endangered plants that grow along the pool edges and the choice of pesticides is limited to those that do not affect non-target species. Other biological control methods such as the introduction of mosquitofish are impossible due to the possible impact on endangered aquatic invertebrate species such as fairy shrimp.

Future Work

Although not studied in this project, benthic animals may be more numerous at the Alba Lane site due to its finer substrate in comparison to the Gobbi Ranch pools. As mentioned earlier, fish and numerous bird species have been seen at the Alba Lane site and may contribute to lower species diversity although much more intensive work is warranted to confirm this hypothesis. It would be interesting to look at aquatic invertebrate populations monthly during the wet season. Of greater value may be a study that compares directly biodiversity in natural versus artificial vernal pool systems. Other future studies could include introduction of threatened or endangered species such as fairy shrimp, red-legged frogs and plants (Sonoma Sunshine, Sebastopol meadowfoam) and a comparison made between natural and artificial vernal pools. Completion of such studies could aid in developing guidelines for future mitigated wetland projects. This ecosystem-based approach may allow for continued urban/suburban development while protecting and conserving vernal pool habitats and the animals and plants that thrive within these unique communities.

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Evaluation of Mosquito and Arbovirus Activity in Orange County During 2000

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ABSTRACT: The Orange County Vector Control District continued its surveillance of mosquito and arbovirus activity throughout 2000 by collecting blood samples from wild birds and sentinel chickens, as well as collecting adult mosquitoes with CDC/CO2, gravid and stable traps. There were no positive mosquito pools, sentinel chicken seroconversions, or human cases in Orange County during 2000. Overall, 3 (0.1%) of the 3,149 sampled house finches and none of the 764 sampled birds of other species tested positive for SLE antibodies. *Culex quinquefasciatus* was the most commonly trapped mosquito, except for a freshwater wetland area of Irvine, where *Cx. tarsalis* was predominant.

INTRODUCTION

The Orange County Vector Control District (OCVCD) encompasses about 780 square miles (all of Orange County) and has almost 3 million residents. Most of the District consists of urban/suburban habitats with a variety of residential mosquito-breeding sources: improperly maintained swimming pools and ponds, debris-choked drainage channels, and other man-made habitats. Interspersed within this development are several natural, mosquito-producing fresh and salt-water wetlands. Three important encephalitis vectors are collected in the county: *Culex tarsalis* Coquillett, *Culex quinquefasciatus* Say, and *Culex stigmatosoma* Dyar. In 2000, the District continued its mosquito and encephalitis virus surveillance program by collecting blood samples from wild birds, sentinel chickens, and adult mosquitoes from a variety of trapping sites.

MOSQUITO SURVEILLANCE

Mosquitoes were collected weekly at 14 sites in the county (Figure 1), using 31 CDC/CO2 traps (Sudia and Chamberlain 1962), and six gravid female ovipositional traps (Cummings 1992). Engorged female mosquitoes were aspirated from an Australian crow trap (McClure 1984) modified to capture wild birds and the mosquitoes that feed on them. Overall, mosquito numbers for all species were much higher in 2000 than the previous year in urban/suburban habitats (Figure 2).

Culex quinquefasciatus was the mosquito species collected most frequently, but varied in abundance by season and habitat. Populations of this species were sampled best with gravid traps in suburbanized areas of the county. Counts peaked in May for the season, and then declined through the winter at all locations (Figure 3).

Culex tarsalis was collected in substantial numbers at only two, relatively small, undeveloped areas of the District (Bolsa Chica and San Joaquin freshwater marshes). At the San Joaquin wetlands, numbers of host-seeking *Cx. tarsalis* were highest in May (165 per trap night) and decreased gradually through the summer months, disappearing by the end of October for the remainder of the year (Figure 4). In contrast, during 1999, *Cx. tarsalis* collections in this habitat were highest in June, averaging only 61 per trap night. Numbers of mosquitoes at this site (*Culex erythrothorax* Dyar being the most abundant) peaked between 500 - 600 per trap night in May and June 2000 (Figure 5) and were more numerous than in 1999.

During the year, 3,627 post-blood fed (gravid or blood engorged) mosquitoes were selected from routine collections (nulliparous adults were not included), and were sent to the University of California-Davis, Center for Vector-borne Disease Research (UCD-CVDR) for testing (Table 1). The submissions included 135 pools of *Cx. quinquefasciatus* and 4 pools of *Cx. tarsalis*. None of these pools tested positive for either SLE or western equine encephalomyelitis (WEE).

Orange County received an average of only 7.4 inches of rainfall during the 1999-00 season (NOAA 2000), approximately the same as the 1998-99 season total (7.2 inches, NOAA 1999). As in most years, mosquito counts rarely correlate with rainfall. Most breeding sources in Orange County are man-made and require water from urban irrigation runoff to support mosquito production during the dry summer months. Year-to-year variations in numbers at the same sites are probably due to reasons other than the amount of rainfall.

SENTINEL CHICKENS

The District maintained one sentinel chicken flock of 10 chickens near a *Cx. tarsalis* - producing freshwater marsh at the San Joaquin Wildlife Sanctuary in Irvine. Blood samples from the chickens were tested biweekly for SLE and WEE antibodies by the California Department of Health Services' Viral and Rickettsial Diseases Laboratory (CDHS/VRDL) from April - November and at the District laboratory for the entire year. None of the chickens or any other sentinel chickens tested

Table 1. Number of mosquitoes and mosquito pools submitted for SLE and WEE virus testing by species and trap type from Orange County during 2000.

Species	No. of Mosquitoes	Gravid Trap Pools	Stable Trap Pools	CO_2 Trap Pools	Total Pools
Culex quinquefasciatus	3,552	107	28	0	135
Culex Tarsalis	75	0	4	0	4
Culex Stigmatosoma	0	0	0	0	0
Totals	**3,627**	**107**	**32**	**0**	**139**

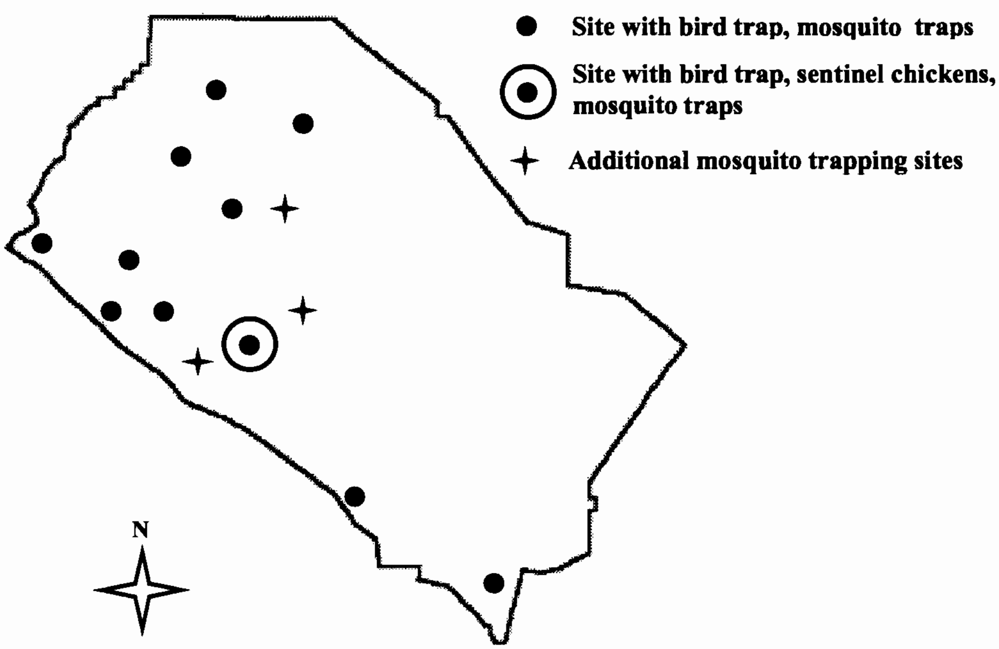

Figure 1. Arbovirus surveillance sites in Orange County, 2000.

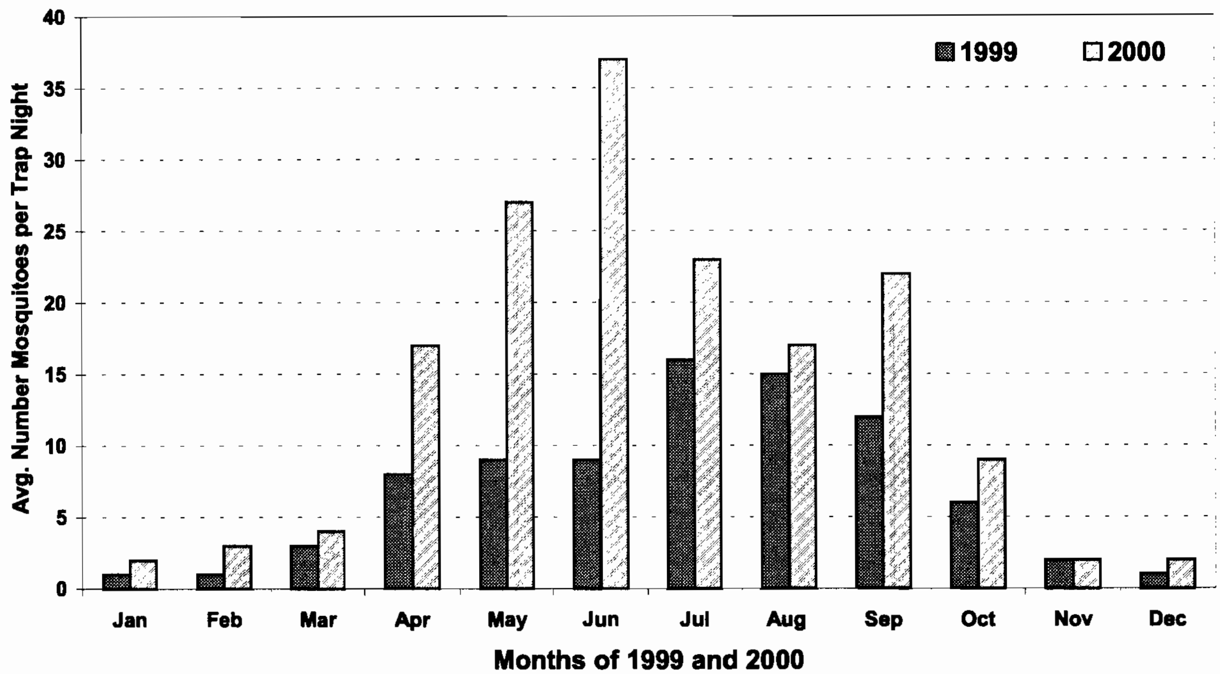

Figure 2. Host-seeking mosquito activity (all species, primarily *Cx. quinquefasciatus* and *Cs. incidens*) at 10 suburban mosquito collecting sites, Orange County, Calif., for 1999 and 2000.

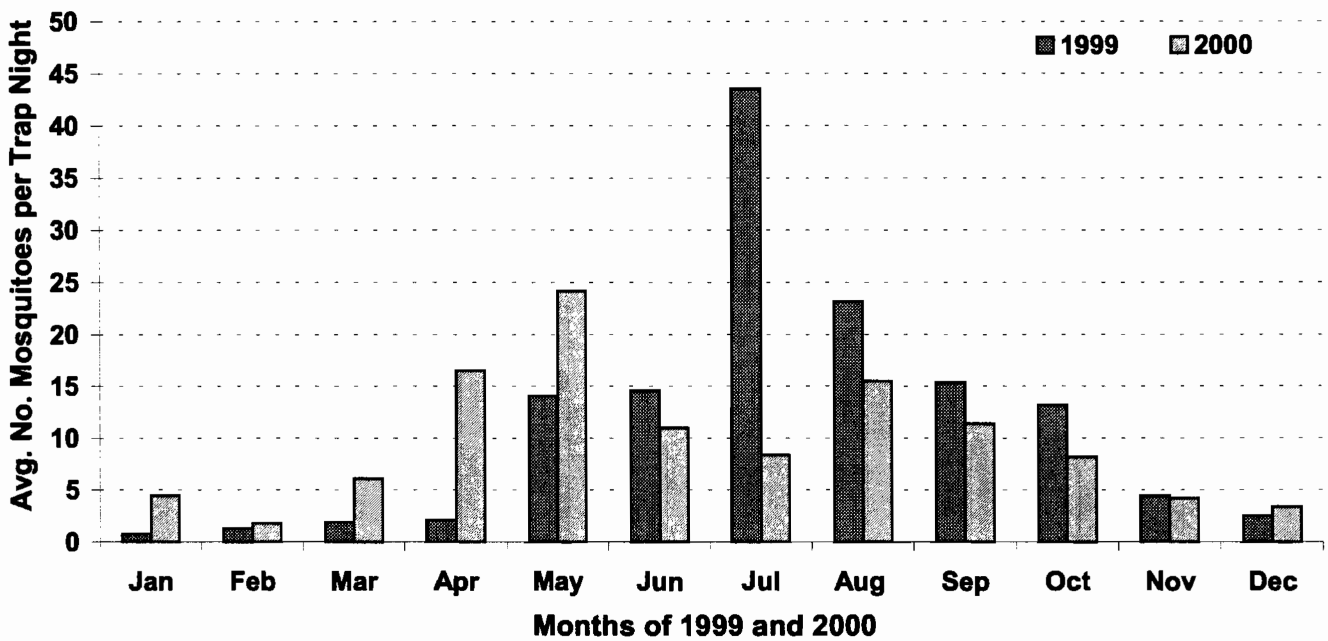

Figure 3. Numbers of gravid *Culex quinquefasciatus* collected from ovipositional traps at six sites in Orange County, Calif., for 1999 and 2000.

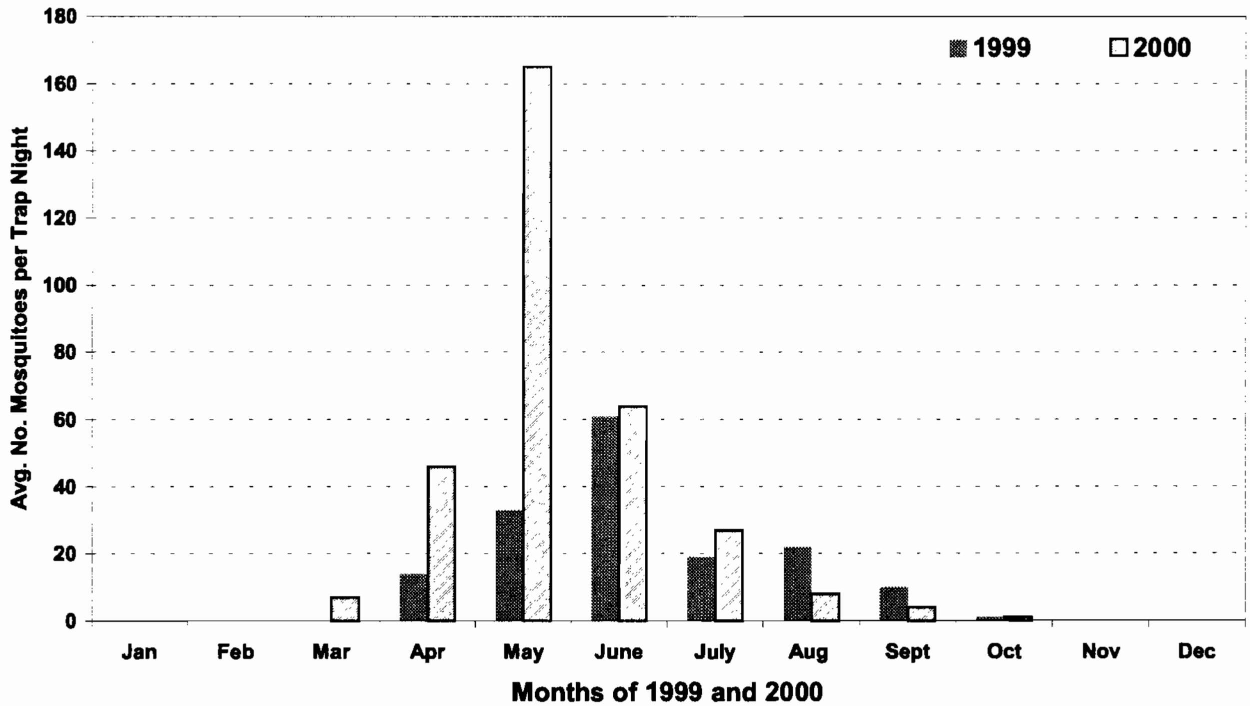

Figure 4. Host-seeking *Culex tarsalis* activity at the San Joaquin Marsh, Irvine, Calif., during 1999 and 2000.

