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Mosquito and Vector Control Association of California

Volume 66 **January 25 through January 28, 1998**

POTENTIAL ARBOVIRAL INFECTIONS OF ANIMALS AND MAN IN CALIFORNIA: A DEDICATION PRESENTATION TO HONOR JAMES L. HARDY

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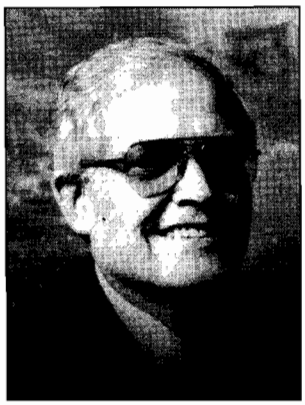

James L. Hardy, 1932-1997

The Mosquito and Vector Control Association of California has dedicated this 66th annual meeting in memory of James L. Hardy who died in 1997. For 35 years, Jim made major contributions to our knowledge on and control of vector-borne diseases in California. I decided it would be fitting to honor him by focusing my talk on what I consider to be a landmark paper he wrote and presented at an international conference in 1992 at Davis (Hardy 1992). That meeting focused on new and emerging diseases in animals in today's world. The paper was titled: "Potential arboviral infections of animals and man in California." An abstract was published in the proceedings of that meeting, but had very limited distribution and few or none of you have nor will see it. The paper summarized over 40 years of data gathered by the Arbovirus Research Unit of the University of California, Berkeley on the epidemiology of some 12 vector-borne viral infections discovered in California since 1945. Jim must receive the major credit for my presentation. I will summarize his paper, use his slides to illustrate the findings, and add some comments on recent discoveries.

We are all familiar with the early research in California on Western equine and St. Louis encephalitis viruses and *Culex tarsalis* (Coquillett), the major vector. These viruses and that vector were important historically and are still a focus in your current surveillance and control programs (Reeves 1990). Since 1945, knowledge has been gained on 12 additional vector-borne viruses that occur in California and their potential to emerge as new disease agents. You should know about these viruses as they may become important to your programs. The public is concerned today with the emergence or reemergence of new diseases and this is reflected in your programs. As examples, hemorrhagic fever and several tick-borne diseases have emerged in California and are now included in your agendas.

We are all familiar with the early research in California on Western equine and St. Louis encephalitis viruses and *Culex tarsalis* (Coquillett), the major vector. These viruses and that vector were important historically and are still a focus in your current surveillance and control programs (Reeves 1990). Since 1945, knowledge has been gained on 12 additional vector-borne viruses that occur in California and their potential to emerge as new disease agents. You should know about these viruses as they may become important to your programs. The public is concerned today with the emergence or reemergence of new diseases and this is reflected in your programs. As examples, hemorrhagic fever and several tick-borne diseases have emerged in California and are now included in your agendas. The scope of your programs will increase still further if additional mosquito-borne viruses emerge as significant disease problems. The 12 new viruses to be discussed today utilize either mosquitoes, *Culicoides* gnats, or ticks as vectors and a variety of animals as their hosts. Additional new viruses continue to be discovered in vectors, birds, rodents and bats and some depend on a vector for transmission.

The research program Dr. Hardy described determined the basic vectors and the range of vertebrate species infected with these 12 viruses and evaluated their possible associations with diseases in man and domestic animals. In the process, thousands of insects and blood samples were collected and tested for either virus or antibodies. Many of the viruses were inoculated into experimental animals or arthropods to determine their host and vector relationships. The advances in knowledge depended on collaboration between the University research unit, vector control agencies, State Department of Health Services, several other University campuses, and the Centers for Disease Control of the United States Public Health Service. I will now illustrate some of the findings.

BASIC MAINTENANCE CYCLES AND VERTEBRATE HOST INFECTIONS

California encephalitis (CE) virus was isolated in 1945 and became a model for future research on newly discovered viruses. The basic maintenance cycle for CE virus was quite different than the cycles for WEE and SLE as it depended on *Aedes melanimon* (Dyar), not *Cx. tarsalis*, as its vector and on jackrabbits, not birds, as the basic animal hosts (Figure 1). This virus was maintained by transovarial (TOT) infection in the vector (i.e., virus passage from infected female vectors through the egg to their progeny). This was a relatively new finding and provided an amazingly efficient reservoir for long term persistence of infection in nature. Infection of humans was incidental and of no importance for virus persistence. CE virus and closely related variants were found to be widespread in the Central Valley, southern and coastal areas of California. An extensive search for evidence that CE virus was associated with encephalitis as a clinical disease identified 3 such cases in humans. Infection without disease was common since as many as 37% of some populations of people resident in the Central Valley had antibodies from prior inapparent infections (Table 1).

In the early 1990's, Jamestown Canyon (JC) virus, a virus related to CE virus, was isolated repeatedly from snow mosquitoes collected in the Sierra Nevada mountains (Hardy et al. 1993). This virus is also known to occur in midwestern and northeastern regions of the United States where it has been associated with encephalitis in humans. This is the basic cycle for this virus in the Sierras (Figure 2). The cycle is very similar to that for CE virus as it is dependent upon TOT in the mosquito for virus maintenance and utilizes large mammals as basic hosts. The virus has an unusually broad geographical distribution in California. We now know this virus occurs from the northern to southern Sierras in snow mosquitoes in *Ae. dorsalis* (Meigen) in salt marshes in coastal California and in *Culiseta inornata* (Williston) in the Central and Imperial Valleys of California.

At this time, there is no evidence that JC virus causes a disease in people or in other animal hosts in California. Infection of people is common in populations resident in the Sierras and in deer and cattle in this environment (Table 2) (Campbell et al. 1992). Infection with this virus should be closely monitored as there is a constantly increasing population that live in or visit the mountainous and coastal areas of California where they may be exposed to infection. If recreational visitors with such infection become ill, it probably will occur after they return home and are miles from the location where infection occurred. Most physicians do not know this virus exists or of its likelihood to cause illness.

Table 1. Evidence of Infection and Disease Associated with California Encephalitis Virus in Man and Domestic Mammals in California.

	NT or HI Antibody Prevalence (%)[1]		
Host	Normal	Sick	Disease reported
Man	11-37	1	3 cases
Domestic mammals			
Dogs	21	nt[1]	
Horses	9-13	4	None
Sheep	7	nt	
Pigs	4	nt	
Cattle	1	nt	

[1]nt=none tested, HI=hemaglutination inhibition

Table 2. Evidence of Infection and Disease Associated with Jamestown Canyon Virus in Man and Domestic Animals in California.

	NT or HI Antibody Prevalence (%)[1]		
Host	Normal	Sick	Disease reported
Man	5-31	3	None
Domestic mammals			
Horses	31-55	nt[1]	
Cattle	73	nt	

[1]nt=none tested, HI=hemaglutination inhibition

Table 3. Evidence of Infection and Disease Associated with Main Drain Virus in Man and Domestic Animals in California.

	NT or HI Antibody Prevalence (%)[1]		
Host	Normal	Sick	Disease reported
Man	nt[1]	<0.1	None
Domestic mammals			
Horses	33-44	28	6 cases
Cattle	2-5	nt	
Sheep	14-21	nt	
Pigs	0-1	nt	
Dogs	11	nt	

[1]nt=none tested, HI=hemaglutination inhibition

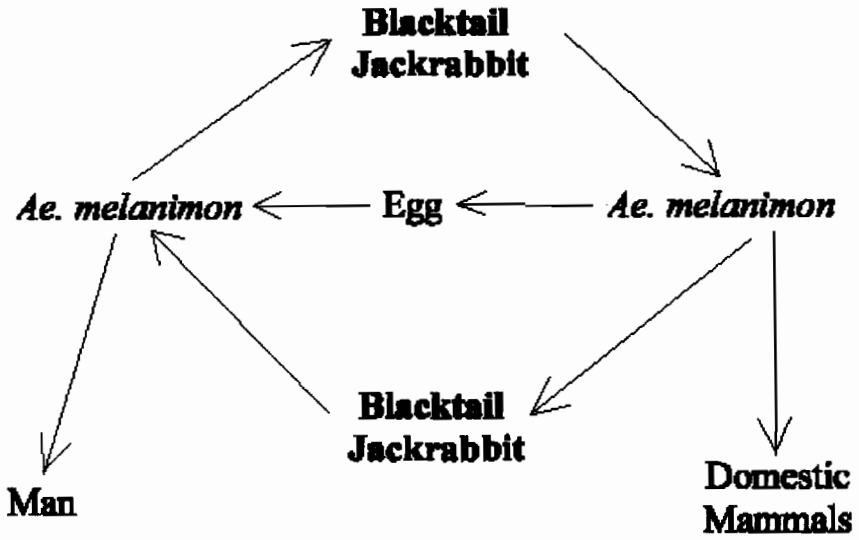

Figure 1. Transmission cycle of California encephalitis virus in California

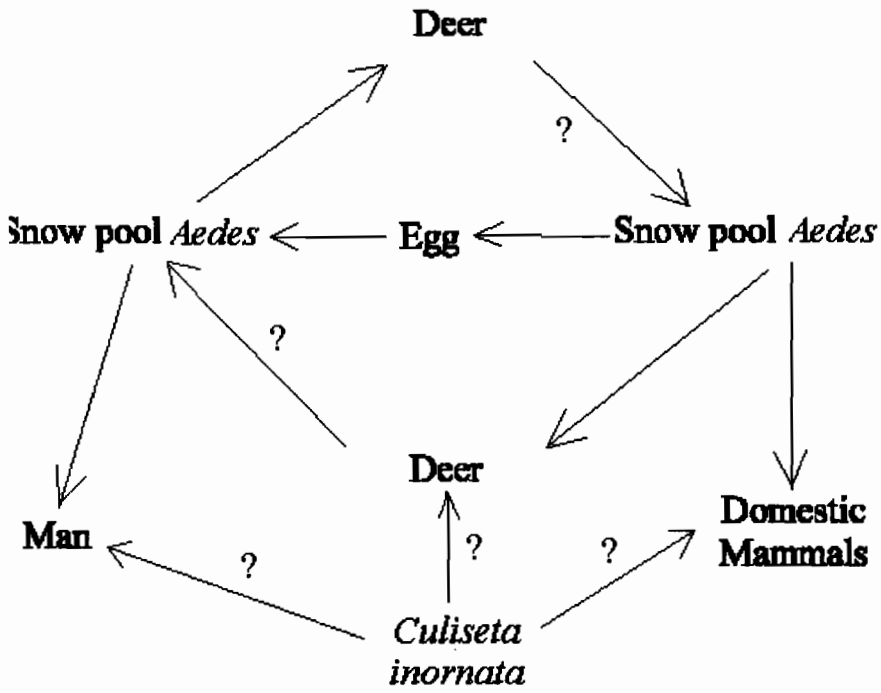

Figure 2. Transmission cycle of Jamestown Canyon virus in California.

A third agent in the CE virus complex is Moro Bay (MB) virus which was first isolated from *Ae. squamiger* (Coquillett) collected from a coastal salt marsh at Moro Bay, California in 1989 (Fulhorst 1996). The basic cycle probably is very similar to that of other viruses in the CE complex as it has TOT in the vector and wild mammals as probable hosts. The virus has a wide distribution as it has been isolated from Moro Bay in the north to San Diego in the south. The very large population that live in this region are exposed as antibodies are found in people and large domestic animals resident in the coastal area. There is no evidence this virus is associated with a clinical disease and this should be the subject of future research.

Let us shift now to consider a very different group of viruses that are associated with *Culicoides variipennis* (Coquillett) as a vector. This gnat is the primary vector of Blue Tongue (BT) disease, a virus infection of veterinary importance in sheep and cattle in California. We have isolated three new viruses Buttonwillow (BW), Lo Kern (LOK), and Main Drain (MD) from this same vector but virologically they are not related to BT virus. The cycles that maintain these viruses seem to be dependent on jack rabbits, cottontail rabbits and *C. variipennis* (Figure 3). Infection of people with these viruses probably is rare, but is common in a wide range of domestic mammals (Tables 3, 4, 5). This distribution of infection is caused by the host preferences of

Table 4. Evidence of Infection and Disease Associated with LoKern Virus in Man and Domestic Mammals in California.

	NT or HI Antibody Prevalence (%)[1]		
Host	Normal	Sick	Disease reported
Man	1	0	None
Domestic mammals			
Horses	5-44	10	1 case
Cattle	1-6	nt[1]	
Sheep	12	nt	
Pigs	0-9	nt	
Dogs	0-28	nt	

[1]nt=none tested, HI=hemaglutination inhibition

Table 5. Evidence of Infection and Disease Associated with Buttonwillow Virus in Man and Domestic Mammals in California.

	NT or HI Antibody Prevalence (%)[1]		
Host	Normal	Sick	Disease reported
Man	nt[1]	0	None
Domestic mammals			
Horses	1-3	0	None
Cattle	0-2	nt	
Sheep	0-4	nt	
Pigs	0-7	Nt	
Dogs	0-6	Nt	

[1]nt=none tested, HI=hemaglutination inhibition

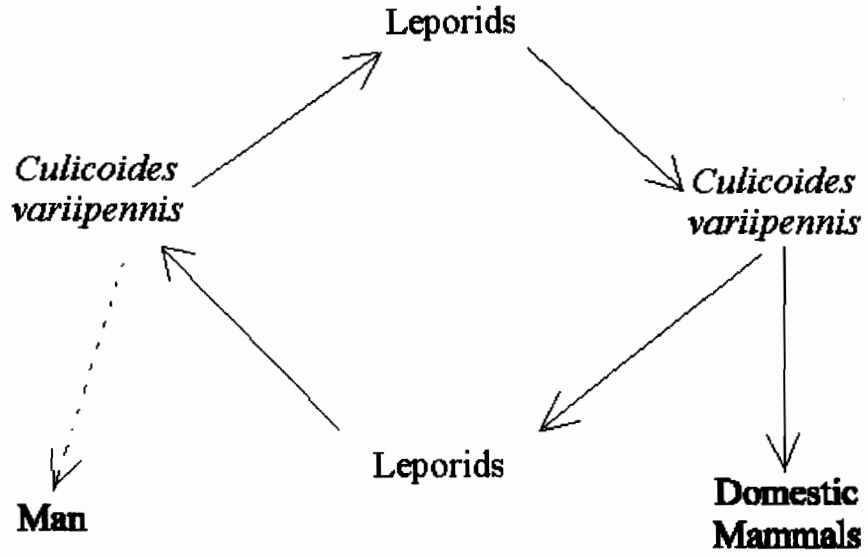

Figure 3. Transmission cycle of Buttonwillow, LoKern and Main Drain viruses in California.