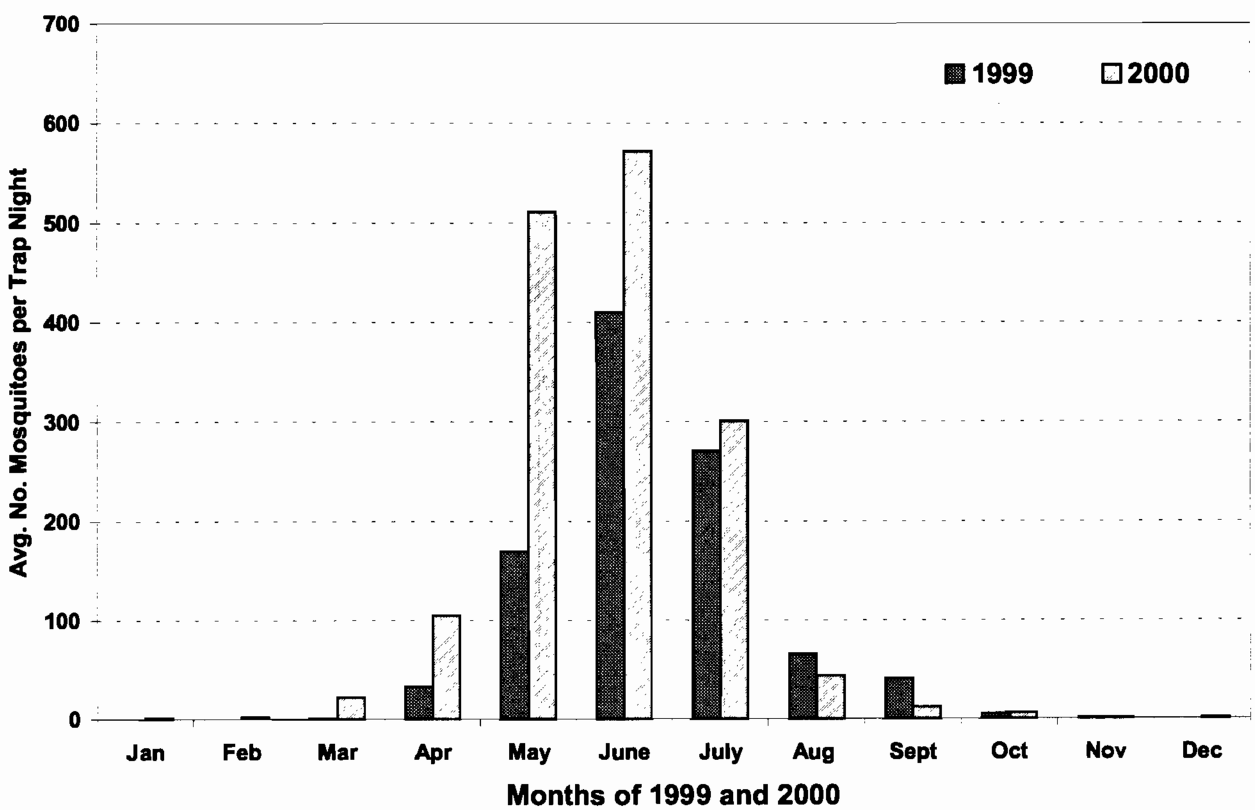

Figure 5. Host-seeking mosquito activity (all species, primarily *Cx. erythrothorax* and *Cx.tarsalis*) at the San Joaquin Marsh, Irvine, Calif., during 1999 and 2000.

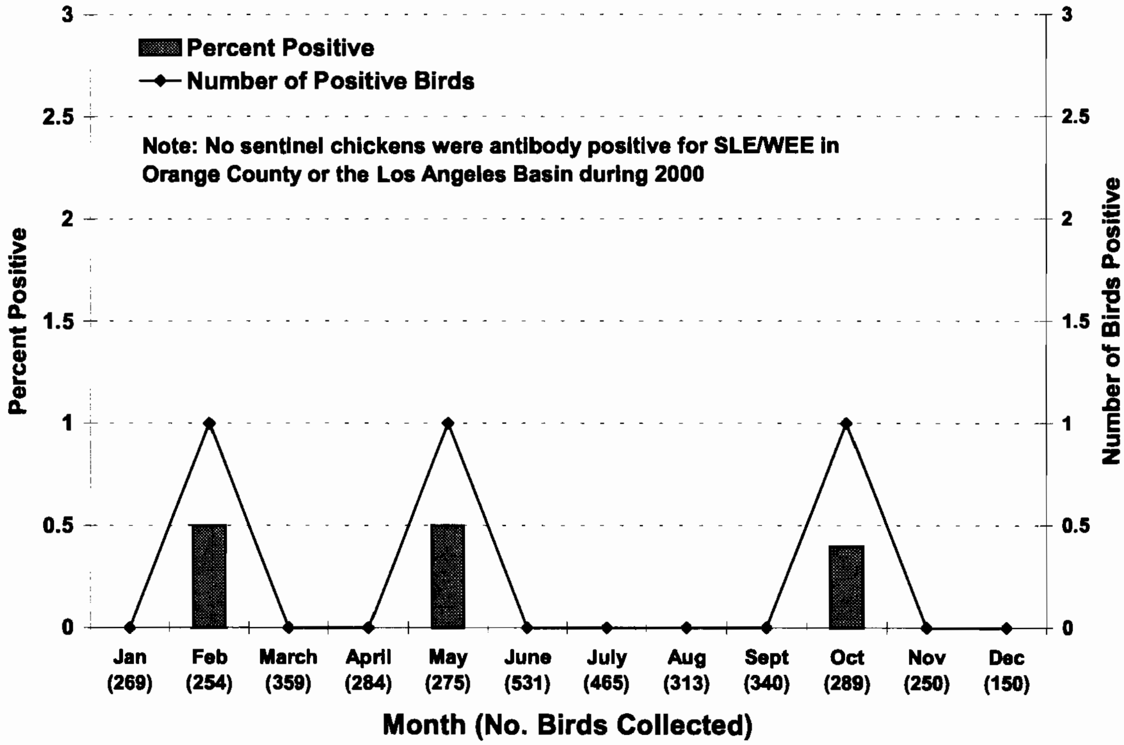

Figure 6. Seroprevalence of SLE antibodies in wild birds (House Finches and House Sparrows) from Orange County, Calif., during 2000.

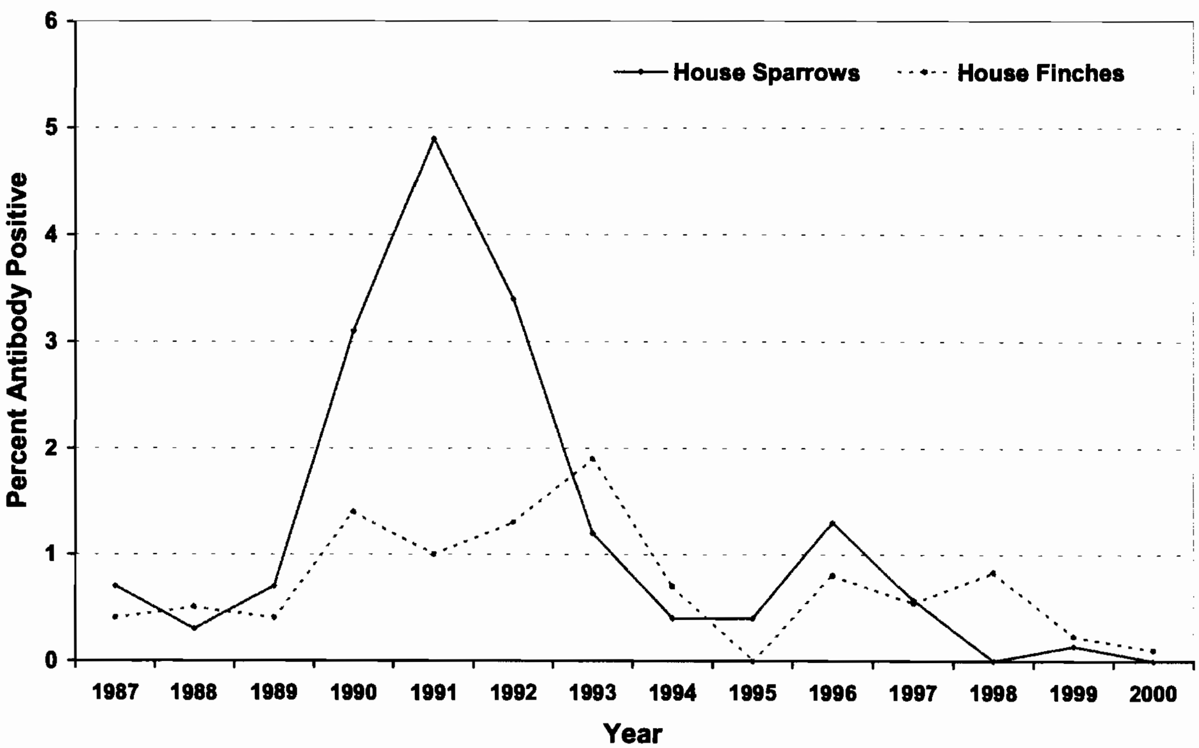

Figure 7. Long-term trends in SLE antibody seroprevalence in wild birds (House Finches and House Sparrows) in Orange County, Calif., from 1987 – 2000.

Table 2. Small bird seroconversions for SLE and WEE antibodies in Orange County during 2000.

Species	No. Samples	SLE Positive	WEE Positive	% SLE	% WEE
House Finch	3,149	3	0	0.10	0
House Sparrow	630	0	0	0	0
Song Sparrow	63	0	0	0	0
White-crowned Sparrow	25	0	0	0	0
Other Species	46	0	0	0	0
Totals	**3,913**	**3**	**0**	**0.08**	**0**

positive for SLE or WEE antibodies.

ENCEPHALITIS ANTIBODY SEROPREVALENCE IN WILD BIRDS

The wild bird encephalitis antibody seroprevalence program focused primarily on two abundant peridomestic passerines, House Sparrows (*Passer domesticus* L.) and House Finches (*Carpodacus mexicanus* Say). Birds were trapped in 11 modified Australian Crow traps at sites also used to sample the adult mosquito population. Six trap sites were located in riparian corridors surrounded by suburban development. House Finches were predominant, whereas House Sparrows were collected almost exclusively at two sites located in urbanized communities with few open areas. Near-equal mixes of House Sparrows and House Finches were seen at only three locations.

Birds were sampled at each site on alternate weeks (5-6 sites/week). Newly captured birds were banded, recorded, bled and released. Blood samples (0.2-ml) were taken from the jugular vein with a 1.0-ml syringe and a 28-gauge needle, dispensed into a 1.8-ml field diluent solution, kept cool and processed at the District laboratory using a hemagglutination inhibition (HAI) assay (Gruwell et al. 1988).

Of the 3,149 House Finches sampled in 2000, three birds (0.1%) tested positive for SLE antibodies. None of the 630 House Sparrows and 134 birds of other species were positive during the year (Table 2). Antibody-positive birds were detected in February, May and October only. These three positive House Finches represented the only evidence of any arboviral activity in the greater Los Angeles Basin during 2000 (Figure 6).

LONG-TERM TRENDS

In the past eight years, 1993 - 2000, the wild bird arbovirus surveillance program has detected a period of relatively low SLE and WEE antibody prevalence, in contrast to three active years from 1990 to 1992 (Figure 7). Approximately 5% (110 birds) of the 2,246 House Sparrows sampled in 1991 were positive for SLE antibodies. Numerous chicken seroconversions were also noted in 1991 and 1992 in the Los Angeles basin (including Orange County), including two confirmed human cases (Bennett et al. 1992, 1993).

In general, mosquito counts in Orange County for 2000 were lower than in previous years. Nevertheless, data from the wild bird serosurveillance program suggest that enzootic transmission of SLE was evident at a very low rate.

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The Impact of Bti on the Survival of the Endangered Tadpole Shrimp *Lepidurus packardi*

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ABSTRACT: We investigated the effects of *Bacillus thuringiensis* var. *israelensis*, a bacterial mosquito control material, on the vernal pool tadpole shrimp *Lepidurus packardi* and larval mosquitoes. Field trials were conducted in ephemeral pools on a wildlife refuge to determine efficacy and residual activity in addition to non-target impacts. The VectoBac[a] 12AS formulation of Bti applied at 1169 ml/ha (16 fl. oz./A) killed 100% of target *Ochlerotatus dorsalis* mosquito larvae at 24 hours post-treatment, but had little effect at 48 hours. There were no detectable adverse effects on survival of the vernal pool tadpole shrimp.

INTRODUCTION

Previous investigators have been concerned with the effect that various vernal pool invertebrate species might have on the efficacy of *Bacillus thuringiensis* var. *israelensis* (Bti) against mosquitoes (Blaustein and Margalit 1991; Fry-O'Brien and Mulla 1996). However, increasing environmental awareness of the value of ephemeral habitats and the subsequent listing of some associated species as either threatened or endangered, has shifted the focus to the effects that contemporary mosquito control materials may have on the non-target organisms themselves. One such species of interest is the vernal pool tadpole shrimp (*Lepidurus packardi* Simon) which was listed as endangered on September 19, 1994.

Lepidurus packardi is found only in California's Central Valley between Shasta County to the north and Tulare County to the south. The only known populations of the tadpole shrimp outside the Central Valley are found in the complex of vernal pools on the Warm Springs Seasonal Wetlands unit of the Don Edwards San Francisco Bay National Wildlife Refuge in Alameda County and on the adjacent Catellus Development Corporation property (Arnold 1997a,b; Goettle 1997).

Prior to 1980, the Alameda County Mosquito Abatement District treated standing water on and around what is currently Refuge property with old style, broad spectrum pesticides to control mosquitoes which can cause discomfort and vector diseases of humans and animals in the surrounding urbanized areas (Hamersky, Rusmisel, pers. com). However, the commercial availability of biorationals like Bti in 1982 (Valent Biosciences, pers. com.) allowed the District to utilize more environmentally friendly, target specific materials to control mosquitoes within its jurisdiction. Although vernal pools have not been treated since the area was designated as a wildlife refuge in 1992, U.S. Fish and Wildlife officials question how this newer material might impact the tadpole shrimp if such applications were to become necessary today. The objective of our project was to investigate whether Bti (VectoBac® 12AS, Valent Biosciences, North Chicago, IL.) applied under operational conditions and rates adversely affects the survival of the vernal pool tadpole shrimp.