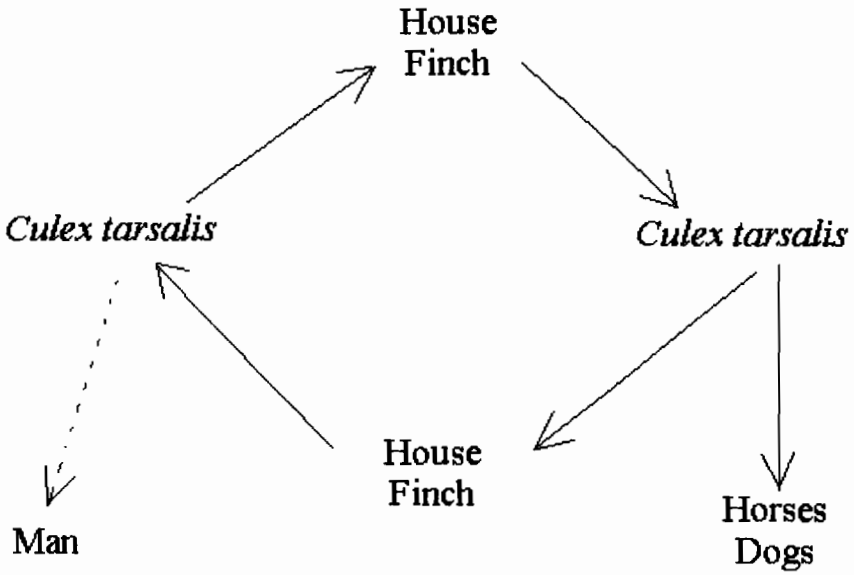

Figure 4. Transmission cycle of Turlock, Hart Park, and Llano Seco viruses in California.

the vector as it rarely or never bites people. It is of considerable interest that MD virus also was isolated from the brain of a horse with encephalitis. In addition, serological evidence was found that MD virus had caused encephalitis disease in five additional horses. LOK virus also was the cause of encephalitis in a horse. BW virus seems to be restricted to the rabbit--gnat cycle for a limited period in the spring when the vector is feeding largely on rabbits. Little is known of the frequency of infection with BW virus in animals. MD and LOK viruses are transmitted throughout the summer when *Culicoides* are feeding equally on rabbits and domestic animals.

We would like to determine if LOK, MD and BW viruses are the cause of significant disease in domestic or feral animals. Such research will require experimental infection of many animals and further study of undiagnosed illnesses in animals in the field. We also think it is important to determine if rabbits are effective hosts for any of the enzootic BT viruses as this has not been determined. The present control program for BT is based on a vaccine and no attention is given to *Culicoides* control: Such control could become part of an integrated control program along with vaccination and would be particularly important if rabbits are found to be a host important for BT virus persistence. *Culicoides* are very common in California. Large numbers are collected in New Jersey or CO2, traps, but up to now have been discarded and not recorded. This procedure may be changed if MD, LOK or BT viruses are found to be pathogens.

We recently found that Northway (NW) virus occurs in California. This virus is widespread in Alaska and Canada as an infection of man, domestic animals and deer. This virus has been isolated from three species of mosquitoes in California: four from *Anopheles freeborni* (Aitken), one from *Ae. sierrensis* (Ludlow), and one from *Cs. inornata.* NW is the only virus so far associated with an *Anopheles* in North America. Antibodies were found in 25% of 337 deer and 44% of 192 horses that lived in mountainous areas of California but were found in only 0.3% of 702 people living at high or low elevations in California. A virus closely related to Northway was isolated recently from *Culiseta particeps* (Adams) collected at Moro Bay. This virus is called Stanfield virus, which was only known previously to occur in *Cs. inornata* collected in Oregon.

I have held off purposely from discussion of three potentially important viruses, Turlock (TUR), Hart Park (HP) and Llano Seco (LS). I believe these viruses may be prime candidates to cause an emerging disease. They are ubiquitous in their distribution at lower elevations in California and all three are similar to WEE and SLE viruses in that *Cx. tarsalis* is their vector and small passerine birds are their hosts (Figure 4). A serious, but unsuccessful effort, has been made to associate these viruses with cases of encephalitis or inapparent infections in people (Ksiasek 1984, Graham 1987).

Infection with TUR virus is rare in people and common in horses and dogs, and its only clinical associations were with five cases of encephalitis in horses (Table 6). Antibodies to HP virus are rarely found in man or domestic animals but the virus has been associated with six cases of encephalitis in humans (Table 7). In tests of sera from domestic animals, only dogs were commonly infected. The last of this trio of viruses is LS. Its maintenance cycle is similar to that for others that involve *Cx. tarsalis*. A wide range of domestic animals and leporids are infected and humans are rarely infected (Table 8). This virus is widespread in the Central Valley of California. No association has been found with any disease.

Two tick-borne viruses have been found in California (i.e., Powassan and Modoc), but have not been studied in detail.

FUTURE CONCERNS

I want to discuss briefly what I believe should be a major concern in California. Dr. Hardy's paper led us to recognize the value of a detailed search that determined a range of new viruses associated with bloodsucking arthropods in California (Hardy 1992). As you know, WEE virus has continued to be active over the past several years in its basic cycle in much of central California and WEE and SLE viruses in the south. In the summer of 1997, the state wide surveillance program identified almost 200 cases of encephalitis or other central nervous system diseases in residents of California, none of which were diagnosed to be either WEE or SLE. The important obvious questions are can we find an alternative cause for all or a part of these cases and are they vector-borne? Dr. Hardy's final scientific paper published in 1997 reported that the WEE viruses that now prevail in *Cx. tarsalis* in California are as

Table 6. Evidence of Infection and Disease Associated with Turlock Virus in Man and Domestic Animals in California.

	NT or HI Antibody Prevalence (%)[1]		
Host	Normal	Sick	Disease Reported
Man	<1	<1	None
Domestic mammals			
Horses	33	9	5 cases
Cattle	0	nt[1]	
Sheep	<1	nt	
Pigs	9	nt	
Dogs	42	nt	

[1]nt=none tested, HI=hemaglutination inhibition

Table 7. Evidence of Infection and Disease Associated with Hart Park Virus in Man and Domestic Mammals in California.

	NT or HI Antibody Prevalence (%)[1]		
Host	Normal	Sick	Disease Reported
Man	0	5	6 cases
Domestic mammals			
Horses	1	2	None
Sheep	0	nt[1]	None
Pigs	0	nt	None
Dogs	57	nt	None

[1]nt=none tested, HI=hemaglutination inhibition

Table 8. Evidence of Infection and Disease Associated with Llano Seco Virus in Man and Domestic Mammals in California.

Host	NT or HI Antibody Prevalence (%)[1] Normal	Sick	Disease Reported
Man	2	<1	None
Domestic mammals			
Horses	32	23	None
Cattle	11	nt[1]	None
Sheep	12	nt	None
Pigs	16	nt	None
Dogs	30	nt	None

[1]nt=none tested, HI=hemaglutination inhibition

Table 9. Summary of the Disease Potential of Arboviruses in "Search of Disease" in California.

Viral Family Genus	Virus	Man	Disease Potential Domestic Mammals
Bunyaviridae			
Bunyavirus	Buttonwillow	No	?
	California encephalitis	Yes	Yes
	Jamestown Canyon	Yes	Yes
	Moro Bay	Yes	Yes
	LoKern	No	Yes
	Main Drain	No	Yes
	Northway	?	Yes
	Turlock	?	Yes
	Stanfield	?	?
Flavivirus	Powassan	Yes	?
Reoviridae			
Orbivirus	Llano Seco	?	Yes
Rhabdoviridae			
Unassigned	Hart Park	Yes	Yes

virulent as they were when many cases were occurring historically. This fact does not explain the current absence of cases of this disease where the virus remains sporadically active (Hardy et al. 1957). One avenue of research still open to us is to select other vector-borne viruses that we now know occur in California and determine if they are the cause of any of these cases. The Center for Vector-borne Disease Research at UC Davis currently plans to reexamine serum samples from the almost 200 cases of CNS from 1997 to determine if any of them were infected with eight of the 12 viruses that we just reviewed (Table 10). We believe the viruses transmitted by either *Cx. tarsalis* or *Aedes* species are likely candidates.