MATERIALS AND METHODS

A Section 7 permit request which would allow incidental take of an endangered species during the course of the research was submitted to the USFWS Endangered Species office in Sacramento in November 1997. Approval was obtained in time to conduct the study in March 1999. The project took place on the Warm Springs Seasonal Wetlands unit of the Don Edwards San Francisco Bay NWR. We found six pools with sufficient *L. packardi* numbers to perform the study, and randomly selected treated and control pools in paired sets (Fig. 1). Because vernal pool tadpole shrimp are endangered and may be declining at this location, we did a pre-experiment survival test to ensure that placing shrimp in sentinel containers

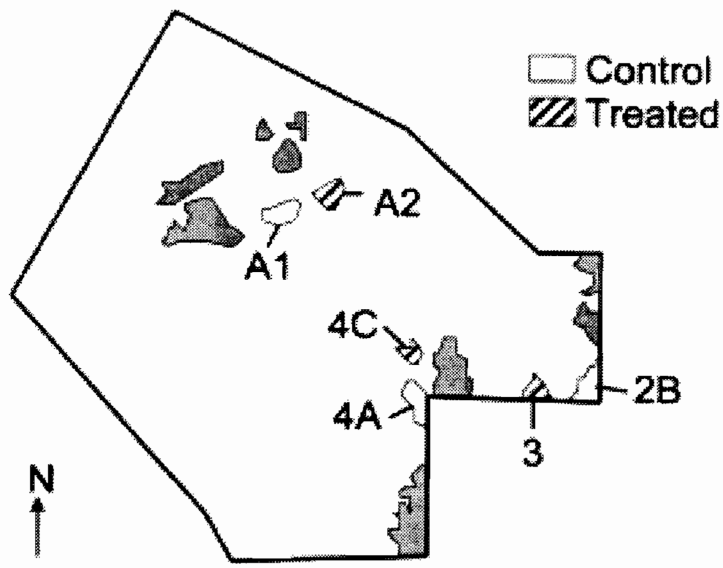

Figure 1. Diagram of Warm Springs Seasonal Wetlands in Alameda County, California. Labels indicate vernal pools used in study. Bti was applied at 1169 ml/ha (16 fl. oz./A) to treated pools.

would not kill them. Three 19 L (5 gal.) buckets with screened windows on the sides, bottom and lid were placed in one pool (A1) so that the container rested on the bottom and extended above the water surface. Five *L. packardi* were put in each container and counted daily for 4 days. There was no mortality for the first 3 days but one shrimp was found dead on day 4 which restricted our investigation to 3 days.

The experiment was set up by placing 2 sets of two sentinel containers in each of the 6 pools. Each set consisted of one, 19 L (5 gal.) bucket which contained five *L. packardi* and one, 4 L (1 gal.) floating bucket which contained 10 late 2nd instar *Ochlerotatus dorsalis* (Meigen) larvae. Both shrimp and mosquito larvae were placed in buckets 24 hours prior to the commencement of the experiment and were counted just before treatment and at 24 and 48 hours post-treatment. VectoBac® 12AS was applied by an environmental specialist from Alameda MAD at 1169 ml/ha (16 fl. oz./A) using a backpack sprayer to the 3 pools designated for treatment. Mosquito larvae were replaced daily as needed to document any residual activity the Bti may have had.

Data were subjected to analysis of variance (ANOVA) using Systat® 7 (SPSS Inc. 1997). Percent survivorship was the combined number in the two buckets per species per pool. Sites were treated as replicates for analysis.

RESULTS

Results of the experiments are summarized in Fig. 2. There was no apparent effect of Bti on tadpole shrimp. All *L. packardi* survived in every pond regardless of treatment and regardless of any mortality in mosquito larvae. However, Bti caused significant mortality of *Oc. dorsalis* (ANOVA $P<0.002$). All mosquitoes survived in all but one control site, but 100% died in the treated sites at 24 hours and 20% died at 48 hours. There was one control site with substantial mosquito mortality from unknown causes (100% at 24 hours and 55% at 48 hours) however, there was still a significant effect of treatment.

DISCUSSION

The *L. packardi* on the Warm Springs unit are of particular interest because they are geographically isolated from the remainder of the population in the Central Valley of California (Linder 1952, Longhurst 1955). Furthermore, refuge personnel are concerned because their surveys indicate that this population appears to be declining (Albertson, pers. com.) so it would be desirable to identify the reasons for this. The U.S. Fish and Wildlife Services' concern with mosquito abatement activities as a possible causative factor may stem from several published reports of adverse effects on another tadpole shrimp *Triops longicaudatus* LeConte (Notostraca: Triopsidae) which is found in fresh water agro-ecosystems like rice and is not endangered (Miura and Takahashi 1974; Walton et. al 1990). The aforementioned studies largely utilized classes of pesticides like synthetic pyrethroids, organophosphates and carbamates which are not currently used by the local mosquito

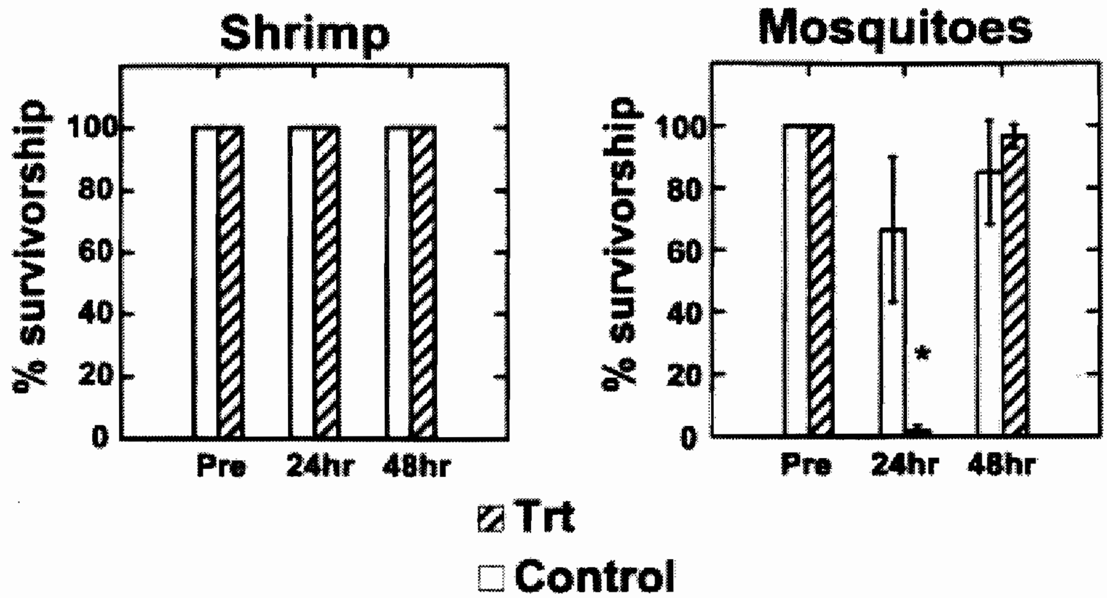

Figure 2. Mean percent survivorship of sentinel tadpole shrimp (*Lepidurus packardi*) and mosquito larvae (*Ochlerotatus dorsalis*) pre- and post- treatment with liquid Bti. Error bars indicate standard error; *indicates significant difference (ANOVA, F (Trt x Day) = 7.49, d.f. = 2,30, P<0.002).

abatement district. Biorationals such as Bti and methoprene are the preferred materials.

Our studies on the VectoBaC® 12 AS formulation of Bti indicate that there is no negative impact on the survival of the endangered vernal pool tadpole shrimp even when there is 100% control of the target organism. These results suggest that Bti is a good candidate material for use in this particular sensitive habitat.

Other factors besides liquid Bti may explain any declines in shrimp populations at this location. Habitat loss which has imperiled vernal pool tadpole shrimp elsewhere (Holland and Jain 1988; USFWS 1994) should not be a factor here since this population is within Refuge boundaries. However, degradation of the mud substrate in individual pools due to plant growth has been observed to decrease other vernal pool species like fairy shrimp (Belk, pers. com.) and should be considered. In addition, Ahl (1991) found the fecundity was drastically reduced in *L. packardi* individuals that were parasitized by an echinostome fluke. Direct predation of shrimp by waterfowl has been observed by Refuge biologists (Albertson, pers. com.) and may reduce *L. packardi* populations. Finally, since the Warm Springs unit is surrounded by industrial areas and is near a major transportation corridor, the presence of some sort of environmental contaminant is also a logical possibility. The unexplained mortality of sentinel mosquito larvae in one control site which was not contaminated from treatment but was adjacent to lifeless pools that contained submerged pipes, supports the latter theory. Our attempts to reproduce the mortality of *Oc. dorsalis* larvae in control pool A1 via a laboratory bioassay using soil and water samples from this particular pool failed, but more extensive chemical testing, which was beyond the scope of our work, merits further investigation.

Acknowledgements

The authors extend their appreciation and thanks to the Alameda County MAD for its contribution of product and application equipment, to S. Schutz for field assistance and invaluable guidance with data analysis, to the personnel at the Don Edwards San Francisco Bay NWR office who assisted with shrimp surveys and finally to the U.S Fish and Wildlife Service and the University of California Mosquito Research Program which provided funding for this study.

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Phototactic Behavior of Insects of Public Health Importance at Parker Dam, San Bernardino County

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ABSTRACT: Of the 23,120 insects collected in six New Jersey light traps with different colored bulbs—violet, blue, green, yellow, red and white, 57% were Diptera and 43% Trichoptera. The Diptera composition by family was Chironomidae 26%, Psychodidae 15%, Culicidae 13%, Ceratopogonidae 2%, and Simuliidae 1%. The Culicidae comprised of several species namely, *Anopheles franciscanus*, *Anopheles freeborni*, *Culex erythrothorax*, *Culex quinquefasciatus*, *Culex stigmatosoma*, *Culex tarsalis*, *Culiseta inornata*, *Ochlerotatus increpitus*, and *Psorophora columbiae*. As a whole, all insects responded to white and yellow, followed by blue, green, red and violet. The response of mosquitoes was highest to white (28%), followed by yellow (24%), blue (13%), violet and red (12%) each, and green (11%). However, data on individual species showed that *An. freeborni* and *Cx. quinquefasciatus* were attracted more to violet than white or yellow.

The data on seasonal distribution showed fluctuations in response to light among various taxa. A majority of taxa showed high populations during the summer, fall and spring seasons, except for the Culicidae which had the highest levels in winter due largely to *Cs. inornata*. Similarly, seasonal responses to different colored bulbs varied among mosquito species. *Cx. stigmatosoma* and *Cx. tarsalis* showed the highest response to blue during the winter and summer, respectively. Based on these data, white, yellow and blue show some potential for use in baited traps in vector management programs.

INTRODUCTION

Communities living along the Colorado River invariably face the problems of vectors and vector-borne diseases. These range from mosquitoes and mosquito-borne encephalitides, to black flies and biting midges. Besides sporadic cases of encephalitis, the encephalitis virus activity has been reported almost every year from the Needles area (Mian 1996).In order to augment and improve vector management practices of these insects, there is a need to learn more about the behavior of these vectors under actual field conditions.

One such behavioral aspect is the attraction of these insects to different colored lights and its use in vector control programs. Studies have shown different responses by mosquitoes to colored lights under different field conditions (Breyev 1963, Brown and Bennett 1981, Jiqun et al. 1984). Attraction of different chironomid midges and mosquitoes species to artificial blue, white and yellow lights under Florida conditions, has been documented (Ali et al. 1986, 1990).

No data are available on the phototactic behavior of local vector fauna along the Colorado River, San Bernardino County. Such information is germane in the development of vector management program in this area. Therefore, a study on the response of various insects to color stimuli was carried out in the Parker Dam area of San Bernardino County in 1991-92. Based on that study, this present paper presents data on the faunal composition and seasonal responses of various dipterous and trichopterous insects to six different colored light bulbs used in New Jersey light traps.

MATERIALS AND METHODS

A study area was selected in the De Silt Wash near the Gene Pumping Plant, Metropolitan Water District of Southern California (MWDSC) Field Headquarters, Parker Dam. The wash has an east-west running water channel on the south side. On the opposite side it has a series of both covered and uncovered horse pens and stalls as well as some 4-H animals such as cows and pigs belonging to the District employees. The vegetation in the wash is a mixture of tamarisks, *Tamarix* species, cottonwoods, *Poplus* species, willows, *Salix exigua*, mulberry, *Morus* sp., reed, *Arundo donax*, along with a mix of sage brush, *Artimesia tridentata*, and creosote bushes. At the eastern end of the stables, there are small stands of cattails, *Typha* sp., and tule.

In an east-west transect, six stainless steel New Jersey light traps were set up along the stables. The traps were hung about six feet above ground. The distance between traps varied from 30 to 65 feet, depending on proximity of the power source. The trap arrangement by color was violet, blue, green, yellow, red and white. Except for the violet (75 watt) and white fluorescent (40 watt), all other light bulbs used were incandescent 25 watt. The light intensity of the bulbs was measured by a light meter (Gossen Lunna-Pro, # 571922, West Germany). The bulb intensity on the lux scale was violet—1.4, Blue—5.5, green—16, yellow—66, red—11, and white—252.2. Due to variations in the bulb intensity, the effect of light intensity on insect responses was not investigated in this study. The bulbs were rotated among the traps in a clock-wise manner every two weeks, allowing each colored bulb a chance of being used in each of the six traps. Insect samples were collected from the traps every other week from about the first week in August 1991 to mid-August 1992. The samples collected in clean vinyl jars with lids were properly labeled before they were transported to the laboratory in San Bernardino for identification purposes.

In the laboratory, each sample was carefully emptied into a 6-in. diameter plastic dish. Only dipteran and trichopteran specimens were sorted out for identification under a stereo binocular microscope. Identification of mosquitoes was to species and sex. Black flies, biting and non-biting midges, and moth flies ware identified to family. Caddis flies were identified up to order. Due to small numbers of some species, the samples were combined by season than on a monthly basis. The seasonal dates used were June 23-Sep. 22 for summer, Sep. 23 - Dec. 22 for fall, Dec.23-March 22 for winter, and March 23-June 22 for spring.

After identification, all data were arranged and analyzed statistically for faunal composition, color preference and seasonal response of each group, using Poly Software International (1993). Data values were compared for each of the aforementioned parameters, using Duncan's multiple range test (DMRT, Duncan 1955).

RESULTS AND DISCUSSION

Based on all samples from the six traps, a total of 23,120 insects were sorted out into Diptera and Trichoptera. Of these, Diptera accounted for 57% and Trichoptera 43%. The percentage breakdown of Diptera included 26% Chironomidae, 15% Psychodidae, 13% Culicidae, 2% Ceratopogonidae and 1% Simuliidae (Table 1). The attraction

Table 1. Attraction of various insect groups to different colored New Jersey light trap bulbs at Parker Dam, San Bernardino County[1].

Order/Family	Total #	%	% Distribution by bulb color					
			Violet	Blue	Green	Yellow	Red	White
Diptera	**13,187**	**57**	7ab[2]	13 a-e	14 a-f	9 a-c	33 f-h	24 a-h
--Ceratopogonidae	474	2	6 a	10 a-c	17 a-f	21 a-h	7 ab	39 h
--Chironomidae	5,880	26	5 a	15 a-f	15 a-f	27 b-h	7 ab	31 d-h
--Culicidae	3,069	13	12 a-d	13 a-e	11 a-d	24 a-h	12 a-d	28 c-h
--Psychodidae	3,485	15	8 a-c	13 a-c	12 a-d	20 a-g	10 a-c	37 gh
--Simuliidae	279	1	4 a	11 a-c	6 a	14 a-f	8 a-c	57 j
Trichoptera	**9933**	**43**	8 a-c	10 a-c	10 a-c	33 f-h	7 ab	32 e-h
Total	**23,120**	**100**	1,726	2,814	2,782	6,426	1,932	7,440
%			7.5	12.2	12.0	27.8	8.3	32.2

[1] Total numbers based on 390 trap nights.