EPILOGUE

I have summarized only one area of research where Dr. Hardy made major contributions. In addition, he traced the pathway of viruses in their mosquito vectors from their ingestion in a blood meal through barriers in the gut until they reached the salivary glands from where they could be inoculated into a host. He did studies on the genetics of the viruses and vectors that effect their disease relationships. He determined the effect of different temperatures on virus growth in their vectors which may reveal possible influences of El Niños and global warming on vector-borne diseases in California. He trained a new generation of virologists, entomologists and epidemiologists in the field of vector-borne diseases. We owe our thanks to Jim Hardy for his dedication to solving many of our problems and he will be sorely missed as we face future problems with vector-borne diseases in California.

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A COMPARISON OF MOSQUITO CONTROL BY TWO LARVIVOROUS FISHES, THE STICKLEBACK (*Gasterosteus aculeatus*) AND THE MOSQUITOFISH (*Gambusia affinis*)

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ABSTRACT

The effectiveness of the nonendemic mosquitofish, *Gambusia affinis*, and an endemic species, the three-spined stickleback, *Gasterosteus aculeatus*, as mosquito control agents were compared. Four treatments were tested in 28 m^2 mesocosms at the Aquatic Research Facility at the University of California, Riverside during the spring and summer in two consecutive years. The ponds were stocked with either stickleback, mosquitofish, or both species. Control ponds were maintained without fish. As compared to the control ponds, the stickleback did not significantly reduce populations of mosquito larvae, whereas the mosquitofish had a significant impact on larval abundance. The extent of mosquito control when both fish were stocked together was similar to that when mosquitofish were stocked alone at 3 kg/ha or 4.5 kg/ha.

There is considerable controversy regarding the introduction of nonendemic mosquitofish (*Gambusia affinis* Baird and Girard) for mosquito control (Rupp 1996, Gratz et al. 1996). The mosquitofish has been successfully used since the turn of the century as a control agent for mosquitoes (Meisch 1985). *Gambusia* is a top-feeding omnivore, consuming phytoplankton, zooplankton, various types of invertebrates, other fish and eggs of indigenous fish (Bay and Anderson 1966, Farley 1980, Hess and Tarzwell 1942, Hurlbert and Mulla 1981, Laird 1977, Miura et al. 1984, Washino and Hokama 1967). The mosquitofish is suspected of causing adverse effects on the population densities of amphibians; however, gut analyses have not yet shown direct consumption of individuals or eggs of these groups (Gamradt and Kats 1996). Dietary analyses have shown that the mosquitofish does not preferentially feed on mosquito larvae but is opportunistic and feeds readily on aquatic organisms in direct relation to their relative abundance (Hess and Tarzwell 1942, Morton et al. 1988). Other studies have shown that both dietary preferences and the impact of *G. affinis* on target and nontarget organisms change seasonally and are correlated to prey densities (Walton and Mulla 1991). Because of its voracious nature and broad diet, the mosquitofish can have a significant impact on local biodiversity.

The use of endemic fishes for mosquito control may avoid or reduce the adverse effects of exotic fishes on aquatic communities. The stickleback (*Gasterosteus aculeatus* L.) is an endemic species which is widely distributed, inhabiting streams, rivers and lakes throughout California (Swift et al. 1993). This fish feeds on small organisms throughout the water column and has been reported to have a preference for mosquito larvae and pupae as food items (Bay 1985). These biological attributes and widespread distribution have led to speculation about the potential success of stickleback for mosquito control (Hubbs 1919). Further simultaneous release of *Gambusia* and fish which feed in a different feeding zones, such as stickleback, may result in an increased level of mosquito control (Woodridge & Davidson 1996). Until now, there has been no definitive study investigating these possibilities.

Our objectives were to 1) evaluate the relative effectiveness of the stickleback and the mosquitofish, 2) evaluate the effectiveness of both fish as mosquito control agents when reared together, and 3) evaluate the potential for coexistence of the two species.

MATERIALS AND METHODS

Two, six-week studies were carried out to examine the relative effectiveness of the two fishes as

mosquito control agents. The first study was carried out in July and August, 1996 and the second study in April and May, 1997. Twelve 4 m x 7 m mesocosms (earthen ponds) at the University of California, Riverside Aquatic Research Facility were utilized for this study. Mesocosms were arranged into two rows and maximum water depths varied slightly, ranging from 56-71 cm. Water levels were maintained by float valves. All natural vegetation was removed by hand. Artificial vegetation was constructed from strips of 0.015 mm plastic sheeting and placed in the corners and the center of each mesocosm. Upon flooding, each mesocosm was enriched with 3.5 kg of rabbit pellets to promote oviposition by mosquitoes.

Four treatments were used in both studies: a) mosquitofish only, b) both fish stocked together, c) stickleback only, and d) control (no fish stocked). Treatments were replicated three times within each study. In order to equalize the variation in mosquito populations among treatments, initial mosquito larval abundance in pre-treatment dip samples was used to assign treatments to the mesocosms.

Mosquitofish were supplied by Northwest MVCD, Corona, CA and sticklebacks were collected with D-ring aquatic nets from the Mojave River near Apple Valley, CA. Fish were transported from each location in ice chests containing water from their original location, which was aerated with portable air pumps. Upon arrival at the UCR facility, fish were acclimated for approximately 24 hours in three 1.25 m^3 cages constructed of a PVC frame covered by fiberglass window screening. The cages were located within a pond similar to those used for the study. Minimal losses (<1%) occurred due to transport and no losses occurred in acclimation procedures.

The treated mesocosms were stocked with fish at rates of kg/ha and 3 kg/ha or 4.5 kg/ha during the 1996 and 1997 studies, respectively. The number of reproductive individuals was standardized among treatments. At the end of each experiment, fish were seined from the mesocosms and a gross wet weight was taken for each population.

Insect populations were sampled weekly with a tow net (mesh size: 153 μm) and twice a week with a dipper (400 ml). On each date, four dips were taken in the corners of each pond and combined using a concentrator cup. Duplicate tow net hauls were taken weekly in each pond. Specimens were preserved in alcohol (final concentration approximately 50%) and processed within 24-48 hours after collection. Individuals within the samples were counted, identified to genera and, when possible, identified to species using Merritt & Cummins (1995). Mosquito larvae were separated into two subpopulations: early (stages I and II) and late (stages III and IV) larval instars. Late instar (stages III - IV) mosquito larvae were categorized to species using Bohart and Washino (1978). Egg rafts were counted along the entire perimeter of each mesocosm, including those laid up to 0.5 m from any edge of the mesocosm during the 1996 study. During the 1997 study, the egg rafts were counted along three one-meter sections of the perimeter. Counts were then extrapolated to determine total number of rafts present on each pond. Egg rafts were sampled twice per week.

To test for significance between treatment means, abundance data were ln-transformed and analyzed using a repeated measures analysis of variance. Data for all species present in samples were analyzed, as well as that of egg rafts and the developmental stage and mosquito species.

RESULTS AND DISCUSSION

Larval mosquito abundance in treatments stocked with *Gambusia* only or *Gambusia* and stickleback together was significantly reduced compared to stickleback treatments and the control mesocosms. There was no significant difference in larval mosquito abundance between the treatment with mosquitofish only and that with both mosquitofish and sticklebacks present (Table 1). *Culex tarsalis* Coq. and *Cx. stigmatosoma* Dyar larvae were most prevalent (combined relative abundance ≤ 90% of larvae collected) and *Cx. quinquefasciatus* Say and *Culiseta inornata* (Williston) were comparatively rare (< 10% of larvae collected). Therefore, the effects on both larval subpopulations can be solely attributed to the presence of the mosquitofish.

In the control treatment, mosquito larval populations declined due to the negative oviposition response of female mosquitoes as the pond waters aged. The number of egg rafts per pond declined by 25% during the two, six-week studies. Mosquito larval abundance in the *Gambusia* treatment and the treatment with both fish differed significantly from the control. Mosquito larval abundance in the stickleback treatment, while not statistically different from the control (Table 1), did not decline

Table 1. Tests for significant differences* in pair wise Comparisons of treatments in 28 m^2 mesocosms.