[2] Values followed by the same letter(s) are not significantly different from one another (DMRT, P=0.05).

of these insects to traps with different colored bulbs varied significantly. The trap with white bulb attracted the highest, 32.2% of the total insects, followed by yellow 27.8%, blue 12.2%, green 12.0%, red 8.3% and violet 7.5%. Similarly, the response of individual taxa followed the same order with the highest responding to white light, followed by yellow and the other colors. The caddis flies, however, responded 32% and 33% to white and yellow lights, respectively.

A total of 3069 mosquitoes were collected in the traps. The mosquito composition by species was *Culex stigmatosoma* Dyar 43%, *Culiseta inornata* Williston 31%, *Culex tarsalis* Coquillett 11%, *Culex erythrothorax* Dyar 9%, *Culex quinquefasciatus* Say 3%, *Anopheles franciscanus* McCracken 2%, *Anopheles freeborni* Atkin1%, and *Ochlerotatus increpitus* Dyar and *Psorophora columbiae* Dyar and Knab <1% each.

The response by species to the light color varied. *Anopheles franciscanus, Cx. erythrothorax, Cx. stigmatosoma, Cx. tarsalis* and *Cs. inornata* all responded higly to white and yellow lights. *Anopheles freeborni* and *Cx. quinquefasciatus*, however, had the highest response to violet light. Due to small sample sizes, no inference could made for *Oc. increpitus* and *Ps. columbiae*. The data also showed no significant difference in response to light color between the sexes for the majority of species, except for *An. freeborni* and *Cx. quinquefasciatus* where the males responded more to violet than white and yellow lights. The response of *An. franciscanus* to white and yellow lights was 34% and 24%, respectively (Table 2).

The data on seasonal abundance of various taxa showed a fluctuating pattern (Table 3). Except for Culicidae, Psychodidae and Simuliidae, all other taxa showed high population levels

Table 2. Attraction of various mosquito species to different colored New Jersey light trap bulbs at Parker Dam, San Bernardino County [1].

Species	Sex [2]	Total #	Total %	% Distribution by bulb color: Violet	Blue	Green	Yellow	Red	White
Ochlerotatus	M	0	0	0	0	0	0	0	0
increpitus	F	2	<1	50 e[3]	0	0	0	0	50 e
Anopheles	M	33	1	9 a	24 bc	18 bc	34 c	0	15ab
franciscanus	F	21	1	5 a	5 a	19 bc	28 c	5 a	38 c
Anopheles	M	5	<1	40 cd	20 bc	20 bc	0	20 bc	0
freeborni	F	15	<1	27 bc	13 ab	13 ab	27 c	13 ab	7 a
Culex	M	71	2	4 a	0	1 a	28 c	4 a	63 e
erythrothorax	F	196	6	4 a	2 a	8 a	16 bc	6 a	64 e
Culex	M	9	<1	89 f	11 a	0	0	0	0
quinquefasciatus	F	86	3	51 de	14 ab	4 a	2 a	12 ab	17 bc
Culex	M	499	16	6 a	14 ab	9 a	33 c	12 ab	26 c
stigmatosoma	F	828	27	18 bc	19 bc	7 a	21 bc	13 ab	22 bc
Culex	M	100	3	9 a	7 a	3 a	16 bc	24 bc	41 cd
tarsalis	F	255	8	14 ab	11 a	8 a	24 bc	11 a	32 c
Culiseta	M	55	2	6 a	7 a	12 ab	29 c	11 a	35 c
inornata	F	893	29	8 a	12 ab	18 c	25 bc	13 ab	24 bc
Psorophora	M	0	0	0	0	0	0	0	0
columbiae	F	1	<1	0	0	0	0	0	0
Total		3,069		375	403	324	730	370	867
%			100	12	13	11	24	12	28

[1] Numbers based on 390 nights.

[2] M-male, F-female

[3] Values followed by the same letter(s) are not significantly different from one another (DMRT, P=0.05)

Table 3. Seasonal distribution of various Diptera and Trichoptera caught in New Jersey light traps with different colored bulbs at Parker Dam, San BernardinoCounty[1/].

Taxon	Season [2/]	Total #	% distribution by bulb color/season Violet	Blue	Green	Yellow	Red	White
Ceratopogonidae	Su	233	7 a-d	16 a-h	14 a-h	12 a-f	6 a-d	45 h-k
	Fa	148	3 a-b	6 a-d	24 a-j	33 a-k	6 a-d	28 a-k
	Wi	22	0	9 a-e	41 e-k	23 a-j	9 a-e	18 a-i
	Sp	71	8 a-d	3 a-b	7 a-d	23 a-j	11 a-f	48 I-k
Chironomidae	Su	1,790	5 a-c	15 a-h	22 a-j	22 a-j	10 a-e	26 a-k
	Fa	1,533	5 a-c	16 a-h	10 a-e	32 a-k	5 a-c	32 a-k
	Wi	948	3 ab	6 a-d	24 a-j	27 a-k	5 a-c	35 b-k
	Sp	1,609	5 a-c	20 a-j	6 a-d	30 a-k	6 a-d	33 a-k
Culicidae	Su	684	32 a-k	19 a-i	8 a-d	9 a-e	10 a-e	32 a-k
	Fa	784	10 a-e	15 a-h	9 a-e	28 a-k	13 a-g	25 a-k
	Wi	897	7 a-d	12 a-f	19 a-i	22 a-j	12 a-f	28 a-k
	Sp	704	10 a-f	7 a-d	4 a-c	37 d-k	13 a-g	29 a-k
Psychodidae	Su	224	11 a-f	22 a-j	19 a-i	15 a-h	15 a-h	18 a-i
	Fa	407	14 a-h	16 a-h	11 a-f	25 a-k	10 a-e	24 a-j
	Wi	320	7 a-d	13 a-g	9 a-e	7 a-d	36 c-k	28 a-k
	Sp	2,534	7 a-d	11 a-f	12 a-g	21 a-j	7 a-d	42 f-k
Simuliidae	Su	73	3 ab	7 a-d	8 a-d	14 a-h	14 a-h	54 km
	Fa	34	6 a-d	26 a-k	15 a-h	29 a-k	9 a-e	15 a-h
	Wi	49	2 a	22 a-j	4 a-c	16 a-h	10 a-e	44 g-k
	Sp	123	4 a-c	6 a-d	2 a	10 a-e	2 a	76 m
Trichoptera	Su	5,757	8 a-d	13 a-g	11 a-f	26 a-k	6 a-d	36 c-k
	Fa	1,207	12 a-f	4 a-c	11 a-f	31 a-k	8 a-d	34 a-k
	Wi	170	15 a-h	13 a-g	26 a-h	15 a-h	13 a-g	18 a-h
	Sp	2,799	5 a-c	6 a-d	10 a-f	49 km	8 a-d	22 a-j

[1/]Data were collected at biweekly interval over 390 trap nights. Data followed by the same letter(s) are not significantly different from one another (DMRT, P=0.05).
[2/] Su=Summer, Fa=Fall, Wi=Winter, Sp=Spring.

during the summer and fall or spring season, in that order. Both Psychodidae and Simuliidae had the highest populations in the spring. Population of Psychodidae was lowest in summer. The low population levels of Psychodidae in the summer might be due to drier conditions, resulting in faster evaporation of sewage ponds and reduced breeding sources downstream. In Culicidae, winter population level was the highest, owing largely to *Cs. inornata*, accounting for 64% of the population.

Seasonal distribution data on mosquitoes revealed that *Oc. increpitus, An. franciscanus, An. freeborni, Cx. quinquefasciatus* and *Ps. columbiae* had population peaks in summer. *Culex stigmatosoma* and *Cx. tarsalis* had population peaks in spring and summer, whereas Cs. *inornata* was more abundant in the fall and winter. The response of all mosquitoes to various color lights was high to both white and yellow color in fall, winter and spring. In the summer, there was a shift in response towards both white and violet, followed by blue. During the winter, mosquito response to white (28%) and yellow color (22%) was followed by response to green light. In general, *An. franciscanus, Cx. erythrothorax, Cx. stigmatosoma, Cx. tarsalis* and *Cs. inornata* were highly attracted to white and yellow lights during the four seasons.

Culex stigmatosoma and *Cx. tarsalis* showed high response to blue color light in winter and summer , respectively. *Culex quinquefasciatus* had the highest attraction to violet color. *Anopheles freeborni,* in small numbers, showed variable attraction to violet, blue and white light during the seasons (Table 4).

Weather data for the study period were obtained through the courtesy of MWDSC Field Headquarters, Parker Dam. Figure 1 shows monthly mean temperature at 0600 h, minimum and maximum temperatures as well as monthly precipitation. Triple digit maximum temperatures were common from June through October. Similarly, monthly mean temperatures were in the 80s during the same period. Whereas nightly minimum temperature during the cold months, December through March, were below 50 °F., the day time temperatures were still in the optimum thermal requirement range of most of the insects in the area. A total of 13.28 in. of rainfall was recorded during the study period. Besides some monsoon rain activity during August and September, the wettest month was March (3.99 in.) followed, in descending order, by February, January and December.

Previous studies have reported mosquito response to relatively darker than light colors. *Anopheles maculipennis* was more responsive to red and violet colors than yellow and white

Table 4. Seasonal distribution of mosquito species collected in New Jersey light traps with different colored bulbs at Parker Dam, San Bernardino County[1/]

Species	Season [2/]	Total #	% Distribution by trap color					
			Violet	Blue	Green	Yellow	Red	White
Anopheles franciscanus	Su	39	8 b-d	21 lm	15 h-j	26 qr	2 a	28 qr
	Fa	7	14 g-j	0	14 g-j	58 a'	0	14 g-j
	Wi	2	0	0	0	50 y	0	50 y
	Sp	7	0	0	33 st	67 b'	0	0
Anopheles freeborni	Su	8	12 f-h	38 u	25 pq	0	25 pq	0
	Fa	2	50 y	0	0	0	0	50 y
	Wi	7	29 qr	0	14 g-j	57 a'	0	0
	Sp	3	67 b'	0	0	0	33 st	0
Culex erythrothorax	Su	35	14 h-j	6 bc	20 lm	23 op	11 e-g	26 qr
	Fa	120	2 a	0	1 a	18 kl	2 a	77 c'
	Wi	65	2 a	3 ab	9 c-e	22 mn	9 c-e	55 za'
	Sp	47	4 b	1 a	4 b	17 l	4 b	70 b'
Culex quinquefasciatus	Su	94	55 za'	14 h-j	3 a	1 a	11 e-g	16 jk
	Fa	0	0	0	0	0	0	0
	Wi	1	0	0	0	100 d'	0	0
	Sp	0	0	0	0	0	0	0
Culex stigmatosoma	Su	431	20 lm	19 kl	7 bc	9 c-e	11 e-g	34 t
	Fa	276	16 jk	21 lm	9 c-e	26 qr	16 jk	12 e-h
	Wi	202	9 c-e	24 no	16 jk	20 lm	18 kl	13 f-i
	Sp	418	8 b-d	9 c-e	4 b	44 r	10 c-f	28 qr
Culex tarsalis	Su	65	15 h-j	25 pq	0	0	8 b-d	52 yz
	Fa	29	10 c-f	4 b	0	48 w	0	38 u
	Wi	60	2 a	10 c-f	27 qr	23 no	10 c-f	28 qr
	Sp	201	15 h-j	7 bc	3 ab	24 o-q	21 l-m	30 rs
Culiseta inornata	Su	4	0	50 y	0	0	50 y	0
	Fa	339	7 bc	17 j-k	14 h-j	28 qr	15 h-j	19 k-l
	Wi	570	7 bc	9 c-e	21 l-m	23 no	11 e-g	29 qr
	Sp	35	14 h-j	17 j-k	6 b-c	43 v	9 c-e	11 e-g

[1/] Data were taken biweekly over 390 trap nights. Data items followed by the same letter(s) are not significantly different from one another (DMRT, P=0.05).

[2/] Su= Summer, Fa= Fall, Wi= Winter, Sp= Spring.

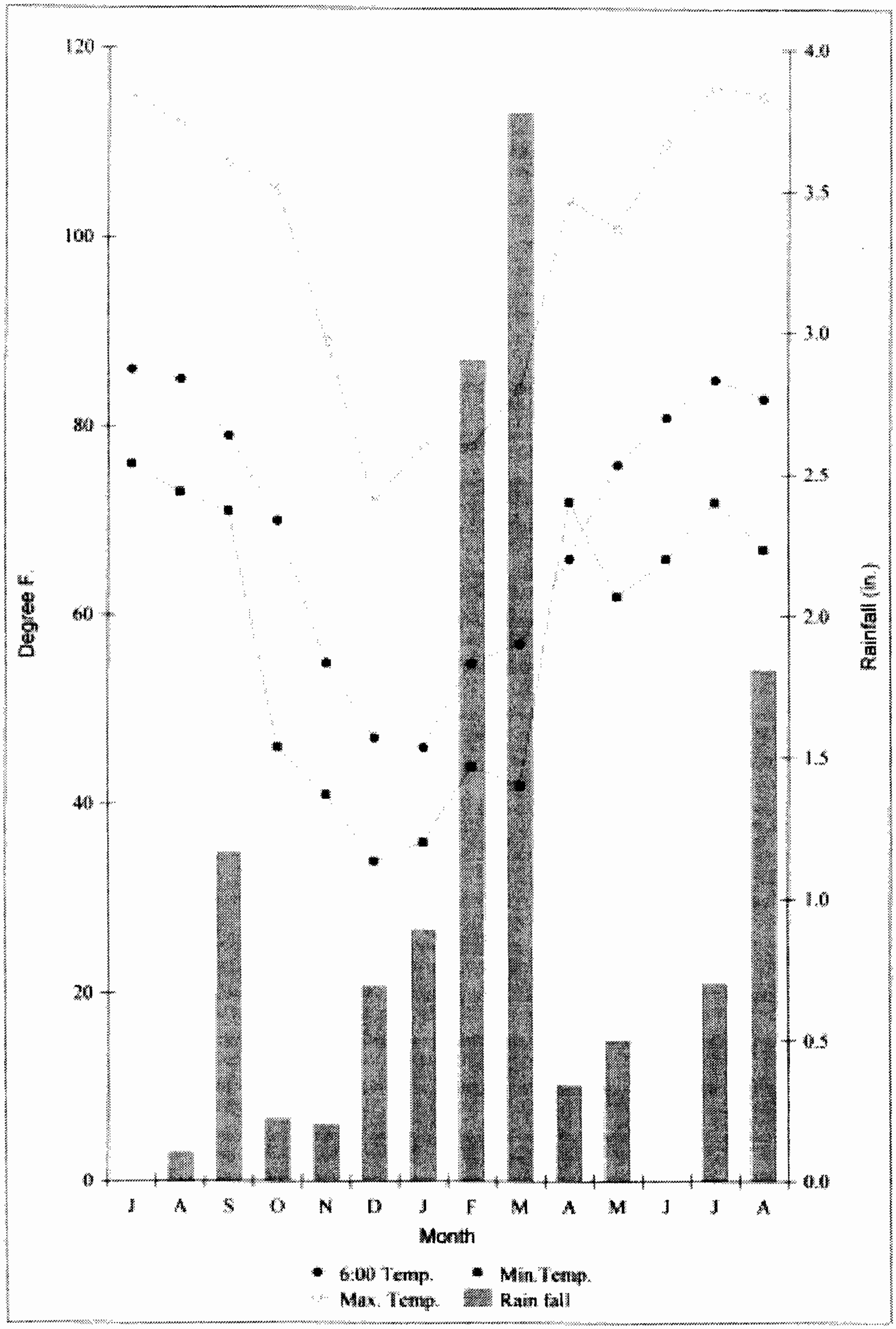

Figure 1. Data on temperatures and precipitation in Parker Dam, during 1991-92.

(Brighenti 1930). *Ochlerotatus cantator* (Coquillett), *Oc. punctor* (Kirby), and *Coquillettidia perturbans* (Walker) were attracted to low intensity colors—blue, black and red (Breyev 1963, Brown and Bennett 1981, Jiqun et al. 1984). Some Florida mosquitoes belonging to the genera, *Aedes*, *Anopheles*, *Culex* and *Psorophora*, were highly attracted to blue colored lights, followed by orange, white, yellow, green and red (Ali et al. 1990). Color was more important than light intensity. More females than males were reportedly attracted to these colors. The results reported in the present study, however, are based on different species and under different geographical conditions. This study provides a year-round data on both faunal and seasonal composition as well as response to visual stimuli of different colors. Moreover, the effect of light intensity on insect response was not studied due to wide variations in wattage of the colored bulbs used in the study.

Acknowledgement

The authors acknowledge the field assistance of various staff members, especially Robert Prochaska (deceased) Donald Gomsi and Lesli Allsup, of the San Bernardino County Vector Control Program. The San Bernardino County Vector Control Program is gratefully acknowledged for the field support of this study. The laboratory part was supported by California State University, San Bernardino, through a summer research fellowship.

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Field Efficacy of Aqua-Reslin®

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ABSTRACT: Four water-based dilutions of Aqua-Reslin® were applied at rates of 0.0035 and 0.0022 pounds permethrin/acre as ultra low volume ground treatments against laboratory-reared *Culex pipiens* mosquitoes and wild-caught, principally *Culex tarsalis*, mosquitoes. Cages of treated mosquitoes were located on a line 100 to 400 feet downwind and perpendicular to the treatment path. All formulations achieved 100 percent knockdown of mosquitoes at two hours post-treatment, and all treated mosquitoes were dead by 24-hours post-treatment.

INTRODUCTION

Aqua-Reslin® is a synergized permethrin formulation (20% permethrin, 20% piperonyl butoxide) labeled by Aventis Environmental Science and designed for ultra low volume (ULV) application. It is labeled for use against mosquitoes, black flies, gnats, biting and non-biting midges, and a variety of other biting flies and nuisance flying insects. This product uses water as a diluent, but is designed to behave as a typical oil-based formulation. Four diluted formulations of Aqua-Reslin were evaluated under field conditions, using a truck-mounted ULV fogger, to determine their efficacy against wild-caught and laboratory-reared mosquitoes.

MATERIALS & METHODS

Field trials were conducted between the times of 7:50 p.m. and 8:50 p.m. on August 14, 2000, in an open, fallow field, approximately 20 acres (8.1 hectares) in size, located adjacent to the Colusa airport in Colusa County, California.

Aqua-Reslin was diluted with water to final permethrin concentrations of 4.0, 5.0, 6.67, and 10.0 percent. The 4.0, 6.67, and 10.0% dilutions were applied at flow rates of 8.1, 4.9, and 3.25 fluid ounces per minute, respectively, to give equal application rates of 0.0035 pounds permethrin per acre (3.92 g AI/ha). The 5.0% dilution was applied at 4.0 fl oz/min to give an application rate of 0.0022 lb permethrin/acre (2.47 g AI/ha).

Per label instructions, application calculations were based on a 300-foot swath using equipment to produce droplets within the range of 8-20 microns (mass median diameter) at a rate not to exceed 0.007 lb permethrin/acre; minimum recommended application rate of Aqua-Reslin is 0.0015 lb permethrin/acre. Prior to the experimental applications, droplet size of the ULV fogger was measured using an AIMS unit provided by the Sacramento-Yolo Mosquito and Vector Control District (Sac-Yolo MVCD).

All product formulations were applied using a truck-mounted ULV fogger. The fogger was manufactured by the Colusa Mosquito Abatement District. This home-built model consisted of an 18 hp Honda electric-start engine with a Roots 45 blower, and an "Ihplat" nozzle operating at 4.5 lb nozzle pressure. Formulations were applied in order of increasing permethrin concentration. The fogging unit was flushed for approximately ten minutes between applications.

Meteorological data were recorded on-site every 15 minutes, beginning approximately one hour before the first trial and terminating 15 min after the final trial. Temperature, relative humidity, wind speed and direction were recorded electronically with a Capricorn II® weather reporter. Ambient air temperature was measured at six and 30 feet above the ground. Applications were made only when a temperature inversion was detected.

Caged mosquitoes (wild and laboratory-reared) were placed on a test line running perpendicular to and downwind of the ULV applicator's path at distances of 100, 200, 300 and 400 feet (30-122 meters).

Mosquitoes used in the bioassays were from two sources. Wild-caught mosquitoes were trapped the previous night in the Colusa area using CO_2-baited EVS light traps. Samples of these mosquitoes indicated that species composition was 94.0% *Culex tarsalis*, 5.5% *Anopheles freeborni*, and 0.5% *Aedes melanimon*. The wild-caught mosquitoes were maintained in cartons supplied with sucrose pads until approximately four hours before the field trials, at which time they were loaded into bioassay cages. Laboratory-reared susceptible *Culex pipiens* were obtained from a colony at Sac-Yolo MVCD and were similarly maintained during the day of the field trials.

Approximately 20 (range 20-26) wild-caught and 30 (range 20-52) laboratory-reared mosquitoes were placed into disposable bioassay cages, constructed of cardboard tubing and nylon netting as described by Townzen and Natvig (1973). A total of 40 cages were used during the evaluation. One cage each of wild-caught and laboratory-reared mosquitoes was placed approximately 3 feet above ground level at each 100-foot interval downwind from the ULV application path. Likewise, cages of control mosquitoes were placed approximately 300 feet upwind of the application path. Cages were left in the test field for approximately 10 min following ULV application to ensure exposure. Following each run, all exposed and control cages were provided with water-soaked cotton pads and placed individually into plastic bags for return to the laboratory and subsequent monitoring. Knockdown observations and mortality data were recorded at two, 12, and 24-hr post-treatment.

RESULTS & DISCUSSION

The ULV applications were conducted under near ideal meteorological conditions. A temperature inversion occurred 30 min prior to the first run and was maintained throughout the study period (Figure 1). A southeast wind of three to six miles per hour (4.8 – 9.7 km/hr) was constant throughout the four runs (Table 1). Droplet size analyses indicated mass mean diameters ranging from 19.1 to 20.8 microns for the four applications.

The 2-hr post-treatment evaluation indicated that each of the four applications achieved 100% knockdown of all exposed laboratory-reared and wild-caught mosquitoes at all distance intervals. A small percentage (< 10%) of "spinners" was noted in several of the treated cages in Runs 2-4, but most exposed mosquitoes already were dead. For mortality reporting purposes, spinners and other mosquitoes lying on the bottom of the cages were considered moribund and counted as dead

Table 1. Meteorological data during four applications of Aqua-Reslin at Colusa Co., CA.

Run	Time	Temp. Inversion(F°)*	Wind Direction	Wind Speed (MPH)
1	1950	0.7	SE	4.0
2	2010	0.8	SE	6.0
3	2026	0.9	SE	3.0
4	2045	1.0	SE	3.0

* Difference between the air temperature at 30 ft above ground and 6 ft above ground.

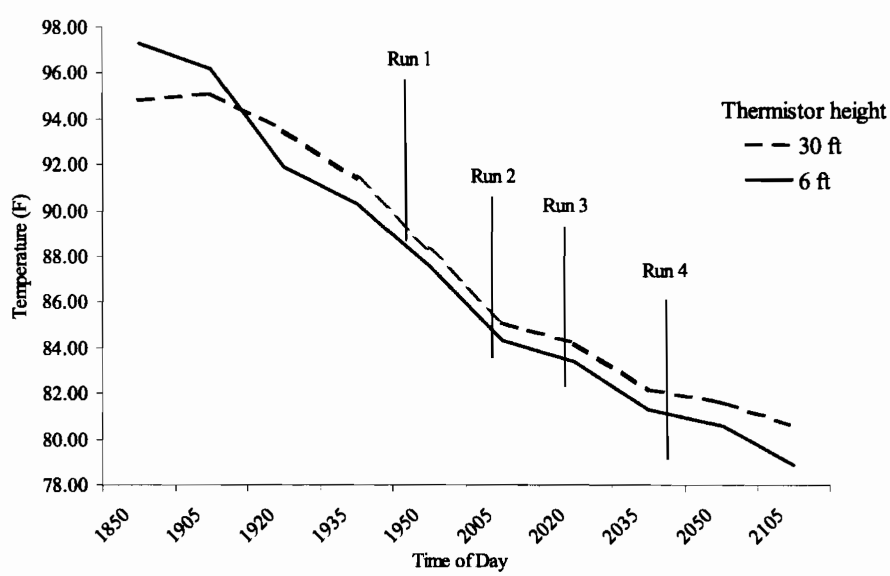

Figure 1. On-site temperature inversion data

Table 2. Percent observed mortality of caged laboratory-reared *Culex pipiens* and wild-caught, principally *C. tarsalis*, mosquitoes following fc ULV applications of Aqua-Reslin.

Run 1 4 % AI at 8.10 fl oz/min

Distance (ft)*	Cage	n	% Observed Mortality 2 hr	12 hr	24 hr
100	Lab	36	100.00	-	-
	Wild	25	100.00	-	-
200	Lab	42	100.00	-	-
	Wild	38	100.00	-	-
300	Lab	20	100.00	-	-
	Wild	20	100.00	-	-
400	Lab	20	100.00	-	-
	Wild	22	100.00	-	-
Control	Lab	25	0.00	0.00	0.00
	Wild	21	0.00	0.00	4.76

Run 2 5% AI at 4.00 fl oz/min

Distance (ft)	Cage	n	% Observed Mortality 2 hr	12 hr	24 hr
100	Lab	41	100.00	-	-
	Wild	20	100.00	-	-
200	Lab	41	100.00	-	-
	Wild	37	100.00	-	-
300	Lab	40	100.00	-	-
	Wild	21	100.00	-	-
400	Lab	46	100.00	-	-
	Wild	22	100.00	-	-
Control	Lab	34	0.00	0.00	0.00
	Wild	20	0.00	0.00	0.00

Run 3 6.67% AI at 4.90 fl oz/min

Distance (ft)	Cage	n	% Observed Mortality 2 hr	12 hr	24 hr
100	Lab	41	100.00	-	-
	Wild	22	100.00	-	-
200	Lab	52	100.00	-	-
	Wild	21	100.00	-	-
300	Lab	39	100.00	-	-
	Wild	23	100.00	-	-
400	Lab	31	100.00	-	-
	Wild	26	100.00	-	-
Control	Lab	38	2.60	2.60	2.60
	Wild	20	0.00	0.00	5.00

Run 4 10 % AI at 3.25 fl oz/min

Distance (ft)	Cage	n	% Observed Mortality 2 hr	12 hr	24 hr
100	Lab	36	100.00	-	-
	Wild	24	100.00	-	-
200	Lab	26	100.00	-	-
	Wild	21	100.00	-	-
300	Lab	37	100.00	-	-
	Wild	20	100.00	-	-
400	Lab	34	100.00	-	-
	Wild	33	100.00	-	-
Control	Lab	33	0.00	0.00	0.00
	Wild	21	0.00	0.00	0.00

* Distance downwind from the ULV application path

mosquitoes. No significant differences in knockdown or mortality were observed between laboratory-reared susceptible mosquitoes and wild-caught mosquitoes. No post-treatment morbidity or mortality was observed in the control cages for three of the four runs. A single mosquito (2.6% mortality) was dead in the laboratory-reared control mosquitoes for Run-3.

No recovery of treated mosquitoes was observed at 12-hr post-treatment. Although a few mosquitoes were observed moving their wings or legs, no spinners were observed.

At 24-hr post-treatment, all treated mosquitoes were dead. The control cages had little or no mortality (range 0 to 5%). Mortality data for all field trials (Runs 1-4) are summarized in Table 2.

The diluted formulations of Aqua-Reslin were applied at the middle to lower middle range of recommended application rates. No differences in mosquito mortality were noted between application rates or flow rates. In all four ULV applications, caged mosquito mortality indicated an effective swath width in excess of that expected from the label information. However, these runs were conducted under near ideal climatic and topographical conditions. Most applications of this product, per label instruction, are intended for residential, industrial, park and other areas where buildings, trees and other landscape features may significantly affect effective swath width. Previously, Inman et al. (1997) reported considerably lower mortality of *Aedes nigromaculis* in Aqua-Reslin field trials, but the authors noted that adverse weather conditions during their trials warranted further evaluation of this product.

Acknowledgements

The authors wish to thank Dan Kiely of Colusa MAD for his technical assistance, Glenn Yoshimura and Sac-Yolo MVCD for use of the AIMS unit and the laboratory-reared mosquitoes, and Fennimore Chemicals for supplying the insecticide.

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Field Trials of Vectolex® (*Bacillus sphaericus*) WDG and CG in Catch Basins in San Mateo County, California

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ABSRACT: Two formulations of VectoLex® were applied to catch basins in San Mateo County during the summers of 1999 and 2000. In 1999, 30 basins each were treated with 0.1, 0.3, 0.4 or 0.5 g VectoLex® WDG (Water Dispersible Granule). One week after treatment, the number of basins containing late stage larvae or pupae decreased by an average of 75%. Basins remained free of late stage larvae or pupae for an average of 1-2 weeks following treatment (range 0-4 weeks). By 3 weeks post-treatment, the proportion of basins containing late stage larvae or pupae returned to pre-treatment levels. In 2000, the efficacy of the WDG formulation of VectoLex® was compared to that of CG (Coarse Granule). No significant difference in the duration of control was observed between these two formulations.

Treatment of catch basins is a major focus of the control program at the San Mateo County Mosquito Abatement District (SMCMAD). Encompassing 12 cities within an area of 163 square miles, this is one of the most highly urbanized districts in California. The SMCMAD has over 10,000 catch basins within its boundaries. Nearly 7,000 of these require regular treatment during warmer months to control mosquitoes. The most common species found breeding in these basins is *Culex pipiens pipiens* L. *Culex p. pipiens* readily enters houses to feed on humans, and accounts for over half of the district's mosquito-related service requests each year. As a vector of St. Louis encephalitis, western equine encephalomyelitis and West Nile virus, this mosquito also presents a potential disease threat to residents of the district (Carpenter and LaCasse 1955).

The SMCMAD has conducted mosquito control in catch basins since the 1950's using a wide array of materials from organochlorines and organophosphates to larviciding oils. Under the current program, Golden Bear Oil® (GB 1111) is applied to basins using a side-mounted spray wand on a right-hand drive vehicle. The SMCMAD relies on seasonal aides to complete this work, and hires 4-5 aides each year to treat basins from June through September. This program has been fairly efficient and cost-effective at reducing service requests, but problems have arisen in recent years. Recruitment of summer hires is more difficult and the district is often unable to hire sufficient staff to treat all catch basins on a weekly basis. In addition, many of the storm drain systems in San Mateo County drain directly into San Francisco Bay. Tighter controls on emissions from these systems in the future may restrict the materials that can be applied to catch basins for mosquito control. These issues have prompted the district to evaluate new materials for our catch basin program. The district seeks a material that can be applied with existing equipment, has minimal toxicity to non-targets, and provides some degree of residual activity.