	Larval Subpopulation	
Treatment	L1/L2	L3/L4
Stickleback vs. Mosquitofish	Yes	Yes
Stickleback vs. Both Fish	Yes	Yes
Stickleback vs. Control	No	No
Mosquitofish vs. Control	Yes	Yes
Both Fish vs. Control	Yes	Yes
Both Fish vs. Mosquitofish	No	No

* Tukey's Test ($P<0.05$) following a significant F-Test.

as in the control ponds. Larval mosquito abundance in ponds containing the stickleback was comparatively unchanged throughout the experiment.

In both studies, lower stocking rates of mosquitofish provided mosquito control similar to higher stocking rates. The stocking rate of fish for all treatments was 2 kg/ha and 3 or 4.5 kg/ha for 1996 and 1997 studies, respectively. In the treatment with both fish, one-half of the total amount stocked was mosquitofish and one-half was stickleback. This difference in stocking rates, and the lack of a significant difference between the mosquitofish only treatment and that with both fish, strongly indicates that the lower stocking rate of mosquitofish provided adequate rates of larvicidal control. This is an important consideration when stocking fish as part of a vector control program and needs further examination.

We observed that the mosquitofish and the stickleback can coexist but noted that the mosquitofish rapidly reproduced and its biomass increased dramatically (30 times the stocking weight). The sticklebacks were able to, at best, maintain their initial gross stocking weight at the end of the study. While both fish produced offspring, a thorough evaluation of the long term persistence of both fish when concurrently stocked was not possible due to the short duration of these studies.

The number of nontarget fauna species or their relative abundance collected in tow net samples did not differ significantly among the treatments. The dominant nontarget organisms differed between the two studies. In the summer months, there were few large insects, while in the spring, chironomids and notonectids were abundant. Ostracods and cladocerans were the most abundant zooplankton throughout both studies.

In summary, the mosquitofish was effective for controlling mosquito larvae while the sticklebacks were ineffective. We further conclude that there was no synergistic effect on mosquito larval control when both fish were stocked together. We observed that these two fish species were able to coexist in artificial mesocosms, and that neither species significantly affected nontarget fauna. As the ponds used in this study are not representative of the variety of ecosystems into which sticklebacks may be introduced, further investigations into the efficacy of sticklebacks as biological control agents in other environments are warranted.

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THE EFFECTS OF LARVAL DENSITY ON THE FEEDING PERFORMANCE OF *Ixodes scapularis*

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ABSTRACT

To test the possibility that different densities of the deer tick, *Ixodes scapularis*, feeding on the white-footed mouse, *Peromyscus leucopus*, may have an effect on the percentage of ticks that can successfully feed to repletion, nine mice were separated into three groups of three mice each and infested with larvae for nine successive days. There was a significant increase in the percentage of engorged larvae that dropped from mice in the medium (25 larvae/day) and high infestation (50 larvae/day) groups over time. The low infestation group (5 larvae/day) did not exhibit a significant increase in the percentage of engorged larvae that dropped. The density of larval infestation did not have a significant effect on the percentage of larvae that dropped off unfed, however, there was a significant decrease in the percentage of flat larvae that dropped in all three infestation groups over time. These results suggest that the presence of high densities of tick larvae may improve feeding performance on *P. leucopus*. This could be a result of immune suppression brought on by tick feeding or by the lack of an effective immune response by the mice.

The behavior of a host species can have dramatic effects on the behavior and survival of the feeding tick. The reaction of *Peromyscus leucopus* (white-footed mouse) to feeding by the tick species *Ixodes scapularis* may be an important factor in determining tick population densities (Galbe and Oliver 1992). Resistance to tick feeding through behavioral, as well as immunological responses may be an important factor in host selection. Unnatural hosts, such as *Cavia porcellus* (guinea pig), exhibit a dramatic immune response to feeding after successive infestations (Allen 1989). Acquired resistance in guinea pigs is manifested by significant reductions in the number of ticks that become engorged and in the weights of ticks that have fed after repeated infestations (Allen 1989). Evidence that resistance may be immunologically controlled is provided by experiments that have utilized immunosuppressive drugs. These drugs prevented the acquisition of resistance in guinea pigs subjected to repetitive infestations (Allen 1989). Resistance may act in a density dependent manner as exemplified by increased tick mortality at higher densities of infestation (Davidar et al. 1989).

The white-footed mouse is the main reservoir of *Borrelia burgdorferi* and the most common host for the immature stages of *Ixodes scapularis*. A natural host, such as *P. leucopus*, apparently does not develop resistance to tick feeding (Galbe and Oliver 1992, Magnerelli et al. 1988). Lack of resistance (tolerance) to tick feeding was apparent in a study by Magnarelli et al. (1988) in which much lower levels of antibody titer to *Borrelia burgdorferi* were detected in *P. leucopus* compared to other mammals such as *Cavia porcellus*.

One possible explanation for tolerance to feeding by *P. leucopus* that the ticks have evolved substances in their saliva that inhibit the immune response of the host (Davidar et al. 1989). The ticks may also have evolved mechanisms to withstand an immune response mounted by the host (Tatchell 1969, Randolph 1979). There may be a threshold level at which an immune response may become severe enough to overcome the tick's defenses (denHollander and Allen 1985). In a sample of 778 wild *P. leucopus*, Main (1982) found a mean of 2.5 larvae/mouse. In this experiment, the number of larvae placed on each mouse far exceeded the number that the mice would acquire in nature. If there is a threshold of tolerance to feeding, as suggested by denHollander and Allen (1985), that level was not reached in this experiment, as exemplified by the increasing percentage of engorged ticks that drop-off throughout the experiment.

The behavior "resistance to feeding" has been assayed by the number of ticks that become engorged, the number of engorged ticks that success-

fully molt into nymphs and the engorgement weights of fed ticks (Allen 1989).

The purpose of this study was to determine the response of *P. leucopus* to a period of successive infestations resulting in a progressive accumulation of larvae on the hosts. If *P. leucopus* does not exhibit resistance to tick feeding, as proposed by Galbe and Oliver (1992), it was hypothesized that there will be no significant difference between different larval infestation densities ranging in the percentage of larvae that can successfully feed.

MATERIALS AND METHODS

White-footed mice were collected by live trapping in and around the Medical Entomology Laboratory in Valhalla, NY, using Sherman live traps baited with oats and peanut butter. The mice were housed in standard mouse cages and provided with rat chow pellets and water, ad libitum. The nine mice used in the experiment were all adults, as indicated by their brown pelage. Five of the mice were male, three female. One died and was discarded before it was sexed. A xenodiagnostic procedure (Anderson et al. 1983) was employed prior to the experiment to determine which mice tested positive for the presence of *B. burgdorferi* spirochetes. All the mice in this experiment tested positive for the spirochetes. *Ixodes scapularis* adults were collected in Westchester County in the spring of 1993 by drag sampling. A white corduroy cloth was passed along the ground to pick up any ticks that may be questing in that area. The larvae used in this experiment were the progeny of adult females that fed on laboratory rats. The larvae were stored in vials at 95% relative humidity, 23°C and 16:8 L:D until they were used. The larvae were approximately 5 weeks old at the time of infestation. Published data indicate that an age of approximately 4 weeks is an optimal age for feeding success (Galbe and Oliver 1992).

Host tolerance to varying infestation levels was assessed in two ways: the percentage of ticks that became fully engorged and the percentage of fed larvae that successfully molted to the nymphal stage. The host animals were infested with larvae by placing them in a housing can (2 lb. coffee can) with a wire mesh bottom, and immobilizing them by pressing the mouse against the wire mesh with a cotton covered can that served as a plunger to hold the mouse against the wire mesh. The unfed larvae were brushed onto each mouse with a paintbrush and the mouse was kept immobilized for 15 minutes. The larvae used on each mouse were a mixture from different egg batches to minimize variation due to the differences in the feeding ability of different egg batches (denHollander and Allen 1985).