VectoLex® (*Bacillus sphaericus*) has been used to successfully control mosquitoes in catch basins in other areas, and may provide a viable alternative in the San Francisco Bay Area (Siegel and Novak 1997, 1999). This material is highly effective against *Culex* and *Culiseta* species in clear or polluted water. Under some conditions, a single application can control mosquito larvae for up to 60 days (see Yap 1990 for a review). The WDG (water dispersible granule) formulation mixes easily with water and can be applied with the same equipment used for dispensing oil and other liquid larvicides. In 1999, the SMCMAD conducted field trials with this material to determine its efficacy and length of activity in catch basins.

MATERIALS AND METHODS

Pesticide Application and Sampling

VectoLex® (*B. sphaericus* strain 2362, serotype H5a5b) was tested in 4 trials at 2 locations during the summers of 1999 and 2000. Three trials were conducted in 1999 using increasing concentrations of the WDG formulation (Table 1). The material was applied to basins in a residential neighborhood in the city of San Mateo. This area encompasses approximately 40 blocks and contains about 187 basins. Many of these basins hold water during summer months and are a steady source of both *Cx. pipiens* and *Culiseta incidens* (Thomson). At the outset of the study (June, 1999) all basins in this area were examined. Sixty-six basins with standing water were included in the study. Approximately 36% of these basins were connected to drain lines. Water in the basins averaged 25.6 square feet (0.73 m^2) in surface area and varied in depth and degree of organic pollution. VectoLex® WDG was applied to basins with a Birchmeyer hand can. In the first trial, basins were sampled immediately before treatment, 48 hours after treatment, and at 1, 2, and 3 weeks post-treatment (PT). In subsequent trials,

Table 1. Application rates for VectoLex® (*Bacillus sphaericus*) in field trials in San Mateo County, California, 1999–2000.

Year	Trial No.	Dates	Formulation	Application Rate /Acre[1]	Calculated Application Rate/Basin[2] g/basin (BsITU/basin)
1999					
	1	Jun 14–Jul 01	WDG	0.5 lb/acre	0.1 (87,100)
	2	Jul 01–Aug 04	WDG	1 lb/acre	0.3 (195,000)
			WDG	1.5 lb/acre	0.4 (260,000)
	3	Aug 04–Sep 01	WDG	1 lb/acre	0.3 (195,000)
			WDG	2 lb/acre	0.5 (325,000)
2000					
	4	Aug 14–Sep 11	WDG	2 lb/acre	0.5 (325,000)
			CG	2 lb/acre	6.5 (325,000)

[1] Based on an average surface area of 25.6 ft^2/basin

[2] Calculated from the following formula: g/basin = (lb/acre X 0.00059 acres/basin) X 0.0022046 g/lb

basins were sampled immediately before treatment and at weekly intervals thereafter. Sampling was done with a standard 1-pint dipper, taking 3 dips from each basin. The presence and stage of immature mosquitoes was recorded, along with water depth and percent surface coverage by debris. Peak mortality from the toxin of *B. sphaericus* occurs 48 hrs after exposure and usually affects the 3rd, 4th, or pupal stages. Early instars generally have not received sufficient exposure and may still be present in treated sites (Mulla et al. 1984). For this reason, basins were considered positive only if they contained late stage larvae or pupae. Basins that were dry or had substantial flow at the time of sampling were not included in calculations for that date.

To determine whether loss of larvicidal activity could be due to settling of the pesticide out of the water column, water in the basins used in the second trial was stirred with a shovel after sampling at 3 weeks PT. The same basins were sampled again the following week. The percent of basins containing late stage larvae and / or pupae at this time was compared with that of the previous week.

Comparison of WDG and CG Formulations

In 2000, the WDG formulation of VectoLex® was compared with the CG (coarse granular) formulation. Thirty basins were treated with WDG at 1.8 lb per acre (0.5 g/basin, 325,000 *Bacillus sphaericus* International Toxic Units (BsITU)). An additional 30 basins were treated with CG at 24.3 lb/acre (6.5 g/basin, 325,000 BsITU). Ten basins were left untreated. This trial was conducted at a 35 square block area in San Mateo approximately 2.5 mi south of the location used in 1999. Basins were sampled immediately prior to treatment and at weekly intervals thereafter. Sampling was conducted in the manner described above.

Statistical Analysis

The chi square test was used to evaluate differences in the proportion of basins containing late stage larvae or pupae after treatment with various concentrations of VectoLex® WDG. Fisher's Exact Test was used to compare control between basins treated with the different formulations of VectoLex®. This test was also used to compare the degree of control achieved in basins treated in different years and at different locations. The duration of control was calculated as the last sampling date after treatment at which no late stage larvae or pupae were found in an individual basin. When late stage larvae or pupae are found, the product is no longer effective. Basins that contained no larvae prior to treatment were excluded from calculations of residual activity. Variation in the duration of control among basins treated at different application rates in 1999 was analyzed by ANOVA.

RESULTS

Application of both the WDG and CG formulations of VectoLex® resulted in a significant reduction in numbers of basins with late stage larvae or pupae by 1 week PT (average 75% + 27% reduction) (Figure 1). However, this degree of control was short-lived. The proportion of positive basins had begun to increase again by week 2 in all trials and by week 3 PT, 35 - 96% of treated basins had late stage larvae or pupae in them (Figure 2).

Trials conducted at various application rates did not differ significantly in degree of control at 1 or 2 weeks PT (Table 2). In trial 4, there was no significant difference in degree of control between basins treated with different formulations of VectoLex® (WDG or CG) (Table 2). Comparison of basins treated with WDG at 2 lb per acre in 1999 and 2000 showed no significant difference in control between the 2 sites or between years (Table 2).

Figure 3 shows the relationship between application rate and duration of control. Increasing the application rate resulted in a slight increase in residual activity. However, the increase was not statistically significant. The duration of control did not differ significantly between basins in different locations, or treated in different years at different sites. Basins treated with different formulations of VectoLex® (WDG or CG) did not differ significantly in duration of control, nor was there any correlation between duration of control and larval density at the time of treatment.

DISCUSSION

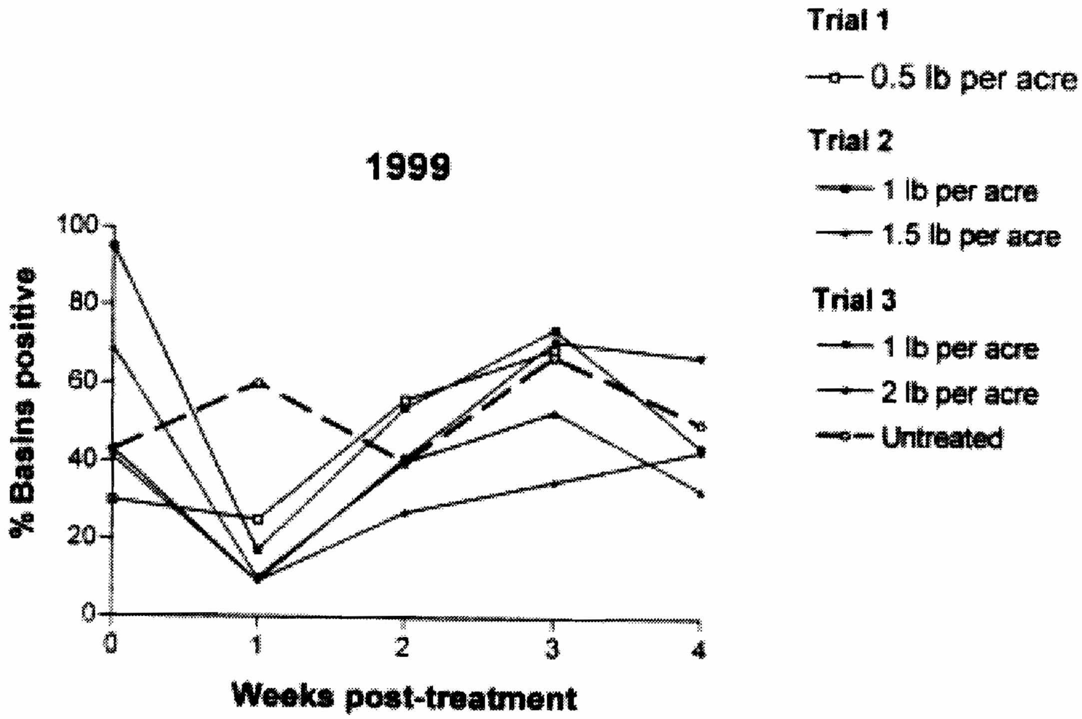
VectoLex® CG and WDG were both very effective at reducing mosquito breeding in catch basins at 1 week PT. Application rates > 1 lb per acre eliminated larvae at over 80% of basins within 1 week of treatment. However, control was not long lasting and by 2 weeks PT the proportion of basins positive for late stage larvae or pupae had returned to nearly that seen during pretreatment sampling. These results differ from previously published reports on the application of VectoLex® to catch basins in other parts of North America. Siegel and Novak (1997) reported an average of 46 days of control in basins treated with 0.68 g VectoLex® CG each (34,000 BsITU/1.2 m^2). In 1999, these authors reported that 98% of 322 basins treated with 1 g/basin VectoLex® CG (50,000 BsITU/basin) remained free of larvae or pupae for 33 days. Basins in the present study were treated with much higher quantities of material (90,000 to 350,000 BsITU/basin), yet the average duration of control was only 1-2 weeks.

To our knowledge, the only published study on the use of VectoLex® in catch basins in California is that of Mulligan et al. (1980). These authors treated catch basins in Fresno with a commercially available powdered formulation of *B. sphaericus* strain 1593-4. Mosquito larvae in the laboratory were periodically exposed to water from treated basins. Water taken from the surface of treated basins at 1 week PT killed 95-100% of larvae. However, only 10% of exposed larvae were killed by surface water taken from the same basins at 2 weeks PT. Water from the bottom of the basins retained activity for up 4 weeks, and mechanical stirring of the sediment at the bottom of basins several weeks after application resulted in over 90% mortality of exposed larvae. The authors concluded that the bacterial spores settled to the bottom of the basins, and therefore became unavailable to larvae. Settling was probably not the factor responsible for the loss of activity in the present study. Stirring of basins in the 1999 location at 3 weeks PT resulted in a slight decrease in the proportion of basins containing larvae. However, the decrease was not statistically significant, and the proportion of basins producing mosquitoes was operationally unacceptable.

Residual activity may also vary widely following treatment of other source types with VectoLex®. Kramer (1990) applied VectoLex® CG to pools in 3 creeks in Contra Costa County. Larval density returned to pre-treatment levels after 1 and 2 weeks in 2 of the creeks, but remained significantly lower for 30 days in a third creek. Differences in duration of larvicidal activity were not correlated with water quality, flow or temperature (Kramer 1990). Similarly, treatment of dairy waste lagoons with VectoLex® G (granular) gave adequate to good control for 1-3 weeks in southern California. However, 3 of 10 ponds had significant resurgence in larval density 2 weeks after treatment (Binding et al. 1996).

Residual activity by *B. sphaericus* can be influenced by a number of factors including water quality, depth, larval species composition and density, solar radiation, and strain and formulation of the pesticide itself (reviewed in Lacey 1990 and Yap 1990). Water quality and depth of the catch basins in the present study varied widely and were not correlated with duration of control. Solar radiation was also not likely to be a cause for the lack of residual control since the basins in question were underground and covered by steel grates. The strain (2362, serotype H5a5b) and formulations of *B. sphaericus* used in this study were the same as those used in the previously mentioned studies (Siegel and Novak 1997, Kramer 1990).

In conclusion, application of VectoLex® to catch basins in San Mateo County gave excellent initial knockdown of mosquito larvae in catch basins. However, this material did not provide long lasting control. Duration of control did not differ significantly between basins treated in different years, locations, or with different formulations of VectoLex®.



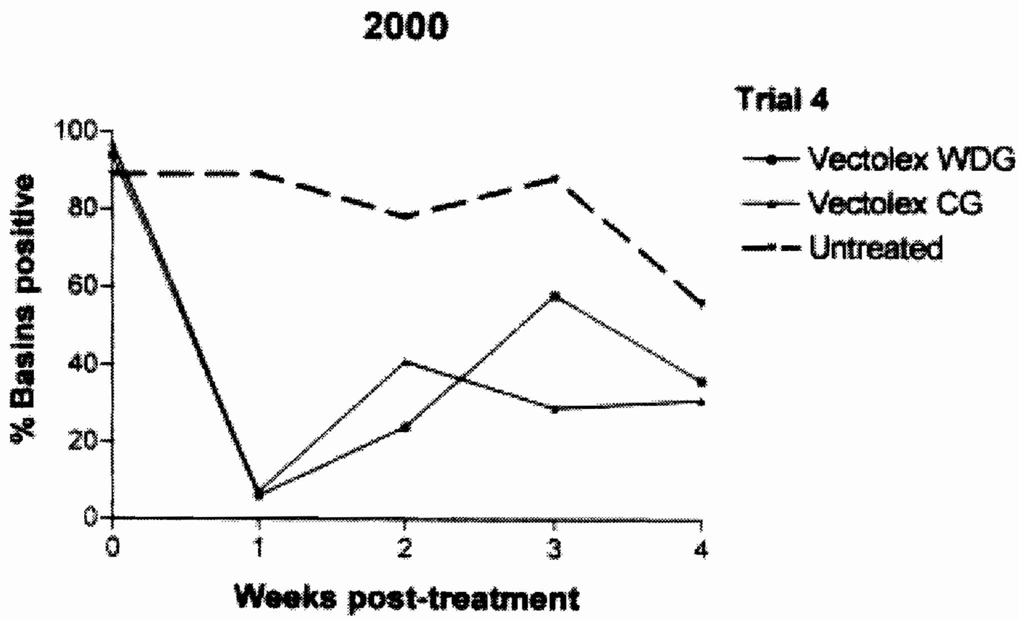


Figure 1. Proportion of catch basins containing late stage mosquito larvae or pupae after treatment with VectoLex WDG in 1999 and 2000, San Mateo County, California.

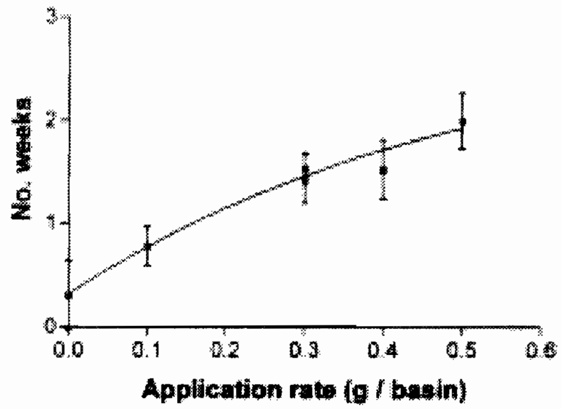


Figure 2. Average number of weeks that individual catch basins remained free of late stage larvae or pupae after treatment with Vectolex® WDG in 1999, San Mateo County, California.

Table 2. Results of statistical analysis on the effect of dosage rates on the efficacy of VectoLex formulations for control of immature mosquitoes in catch basins in San Mateo County, 1999-2000.