Nine mice were separated into three groups of three mice each and infested with larvae for nine successive days. Three of the mice were infested with 5 larvae/day, three were infested with 25 larvae/day and the last three were infested with 50 larvae/day. All host exposures were conducted in the afternoon except for the day-10 infestation, which was conducted in the mid-morning. The mice were kept individually in cans and hung over pans of water for the duration of the experiment. The pans were checked twice a day for unengorged and engorged larvae. All ticks were removed from the pans and cleaned with distilled water, air dried and stored in vials at 95% relative humidity, 23°C and a 16:8 L:D photoperiod until molting.

All percentages were arcsine transformed (Sokal and Rohlf 1969) and tested for normality (K-S test). The data was examined by ANOVA to determine if differences in drop-off percentages were dependent on the number of days that the experiment was conducted. Statistical significance was determined at a P value of < 0.05.

To determine which mice were infected with *B. burgdorferi*, a xenodiagnostic procedure was employed (Anderson et al. 1983). In this procedure, uninfected flat larvae were placed on a potentially infected host and allowed to feed to repletion. Direct fluorescent antibody staining methods were utilized on the engorged larvae that successfully molted. The nymph's abdomen was punctured and the midgut contents were spread thinly onto one well of a 12-well slide. The slides were incubated overnight at 37°C, then fixed in acetone for 10 minutes and allowed to air dry. The slides were overlaid with fluorescein-conjugated IgG fraction (rabbit anti-*Borrelia burgdorferi*, lot # 14709 diluted 1:99 with distilled water. The slides incubated at 37°C for one hour and then washed with distilled water. The slides were examined with a Nikon FA scope.

RESULTS

There was a significant increase in the percentage of engorged larvae that dropped from mice in the medium (25 larvae/day) and high infestation (50 larvae/day) groups (Table 1). The low infestation group

(5 larvae/day) did not exhibit a significant increase in the percentage of engorged larvae that dropped (Figure 1). The density of larval infestation did not have a significant effect on the percentage of larvae that dropped off unfed. There was a significant decrease, however, in the percentage of flat larvae that dropped in all three infestation groups over time (Figure 2).

Table 1. Percentage of larvae recovered from each infestation group.

Density of larval infestation	Flat larvae dropped*	Engorged larvae dropped*	Proportion fed
5	4.4 ± 1.6	2.0 ± 1.9	0.13
25	25.2 ± 6.1	12.9 ± 11.6	0.17
50	43.1 ± 12.4	6.5 ± 18.9	0.11

* Mean ± standard deviation.

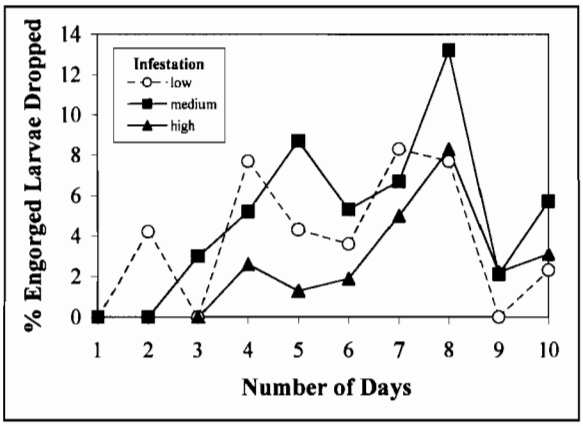

Figure 1. % Engorged Larvae Dropped.

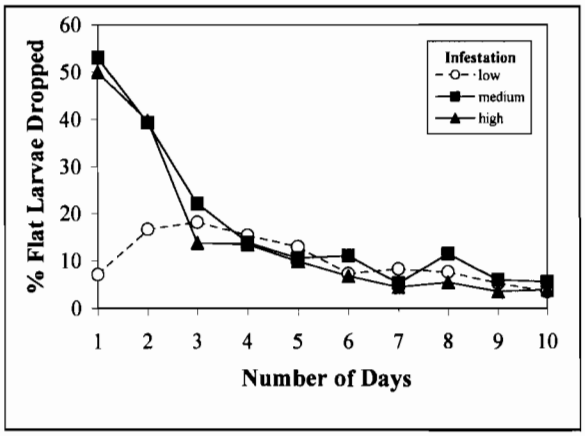

Figure 2. % Flat Larvae Dropped.

DISCUSSION

These results suggest that the presence of high densities of tick larvae may improve feeding performance on *P. leucopus*. This could be a result of immune suppression brought on by the tick feeding or by the lack of an effective immune response by the mice.

An immune response to ticks would result in the accumulation of antibodies in the blood of the mice and a subsequent decrease in the percentage of ticks that successfully feed to repletion (Anderson 1978). The severity of the immune response is a function of the number of parasites on the host (Anderson 1978). In this experiment, if the mice displayed an immune response, one would expect the high infestation group to exhibit a decrease in the percentage of ticks that successfully fed. The increase in the engorgement percentages for the high and medium infestation groups indicates tolerance to feeding by *P. leucopus* in these experiments.

The results of this experiment suggest that not only is *P. leucopus* tolerant to feeding by *Ixodes scapularis*, but that feeding performance actually improves at higher densities of larval infestation. These results are in agreement with those of Davidar (et al. 1989), which suggest that improved feeding at higher densities may give a selective advantage to the temporal clumping of ticks on the host. The diurnal drop-off of replete ticks, resulting in the concentration of ticks within the host's nest (Mather and Spielman 1986), and the limited horizontal movement of *Ixodes scapularis* (Fish and Falco 1991), may serve to maintain *I. scapularis* in high density patches in the environment.

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A MASS REARING PROJECT FOR MOSQUITOFISH AT THE GLENN COUNTY MOSQUITO AND VECTOR CONTROL DISTRICT

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An existing two-acre pond at a local fiberglass insulation manufacturing plant was modified over a nine-year period to optimize the extensive aquaculture of mosquitofish, *Gambusia affinis* (Baird & Girard). Unfortunately, fish introduction into this pond did not initially produce a significant number of progeny to employ for District's vector control operations. Chemical wastes from the manufacturing process and routine chlorination resulted in an aquatic environment that was not conducive to the propagation of this hardy fish or even its long-term survival. The pond was essentially free of either phyto- or zooplankton, which are necessary for both the establishment of a food web and protective cover for mosquitofish.

After this initial setback, the cooperation of the plant environmentalist, Ron Greenburg, was secured, which resulted in the elimination of undesirable chemicals such as solvents, oils, and other chemical wastes from the various drains feeding the pond. Water quality tests had also revealed that aeration of the pond water would be essential for a suitably aerobic environment. The company again demonstrated its willing cooperation by installing a system that consisted of four submerged air diffusers supplied by a Rootes-style centrifugal blower powered by a three horsepower electric motor. Later this system was improved by the incorporation of vertical water spray nozzles. Chlorination levels were then reduced to protect the fish and the food web organisms.

Another local rearing site at a milk producer's plant had always been phenomenally successful in the culturing of mosquitofish through the intentional introduction of waste milk solids and butterfat. These wastes greatly increased water nutrient levels and thereby served as a direct and indirect food source, so an attempt was made to simulate the conditions there by obtaining waste cheese materials from a local cheese factory and introducing them into the waters at the insulation plant's pond. The cheese factory donated about 300 pounds of cheese for this purpose each week and it was placed into wire mesh baskets, which were floated in the pond and refilled on a two- to three-week schedule. Six larger, live poultry transport cages later replaced the wire mesh baskets and were positioned at both ends of the pond.