Factor Evaluated		No. basins with late stage larvae or pupae		Test Used	Test Statistic	P value[1]
		+	-			
Dosage						
1 week PT						
	.5	6	18			
	1	8	53			
	1.5	2	9			
	2	4	49	Chi²	$x^2 = 4.782$, 3 df	0.1885 (NS)
Dosage 2 weeks PT						
	.5	10	8			
	1	28	2			
	1.5	6	9			
	2	13	38	Chi²	$x^2 = 7.416$, 3 df	0.0598 (NS)
Formulation 1 wk PT						
	WDG	2	29			
	CG	2	28	Fisher's Exact		0.2630 (NS)
2 wks PT						
	WDG	7	22			
	CG	12	17	Fisher's Exact		1.0 (NS)
3 wks PT						
	WDG	5	12			
	CG	14	10	Fisher's Exact		0.1120 (NS)
4 wks PT						
	WDG	4	9			
	CG	5	9	Fisher's Exact		1.0 (NS)
Location & Year						
1 wk PT						
	1999	2	20			
	2000	2	29	Fisher's Exact		1.000 (NS)

[1] NS = not statistically significant

Table 3. Average length of time individual basins remainded free of late stage mosquito larvae or pupae following treatment with various amounts of VectoLex ® WDG in San Mateo County in 1999.

Trial No.	Amount Applied (g/basin)	Weeks of Control Mean	SD	N
1	0.1	0.8	0.570	9
2	0.3	1.5	0.761	26
3	0.3	1.44	0.570	25
2	0.4	1.53	1.050	13
3	0.5	2.00	1.120	17

Increasing the application rate did not significantly extend the duration of control.

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Susceptibility of *Culex* Mosquitoes Breeding in Dairy Ponds to *Bacillus sphaericus*

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ABSTRACT: During the heavy breeding seasons of 1996, 1997 and 1998, *Culex* larvae were collected weekly or biweekly from dairy wastewater lagoons in the Chino Basin of the Northwest Mosquito and Vector Control District (NWMVCD), Riverside County, California. These breeding sites were treated with Golden Bear GB 1111 larvicidal oil alone in 1996 and with both the oil and VectoLex CG (*Bacillus sphaericus* 2362) in 1997 and 1998. VectoLex was not applied to these ponds prior to 1997. The species composition of the collected larvae from the ponds consisted of mostly *Culex quinquefasciatus* Say and *Cx. stigmatosoma* Dyar, while *Cx. tarsalis* Coquillett occurred in low numbers. To determine the susceptibility profile of field larvae pre- and post-treatments with VectoLex, laboratory bioassays were carried out using VectoLex TP. In 1996 when *B. sphaericus* treatment was not made, *Culex* larvae from the ponds were highly susceptible to *B. sphaericus*. Compared with the baseline data from bioassays in 1996, the susceptibility at the LC_{50} and LC_{90} levels was lowered somewhat in 1997 as shown by the shift in LC_{50} and LC_{90} distributions to the higher ranges. In 1998 it was essentially the same as in 1997, although a slight increase in susceptibility was noted as indicated by the shift of LC_{50} and LC_{90} distribution to the lower ranges. No significant reduction in *B. sphaericus* susceptibility in the monitored *Culex* populations was detected after 2 years of joint application of VectoLex CG and larvicidal oil in 1997 and 1998. The heterogeneity of the *Culex* populations was reduced by the treatments in 1997 and 1998, as indicated by the shift in distribution of LC_{90}/LC_{50} ratios to the lower range from 1996, 1997 to 1998.

INTRODUCTION

Bacillus sphaericus Neide is a rod–shaped, aerobic, spore–forming bacterium commonly found in nature. To date, over 300 strains have been isolated and identified, among which at least 16 strains have shown moderate to high levels of mosquitocidal activity. The strains 1593M and 2362 (both serotype H5a5b) and 2297 (serotype H25) have been the subject of more intensive investigations than the other strains (Charles et al. 1996). The highly toxic strains are capable of producing parasporal inclusion bodies during sporulation, which contain the binary mosquitocidal protoxin crystals. Upon ingestion by susceptible mosquito larvae, the protoxins are activated by proteolytic enzymes. The binary toxins act synergistically to induce a series of pathological processes resulting in death of the target larvae (Baumann et al. 1991, Porter et al. 1993).

A number of *B. sphaericus* preparations has been made available for testing and evaluation against mosquito larvae since the late 1980s. Large scale field trials on the formulations of various strains of *B. sphaericus* have been conducted in different countries (Lacey 1990, Yap 1990, Karch et al. 1992, Hougard et al. 1993, Sinègre et al. 1993, Regis et al. 1995, Mulla et al. 1997, 1999, 2001, Skovmand and Sanogo 1999). The advantages of this control agent, such as high efficacy and specificity against some mosquito species, as well as environmental safety have been well documented. However, resistance to *B. sphaericus* in both laboratory and field populations of *Culex* mosquitoes has been reported. When 4^{th} instars of *Cx. quinquefasciatus* Say were subjected to selection by *B. sphaericus* strain 2362 at LC_{80} every generation, the selected colony began developing resistance by the 20^{th} generation (8.1–fold at LC_{50} level), which increased gradually and reached 37–fold by the 80^{th} generation (Rodcharoen and Mulla 1994). In a colony from a previously untreated field population of the same species from Bakersfield, California, the 4^{th} instar larvae demonstrated a high level of resistance > 7,000–fold at the LC_{50} level at the 12^{th} generation, as a response to a high selection pressure at LC_{94-98} every generation (Wirth et al. 2000). In contrast, the same species from a different geographical area in Chino Basin, Riverside County, California, exhibited a different level of response to moderate selection pressure. These larvae, under successive selections at LC_{80}, developed a slight degree (4.4–fold) of resistance at the LC_{50} level in the 3^{rd} generation, then the resistance level fluctuated between 10– and 30–fold during the subsequent selections up to 80 generations (Rodcharoen and Mulla 1994).

The development of resistance in field populations of the

Culex pipiens complex in different geographical areas has also been documented recently, where *B. sphaericus* formulations have been used in large scale control programs for various durations (Sinègre et al. 1994, Rao et al. 1995, Adak et al. 1995, Silva–Filha et al. 1995, Yuan et al. 2000). The first case of high level (16,500–fold) of field resistance was reported in *Cx. pipiens pipiens* after 18 treatments in 8 years using Spherimos strain 2362 in Port Saint Louis, southern France (Sinègre et al. 1994). Other cases were also reported from different parts of the world. In Kochi, India, for instance, field resistance to *B. sphaericus* was observed in a population of *Cx. quinquefasciatus* after 35 rounds of spraying using Biocide–S strain 1593M over a 2–year period. Larvae from the sprayed area had LC_{50} and LC_{90} values that were 146 and 180 times greater than corresponding values for a susceptible strain from an unsprayed locality. As a response to the moderate selection pressure at every generation in the laboratory, field collected larvae developed a resistance of 6,223– and 31,325–fold at LC_{50} and LC_{90} levels respectively at the 18th generation (Rao et al. 1995). In the multicentric trials using the commercial preparation of *B. sphaericus*, Spherix (Russian strain B–101, Serotype H5a5b) to control *Cx. quinquefasciatus* in India, this formulation was initially effective at 1 g/m^2 for 2–4 weeks.

After 20–25 rounds of continuous application within one year, up to 155– fold resistance at the LC_{90} level in the treated field population was noted. A homozygous *B. sphaericus* resistant strain of *Cx. quinquefasciatus* was isolated from a population in the treated area and maintained in the laboratory for 6 generations without selection pressure. This colony demonstrated a resistance level as high as 52,000–fold at LC_{50} level when bioassayed with Spherix (Adak et al. 1995). In Recife, Brazil, a field population of *Cx. quinquefasciatus* was intensively treated with *B. sphaericus* strain 2362 whole culture or Spherimos for 25 months (37 treatments). This population was found to be approximately 10–fold less susceptible than control field populations (Silva–Filha et al. 1995). Most recently, a high level of resistance to *B. sphaericus* C3–41 in *Cx. quinquefasciatus* (22,672–fold at LC_{50} level) was reported in southern China, where a flowable formulation of this strain had been continuously used for 8 years to control *Cx. quinquefasciatus* (Yuan et al. 2000).

In 1991, VectoLex, a *B. sphaericus* product of Abbott Laboratories, North Chicago, Illinois, was registered by the US Environmental Protection Agency for mosquito control in the United States. This product was registered in California in 1996. Based on the published information to date, the susceptibility to *B. sphaericus* in *Culex* mosquito populations of various geographical origins is quite variable, and some mosquito populations became resistant to *B. sphaericus* preparations much faster than others (Rodcharoen and Mulla 1994, Sinègre et al. 1994, Rao et al. 1995, Adak et al. 1995, Silva–Filha et al. 1995, Yuan et al. 2000, Wirth et al. 2000).

To document the profile of *B. sphaericus* susceptibility and impact of application of *B. sphaericus* formulations on it, we initiated these studies on *Culex* larval populations breeding in dairy wastewater lagoons in the Chino Basin of the Northwest Mosquito and Vector Control District (NWMVCD), Riverside County, California. The data of *B. sphaericus* susceptibility were collected during the heavy breeding season in 1996, when no *B. sphaericus* formulation was used, and in 1997 and 1998, when Vectolex CG and Golden Bear GB 1111 larvicidal oil were jointly applied for mosquito control.

MATERIALS AND METHODS

Study area and treatment

The study area comprised 1,036 ha in the Chino Basin located in Riverside County, California (NWMVCD jurisdiction). More than 20 dairy farms are in operation, each of them having 1–2 wastewater lagoons which are used for holding highly polluted barn–wash water. These lagoons or ponds support breeding of heavy populations of *Culex* mosquitoes. The ponds were inspected weekly for the presence of mosquito larvae during the breeding seasons of 1996, 1997 and 1998 by the technical staff of the NWMVCD. Sources were treated by district personnel on a weekly basis in 1996 using only Golden Bear GB 1111 larvicidal oil. After VectoLex was registered in California in 1996, a corn grit formulation (CG) along with the larvicidal oil were used in 1997 and 1998. Larvicidal oil was only applied if and when pupae were present. In the absence of pupae, VectoLex CG was used. VectoLex CG was applied at 5.8–11.6 kg/ha, and larvicidal oil at 19.0–47.3 liter/ha.

Field susceptibility surveillance

Mosquito collection and handling: Culex larvae were collected from the treated dairy ponds weekly or biweekly during June to November of 1996, 1997 and 1998, with a 350 ml dipper and concentrated through a screen concentrator cup, then transferred to a plastic bucket (2 L in volume). The volume of habitat water in the bucket was less than 4/5 of the bucket's capacity to ensure the availability of air for larvae. The buckets holding the collected larvae were placed in an ice chest and brought to the laboratory where the larvae were transferred to enamel pans each containing 2 L of tap water, using a fine screen strainer. The larvae in each pan were fed 2 g rabbit pellets (Brookhurst Mill, Riverside CA, crude protein e" 17%). The larvae were held for at least 24 h before setting up the bioassay, during which time the dead and moribund larvae were removed. Species composition was determined for each sample. The late 3rd and early 4th instars were used in bioassays. The remaining larvae were kept for repeated tests, if necessary, until satisfactory dosage response lines were obtained. During the holding period, the pupae from the unused collections were removed and discarded daily to prevent emergence.

Bioassay: The test material was Vectolex TP, technical powder of *B. sphaericus* strain 2362 (ABG–6184, Lot# 13–194–W5) with a potency of 2,000 ITU/mg, which was received on 3/21/1996 from Abbott Laboratories, North Chicago, Illinois. A quantity of 200 mg technical powder was suspended in 20 ml distilled water by vigorous shaking to ensure the homogeneity of the 1% (W/V) suspension, which was then diluted serially 10–fold in amounts of 20 ml, obtaining suspensions with concentrations of 0.1%, 0.01%, 0.001% and 0.0001%. The needed aliquots of the diluted suspensions were added to 120 ml waxed paper cups each containing 100 ml tap water and the test larvae (see below).

To find the activity range for each sample, a preliminary test was conducted using 3–5 concentrations ranging from 0.0005 to 0.1 ppm of VectoLex TP and an untreated control. Each treatment was replicated 3 times. Twenty five late 3rd or early 4th instar larvae were transferred to 100 ml tap water in each 120 ml test cup. The necessary aliquot of VectoLex TP suspension was then added to each cup. One drop of 10% larval food (powdered rabbit pellet and Brewer's yeast in the ratio of 3:1) was given to each test cup. In total, 225–375 larvae in the preliminary test were used in bioassay for each field sample. The bioassay was carried out in a holding room maintained at 27°–29°C. Mortality was recorded at 48 h after treatment, and moribund larvae were considered dead. If the mortality in untreated control was greater than 5%, the test was discarded. Using the concentration range from the preliminary test as a reference, a final test was set up using 3–5 concentrations yielding 10–95% mortality and 9 replicates were made for each concentration and control. A total of 675–1125 larvae for each sample were used in bioassay. At the same time, the microbial product VectoLex TP stored at 4°C was bioassayed periodically against a susceptible reference colony of *Cx. quinquefasciatus* to evaluate the activity level of the microbial material.

Data analysis: The data of the dosage/response relationship were subjected to probit regression analysis using POLO–PC (LeOra Software 1987). The LC_{50}, LC_{90} and their 95% confidence limits as well as LC_{50}/LC_{90} ratios were obtained.

RESULTS AND DISCUSSION

Field treatments

To collect baseline data of *B. sphaericus* susceptibility in natural *Culex* populations, during the months of April through November of 1996, the ponds on 18 dairy farms were sampled. Treatments for mosquito control were made with Golden Bear GB 1111 larvicidal oil alone. The frequency of treatment and amounts applied were based on abundance of mosquito larvae and pupae. No *B. sphaericus* treatments were made anywhere in the region in 1996 and previous years.

During April to November in 1997 and 1998, the study area was treated with both the microbial larvicide VectoLex CG and the larvicidal oil GB 1111. Larval samples were collected and used in bioassays with VectoLex TP to detect changes of *B. sphaericus* susceptibility in the natural populations. The treatment regimens (larvicides, treatment frequency and amounts applied) were different among the habitats, depending on the density and age composition of immature mosquitoes. In 1997, ten wastewater lagoons were sampled. In total, 76 treatments with VectoLex CG and 153 treatments with larvicidal oil were made in eight of these ten ponds. The quantities applied in each treatment varied from 0.5–16.0 kg for VectoLex CG and 3.8–94.6 liters for the oil. In total, 340.6 kg of VectoLex CG and 2297.7 liters of larvicidal oil were applied in these eight ponds. Two other ponds received a total of 26 treatments using larvicidal oil alone. At each treatment, 3.8–30.3 liters of oil were applied, amounting to 238.5 liters of larvidical oil being applied to these two ponds. In 1998, ten ponds in the same study area were selected and monitored for susceptibility to *B. sphaericus*. Among these ten ponds, seven ponds received 27 treatments of VectoLex CG and 105 treatments of Golden Bear GB 1111 larvicidal oil. The quantities applied in each treatment were 0.5–11.3 kg of VectoLex CG and 3.8–45.4 liters of the oil. In total, 92.5 kg of VectoLex CG and 1340.0 liters of larvicidal oil were applied to these seven ponds. The other three ponds received a total of 22 treatments of oil alone with 3.8–30.3 liters of oil being applied per treatment. All together, 189.3 liters of oil were applied in these three ponds.

Field susceptibility surveillance

During the breeding seasons of 1996, 1997 and 1998, a total of 32, 30 and 35 samples of *Culex* larvae were collected weekly or biweekly from the dairy wastewater lagoons respectively. Among the collected larvae, *Cx. stigmatosoma* Dyar and *Cx. quinquefasciatus* were the dominant species, representing 53–87% and 13–47% of the total population respectively in 1996, 10–94% and 6–89% respectively in 1997, and 18–79% and 11–82% respectively in 1998. *Cx. tarsalis* Coquillett was found at a low percentage, less than 12% in the samples collected during these 3 years. The species composition is attributable to the highly polluted water in the ponds resulting from dairy operations. The former two species prefer to lay their eggs and breed in polluted water, while *Cx tarsalis* chooses relatively clear water as oviposition sites (Gjullin and Johnson 1965, Rodcharoen et al. 1997).