Planktonic organisms have been periodically introduced by the transfer of pond water from the milk plant ponds. This has helped establish suitable ecological base for the pond's food web. Planktonic algal growth rapidly creates water column turbidity, which also serves as cover for mosquitofish progeny and has thus reduced undesirable cannibalism by adults.

Fish production from this pond has varied over the years, with the first successful harvest year of 1990 that yielded 2,076 pounds over a six-month period (March to September). Monthly harvests averaged 346 pounds, but monthly totals ranged between 160 and 492 pounds. Subsequently, further engineering modifications to this culture system have been made. A new spray aeration system consisting of three Aqua-Lator Fountains having a combined output of 270,000 gallons of water per hour has replaced the older aeration system. More recently in 1996, 261 pounds of fish were used to restock this pond, and in May 1997, harvests were again initiated. Over a three-month period commencing in May 1997, 1038 pounds of fish were seined for District needs. Again, monthly harvests averaged 346 pounds and varied between 121 and 590 pounds.

INFESTATION OF THE SOUTHERN ALLIGATOR LIZARD BY *Ixodes pacificus* AND ITS SUSCEPTIBILITY TO *Borrelia burgdorferi*

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Subadults of the western black-legged tick *Ixodes pacificus* utilize a wide variety of hosts including reptiles, birds and mammals. Lizards appear to be much more important as hosts of this tick than either birds or mammals that occupy the same habitats. At present, the only lizard that has been intensively studied as a host of *I. pacificus* is the western fence lizard *Sceloporus occidentalis.* This study investigates the host potential, duration of tick attachment and the reservoir competence of the southern alligator lizard *Elgaria multicarinata* for the Lyme disease spirochete *Borrelia burgdorferi.* Fourteen lizards were collected from two sites on either side of the northern Central Valley of California. Seven lizards were collected from Drivers Flat in Placer County and seven were taken from Cache Creek Canyon in Yolo County. Each lizard was held in an individual cage while attached ticks fed fully and dropped-off. Overall mean abundance of *I. pacificus* on all 14 lizards was 34.1 (range 3- 63) for larvae and 11.0 (range 1-28) for nymphs. Attached *I. pacificus* larvae and nymphs required 12.6 (range 1-37) and 14.4 (range 5-44) days to feed fully, respectively. After fed ticks had molted, the resultant nymphs and adult ticks were tested for the presence of *B. burgdorferi. Borrelia burgdorferi* spirochetes could not be isolated from either the blood of lizards or lizard-fed ticks and efforts to cultivate spirochetes on lizard sera were unsuccessful. We conclude that, as with *S. occidentalis*, the southern alligator lizard is not a competent reservoir for *B. burgdorferi*, though it is an important host for *I. pacificus* subadults.

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NUISANCE AQUATIC MIDGES, AN EMERGING PROBLEM IN SOME URBAN AREAS OF LOS ANGELES COUNTY

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ABSTRACT

We initiated basic and applied studies on the nuisance aquatic midge problem in the Ballona Creek drainage system in the western part of Los Angeles County along the Pacific Coast in California. All channels and waterways supported a variety of aquatic midge species. Areas of concrete-lined bottom with thin layers of attached algae and algal masses in the waterways contained dense numbers of *Cricotopus* larvae. Algal growths on the concrete surface encrusted with sediments as well as algal mats growing on the sides of channels supported both *Cricotopus* and *Dicrotendipes* midge larvae. Thick deposits of sand with muck, silt and muck in the intertidal zone (brackish water) area supported *Tanytarsus* sp. 1 larvae only. Observations on the nuisance level of midges in 1997 at night in residential areas showed this latter species to be the main problem. Other species were contributing little to the nuisance problem during the present season. Changing conditions may allow other less prevalent species to become dominant and cause nuisance problems, and therefore additional observations are warranted.

A system of storm drains and flood control channels carry surface and storm water from the west and central parts of Los Angeles County dumping it in the Pacific Ocean. The main channel draining this area is known as Ballona Creek. Several other channels such as Sepulveda and Centinela dump storm and surface water into the lower end of Ballona, which then empties into the Pacific Ocean. Other small drains and channels also empty into Ballona Creek. Ballona Creek is paved with concrete in its entirety except about a 2-mile stretch at the lower reaches starting at Centinela bridge and extending to the Pacific Ocean. The other major channels are also concrete lined.

Ballona, as well as the other channels, traverse through urban areas of Western Los Angeles County. The channels are in the midst of high value residential, commercial and industrial properties. The midge nuisance problem has been a perennial one for almost 30 years in and around Mar Vista, Playa del Rey and other coastal communities. In the 70s, Mr. Norm Hauret, then manager of the L.A. County West Vector Control District, related to the senior author that there was a nuisance midge problem created by the drainage system. However, no funds were made available to tackle this problem, and until last year, very little if any information was at hand to manage midge infestations in this system. The best that the District could do was to treat the channels in the problem areas with mostly organophosphate larvicides labeled for use on midges. In recent years, B.t.i. and methoprene treatments have replaced organophosphate insecticides in the control program. The extent of control provided by these treatments is not known.

The Research Project: In 1996, at the urging of L.A. County West Vector Control District and combined with support from concerned citizens in the area, the Los Angeles County Dept. of Public Works contacted the senior author at the University of California, Riverside, to develop a research project leading to the possible control of midges in the lower reaches of the Ballona drainage system. A research project was developed and funded by the Department of Public Works and was launched in cooperation with the L.A. County West Vector Control District in April, 1997.

The objectives of the project as stipulated by the Department of Public Works were: Determine effectiveness of channel clean out. At present the Public Works Department drags, cleans and stirs up water in the paved portion of Ballona Creek every six weeks

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in the summer months. This strategy is based on our studies of clean out of Coyote Creek in Los Angeles County carried out some 20 years ago. It is not known whether this practice leads to reduction of midge production in Ballona Creek. 2) Consider paving the unpaved portion of Ballona Creek as a control measure where the nuisance problem in surrounding properties is more intense. The citizens in the area believe that the midge nuisance problem is confined to the unpaved portion and are pressuring Public Works to line that section with concrete, with a cost estimate of $15,000,000 (1995). The question posed by Public Works is whether paving would solve the midge problem. 3) Identify problem midge species and determine the ecological conditions, which create this nuisance problem. 4) Evaluate the efficacy of methoprene treatments in the Ballona Creek system. Methoprene is one of the control agents which is currently used by the L.A. County West Vector Control District in the drainage system, but the level of control realized is not yet established. 5) Evaluate the efficacy of B.t.i., as this is another material used for midge control in this environmentally sensitive area. As with methoprene, the effectiveness of B.t.i. is not known for this drainage system.

Studies Launched: We embarked upon detailed studies on the nature and scope of the midge problem in the lower reaches of the Ballona drainage system. At weekly intervals, benthic and drifting larval populations were sampled using a scoop sampler for deposits in the water, a Surber sampler in the running water, plus collections of attached and floating algae. Additionally, we made observations on resting populations during the daytime and visited residences at night to evaluate the extent of infestations and the species causing the nuisance problem. We also identified and counted adult midges collected in NJ light traps operated by the Vector Control District.

Observations on Abundance and Pestiferous Nature of Adult Midges: On the night of June 17, 1997, we visited several homes in the housing tract west of Centinela Blvd. bordered by Ballona Creek on the north and Centinela Channel on the south. This housing tract has experienced heavy outbreaks of nuisance aquatic midges in the past several years and is experiencing similar outbreaks, although of lesser magnitude in 1997. The purpose of our visit at night was to assess the intensity of adult midge activity around homes, lights, and on streets by observing, collecting and identifying the problem species. Although 20 or more species of midges are breeding in Ballona Creek and other channels adjacent to the impacted area, we are of the opinion that only a few of these species actually create the nuisance problem. By identifying and knowing these species, it will be easier to develop and administer management strategies more effectively.