To determine the susceptibility of larvae to *B. sphaericus*, field-collected larvae were used in bioassay with VectoLex TP using the procedures described above. During the 3-year study, differences in the susceptibility to *B. sphaericus* were indicated not only among the samples collected from different dairies, but also among those taken from the same dairy on different dates in a given year under a specific treatment regimen. Among the ranches Dyt2, Harrison, Hettinga, Leal, Midhill, Providence and Schakel which were sampled most frequently during the study period, the LC_{50} and LC_{90} values were quite variable temporally and spatially in a given sampling year (Tables 1 and 2). In 1996, among the three frequently sampled ranches, Harrison, Midhill and Providence, the LC_{50} and LC_{90} levels varied within 2.0–4.0 fold and 1.6–3.9 fold, respectively. The average LC_{50} and LC_{90} values ranged from 0.005–0.008 ppm and 0.018–0.026 ppm respectively. In 1997, among the four frequently sampled ranches, Dyt2, Hettinga, Midhill and Providence, the LC_{50} and LC_{90} levels fluctuated within 1.8–3.4 fold and 2.0–3.6 fold respectively. The average LC_{50} and LC_{90} levels ranged from 0.006–0.016 ppm and 0.018–0.072 ppm respectively. In 1998, the LC_{50} and LC_{90} levels varied within 1.2–3.5 fold and 1.1–4.8 fold respectively, among the five frequently sampled ranches, Dyt2, Hettinga, Leal, Midhill and Schakel. The average LC_{50} and LC_{90} levels ranged from 0.004–0.045 ppm and 0.013–0.110 ppm respectively (Tables 1 and 2).

In 1996 when no *B. sphaericus* formulations were applied, 32 samples were subjected to bioassays against VectoLex TP. The overall LC_{50} and LC_{90} values for these samples from the lowest to the highest fluctuated within about 10–fold (0.001–0.010 ppm) and 11–fold (0.005–0.054 ppm), respectively. The LC_{50} values of 50% of the samples were < 0.005 ppm and the other 50% were within 0.005–0.015 ppm. The LC_{90} values of 25% of the samples were < 0.015 ppm and the other 75% were within 0.015–0.075 ppm (Fig. 1). The ratios of LC_{90}/LC_{50}, a parameter of population heterogeneity, ranged 2.0–17.5. As to the distributions of LC_{90}/LC_{50} ratios, 25%, 44% and 31% of them were < 3, 3–5 and > 5 respectively (Fig. 2). This wide range distribution of ratios of LC_{90}/LC_{50} indicates a presence of some *Culex* populations with high level of tolerance to *B. sphaericus* preparations.

Among the 30 samples collected in 1997 when the mosquito populations were treated with both VectoLex CG and larvicidal oil , the LC_{50} and LC_{90} values from the lowest to the highest varied within about 9–fold (0.003 – 0.027 ppm) and 9–fold (0.014–0.121 ppm) ranges, respectively, which were similar to those in 1996. However, compared with the data in 1996, the distributions of LC_{50} and LC_{90} values shifted somewhat to the higher ranges. The LC_{50} values of 3% samples were < 0.005 ppm, 77% of the samples were within 0.005–0.015 ppm, and 20% of the samples were within 0.016–0.050 ppm. The LC_{90} values of 3%, 80% and 17% of the samples were < 0.015 ppm, 0.015–0.075 ppm and 0.076–0.150 ppm, respectively (Fig. 1). The ratios of LC_{90}/LC_{50} ranged 2.0–8.1, 30% of them < 3, 43% within 3–5 and 27% of them > 5 (Fig. 2). This distribution of LC_{90}/LC_{50} ratios shifted slightly to the lower ranges compared with the data in 1996.

As for the 35 samples collected in 1998 when both VectoLex CG (smaller quantities than in 1997) and larvicidal oil were applied, the LC_{50} and LC_{90} values from the lowest to the highest fluctuated within wider ranges, 19–fold (0.0026 – 0.049 ppm) for LC_{50} and 21–fold (0.0054–0.115 ppm) for LC_{90}. The LC_{50} values of 20%, 57% and 23% of the samples were < 0.005 ppm, 0.005–0.015 ppm and 0.016–0.050 ppm, respectively. The LC_{90} values of 20%, 57% and 23% of the samples were < 0.015 ppm, 0.015–0.075 ppm and 0.076–0.150 ppm, respectively (Fig. 1). The distributions of LC_{50} and LC_{90} values in 1998 shifted slightly to the lower ranges compared with those in 1997. The ratios of LC_{90}/LC_{50} were in the range of 2.1–7.3. The LC_{90}/LC_{50} ratios of 34%, 52% and 14% of the

Table 1. Range of LC_{50} and LC_{90} values (ratio of the highest/the lowest LC_{50} or LC_{90}) in *Culex* larvae from a few of most frequently sampled ranches in 1996, 1997 and 1998, as bioassayed with VectoLex TP†.

Ranches	1996			1997			1998		
	No. of bioassays	LC^{50}	LC^{90}	No. of bioassays	LC^{50}	LC^{90}	No. of bioassays	LC^{50}	LC^{90}
DYT2	—	—	—	7	3.4	3.2	5	1.8	1.8
Harrison	4	2.0	2.1	—	—	—	—	—	—
Hettinga	—	—	—	6	3.2	3.6	8	2.9	3.3
Leal	—	—	—	—	—	—	4	1.2	1.1
Midhill	5	4.0	1.6	5	1.8	3.0	6	3.5	4.8
Providence	5	3.8	3.9	4	2.0	2.0	—	—	—
Schakel	—	—	—	—	—	—	5	3.0	3.2

† *Bacillus sphaericus* strain 2362, technical powder, ABG–6184, Lot# 13–194–W5, 2,000 ITU/mg, received on 03/21/1996.

Table 2. Average LC_{50} and LC_{90} values in *Culex* larvae (mainly *Cx. quinquefasciatus* and *Cx. stigmatosoma*) from a few of most frequently sampled ranches in 1996, 1997 and 1998, as bioassayed with VectoLex TP†.

Ranches	1996			1997			1998		
	No. of bioassay	LC^{50} (SE)	LC^{90} (SE)	No. of bioassay	LC^{50} (SE)	LC^{90} (SE)	No. of bioassay	LC^{50} (SE)	LC^{90} (SE)
DYT2	—	—	—	7	0.016 (0.003)	0.072 (0.010)	5	0.006 (0.001)	0.025 (0.003)
Harrison	4	0.006 (0.001)	0.018 (0.004)	—	—	—	—	—	—
Hettinga	—	—	—	6	0.011 (0.002)	0.061 (0.010)	8	0.015 (0.001)	0.070 (0.009)
Leal	—	—	—	—	—	—	4	0.045 (0.002)	0.110 (0.002)
Midhill	5	0.005 (0.001)	0.023 (0.002)	5	0.006 (0.001)	0.019 (0.004)	6	0.004 (0.001)	0.013 (0.003)
Providence	5	0.008 (0.002)	0.026 (0.008)	4	0.006 (0.001)	0.018 (0.003)	—	—	—
Schakel	—	—	—	—	—	—	5	0.008 (0.002)	0.027 (0.005)

† *Bacillus sphaericus* strain 2362, technical powder, ABG–6184, Lot# 13–194–W5, 2,000 ITU/mg, received on 03/21/1996.

samples were < 3, 3–5 and > 5 respectively (Fig. 2), which indicated a slight shift to the lower ranges compared with those in 1997.

During the assessment of field-collected samples, no significant reduction in activity of VectoLex TP used in the bioassays was detected. This was established by using a susceptible colony of *Cx. quinquefasciatus* as a reference, which was used in 6 bioassays against VectoLex TP from April 1996 to October 1998. The LC_{50} and LC_{90} values ranged from 0.024 to 0.036 ppm and from 0.071 to 0.097 ppm, respectively. These differences in LC values is normal in experiments done on different times or within a narrow span of time.

As to the changes in susceptibility pattern in terms of LC_{50} and LC_{90} during the 3 sampling years, a slight increase in tolerance to *B. sphaericus* was noted in 1997 compared with the baseline data from the bioassays in 1996, as shown by the shift of LC_{50} and LC_{90} distributions to slightly higher ranges (Fig. 1). The susceptibility in the samples collected in 1998 from the same areas was essentially the same as in 1997, even though a slight increase in susceptibility was noted as indicated by the shift of the distribution of LC_{50} and LC_{90} values to the lower ranges (Fig. 1). It is obvious that no significant reduction in *B. sphaericus* susceptibility was detected in 1997 and 1998 after 2 years application of VectoLex CG along with larvicidal oil against natural *Culex* populations

Comparing the LC_{90}/LC_{50} value ratios in these successive 3 years of sampling, the distributions of the ratios gradually shifted to the lower range from 1996, 1997 to 1988 (Fig. 2). It is suggested that the heterogeneity of the *Culex* population in the treated area was reduced by the treatments.

In previous studies, moderate to high levels of resistance of colonized *Cx. quinquefasciatus* to *B. sphaericus* preparations have been achieved by exposing the larvae to LC_{80} (Rodcharoen and Mulla 1994) or LC_{94-98} (Wirth et al. 2000) selection pressures every generation. As a result of field applications of *B. sphaericus* formulations for various durations, high levels of resistance have been reported against *Cx. pipiens* in southern France (Sinègre et al. 1994) and against *Cx. quinquefasciatus* in India (Rao et al. 1995, Adak et al. 1995). Low level and reversible resistance have occurred in natural populations of *Cx. quinquefasciatus* in Brazil (Silva–Filha et al. 1995). Most recently, high level resistance in *Cx. quinquefasciatus* was reported to occur in southern China after 8–year application of *B. sphaericus* formulation (Yuan et al. 2000). From our current studies, no significant change in *B. sphaericus* susceptibility in natural *Culex* populations was detected after joint application of VectoLex CG and larvicidal oil for 2 years.

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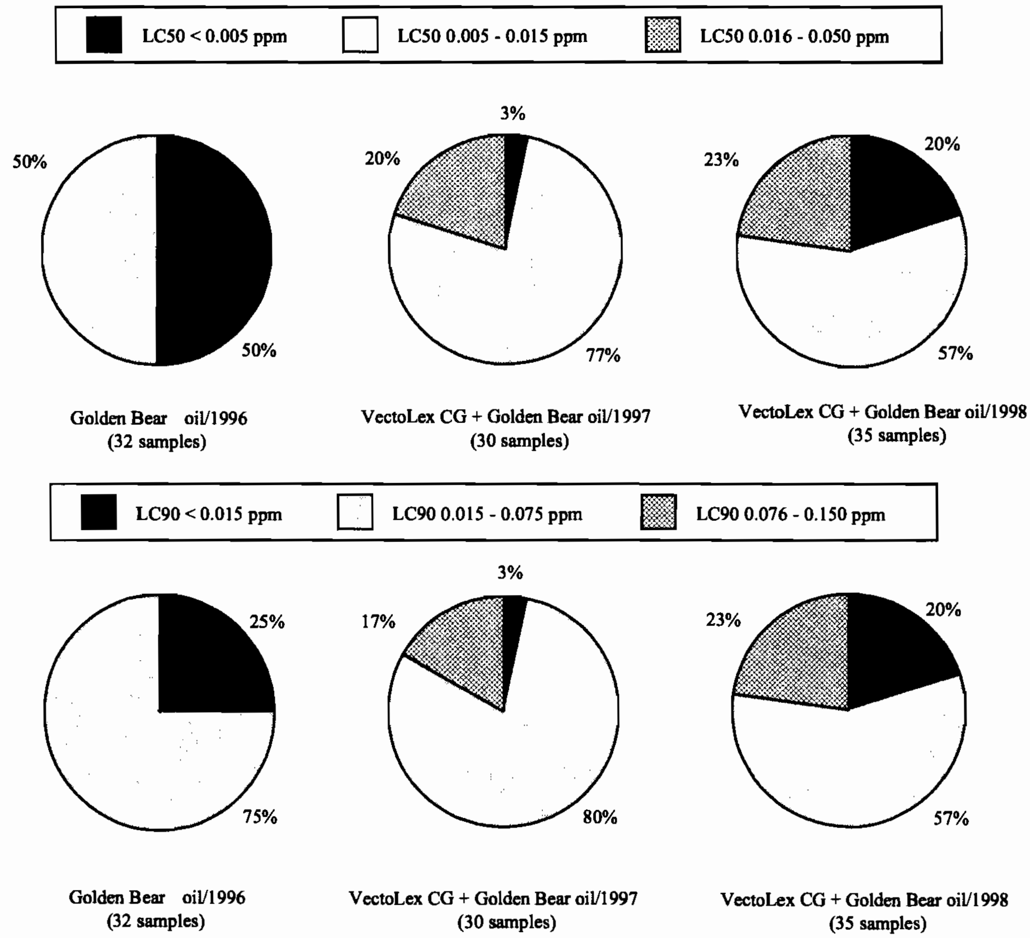

Figure 1. Distribution of the LC_{50} and LC_{90} values (%) in *Culex* larvae collected from the breeding sites treated with Golden Bear GB-1111 larvicidal oil alone in 1996 and oil with VectoLex CG in 1997 and 1998 in Northwest Mosquito and Vector Control District, Riverside County, California. The larvae were bioassayed with VectoLex TP (*B. sphaericus* strain 2362).

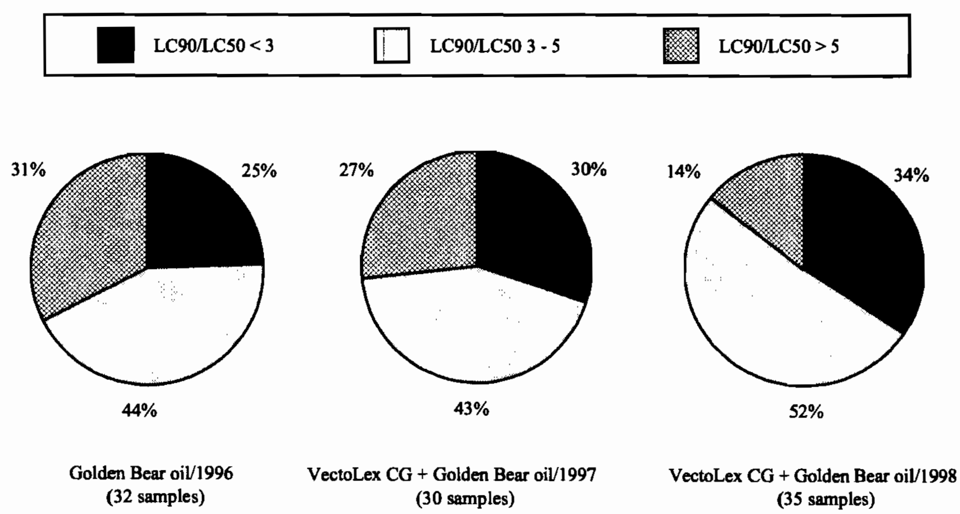

Figure 2. Distribution (%) of LC_{90}/ LC_{50} ratios in *Culex* larvae collected from the breeding sites treated with Golden Bear GB-1111 larvicidal oil in 1996 alone and oil with VectoLex CG in 1997 and 1998 in Northwest Mosquito and Vector Control District, Riverside County, California. The larvae were bioassayed with the microbial product VectoLex TP (*B. sphaericus* strain 2362).

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