At 20:00h (sunset), we arrived at the residences and the people living there told us that midges do not become active until 21:00h pm or later. At this time, the weather was cool with a thick cloud cover and a diminishing breeze blowing from the Pacific Ocean in an easterly direction. We went around the neighborhood and took sweepings from shrubbery and tree branches adjacent to the channels. There were quite a few adult midges resting in the foliage. Most of the adults belonged to the genus *Tanytarsus* (new species, *Tanytarsus* sp. 1). The identifications were confirmed by Dr. James E. Sublette and Mr. Martin Spies. We also noted masses of dead adult midges entangled in spider cobwebs on screens and fascia of buildings.

Soon after sunset (ca. 20:30h), we observed huge swarms containing (5,000-10,000 individuals) in backyards of houses bordering Centinela Channel. Swarms rising above the roofs of houses were waving like twisters. There were many midges flying at ground level, and as one walked, midges would hit the face, and get into eyes, mouth and ears.

At about 21:00h, when the breeze ceased completely, there was a flurry of adult midge activity and large swarms formed around streetlights. Dead and living midges eventually covered automobiles parked on the street and driveways under adjacent lights. A fairly large number also were flying in the air. The airborne midges were sufficiently numerous to be felt just by walking in the street as they impacted the face and other exposed parts of the body.

Detailed observations were made at several of the residences adjacent to Ballona Creek. There were large numbers of midges flying and swarming around lights, even though the residents told us that this night midge numbers were relatively tolerable. Their numbers, however, were much smaller than previous nights because of an approaching cold front that resulted in cooling evening temperatures. Nonetheless, we noted very large numbers of midges (mostly *Tanytarsus* sp.1) crawling and clinging as this species has the characteristic to stick to screens, stucco, fascia and other surfaces of homes and automobiles in the neighborhood. Midge activity also was noted to

increase markedly when indoor or outdoor lights were turned on. Another important feature of these midge infestations was the ease at which they readily entered in mass into houses during entry and exit. Being tiny, some of these midges can gain access through the window screens.

We noted both dead and live midges on window-sills and in window guides. Many were stuck on stucco, fascia, windows, window screens, etc. Additionally, spider cobwebs entrapped many of the midges and these cobwebs appeared as gray spots or blotches on stucco, window screens and other structural surfaces.

Some of the houses we visited had all the lights turned off with only a dim light or two turned on in the house.

Comments by the Residents: The comments made by some of the residents that we contacted are as follows:

"The midges have been really bad for the past three or four years. This year they started early."

"It is not known as to why they have become more numerous in the last several years."

"Tonight (June 17, 1997), the midge infestations are tolerable and nothing compared to other nights (even though we found the population of midges to be heavy and not tolerable".

"The adult midges swarm, attack you, and hit you in the face if you are outside. When we have a barbecue or we have a party, as soon as the dusk period approaches, midge swarms and activity begin. Everyone has to run inside, close all doors and windows, and turn the lights off." (We noted this to be true, as it was difficult to enjoy the outdoors at dusk or later.)

"Accumulation of dead midges along with cobwebs is unsightly. We have to vacuum dead midges and spider webs quite often. Brushing will not do the job because the dead midge bodies will leave permanent indelible spots on house surfaces. Special vacuum attachments have to be concocted to reach the eaves and upper walls of one and two-story houses."

"Midges enter houses and swarm and hover in kitchens, living rooms, dining rooms, and other lit areas. They enter fluorescent and incandescent light fixtures. On midgy nights, we could not eat in the dining area with the lights on. We had to eat in the dark, which does not leave a good impression with the guests."

CONCLUSIONS

It is not appropriate to make any definitive conclusions based on a single night's observation. Observations of this type have to be made on several occasions to cover the full spectrum of weather conditions, seasonal midge species compositions, and spatial distributions. However, the following observations are an indication of the nature and scope of the problem.

Midge infestations, even though lower compared to previous nights, seemed to be heavy and substantial at some premises. Additional night observations also indicated midge presence and swarms.

Statements and assertions made by the residents are largely factual.

From creek and channel sampling, we know that heavy midge breeding is taking place in the waterways, but not all species, at least during this season, were a nuisance problem.

Although 20 or more species of midges inhabit the waterways in the Ballona Creek area, we found only two or three species to be problematic in the residential area. Of these, *Tanytarsus* species (possibly two species) are the primary source of complaints, followed by *Chironomus* spp., *Dicrotendipes* spp. and others.

These midges are highly attracted to bright lights, both inside and outside. Such extraneous light sources compete with the New Jersey surveillance light traps set in residential yards. Even though the light traps may only catch a small number of midges, they are indicative of the types of midges invading the affected residential area.

We are beginning to see a close relationship between certain midge breeding areas and the resultant infestations. Not all the species breeding in the creek or channels are associated with the problem. This aspect needs further corroboration in time and space, as seasonal and ecological changes may alter abundance and render less bothersome the more numerous species.

We visited the midge-impacted areas two or three additional nights during the summer season, but found that adult midge activity was quite low during the dusk periods. Even on these low activity nights, *Tanytarsus* sp. 1 constituted 80-95% of the population. The remaining species found around the resi

dential premises belonged to the genera: *Chironomus*, *Dicrotendipes* and a few *Tanypus* sp. Interestingly, *Cricotopus* species, although the most dominant species breeding in the waterways above the intertidal areas, were absent from residential establishments. This latter group probably remains in the creeks and/or are not readily attracted to lights. Again, repeated observations are needed to document the problem causing midge species over space and time.

Problem Species: Observations at night in residential areas and sweeping of resting sites of midges during the daylight hours and collection of light traps showed the dominant midge to be a new species of *Tanytarsus* designated as *Tanytarsus* sp. 1 (James Sublette and Martin Spies 1997, personal communication). Assessment of the breeding sites of this midge showed that it only breeds in mud, muck, and encrusted sandy substrates with muck in the intertidal zone of the lower portions of both Ballona Creek and Centinela Channel. An unidentified *Tanytarsus* species was also recovered during the spring from deposit substrate in Sepulveda Channel where water has higher salinity than the freshwater in Ballona Creek and Centinela Channel. The intertidal zone in the latter channel where *Tanytarsus* sp. 1 breeds almost exclusively is near the midge impacted residential developments. The remaining stretches of the creek and channels, although supporting heavy populations of *Cricotopus* spp., were not contributing to the nuisance problem at this time. In some niches in the creek and channels, considerable numbers of *Chironomus* and *Dicrotendipes* were also prevailing, but we did not notice these to be a major cause of the problem during the 1997 summer period. The halobiont *Tanytarsus* sp. 1 seems to be the species responsible for the problems in the affected residential establishments. Because of the lack of factual information on this species, it is not known how long this species has been the main cause of nuisance and misery in this area. Again, it should be emphasized that other species of midges may become pestiferous due to changing seasonal and ecological conditions.

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OPTIONS FOR FILTH FLY SUPPRESSION

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Flies are not a bad thing. They serve as exemplary recyclers and primary decomposers, synergizing microbial degradation of organic material, both animal and vegetable in origin. However, adult flies have the unattractive habit of invading our homes, regurgitating and defecating on our food, and leaving fly specks where they perch. Adult flies are considered pestiferous, both because of their unsanitary habits and due to their mere presence. While filth flies can serve as vectors, their significance in disease transmission in developed countries is minimal, due to environmental factors such as closed sewer systems and screens on windows and doors. Preventing flies from having access to potentially infective material limits their ability to acquire infectious agents. Though flies are of little consequence as disease vectors in the United States, they are objects of control efforts due to their pest status.

In California, only a few insecticides are registered for fly control. The most ubiquitous are the pyrethrins, the insecticide of choice found in spray cans in virtually every home. Likewise, allethrin and resmethrin are used in aerosols for quick knockdown of flies indoors. Permethrin is the most commonly used pyrethroid, combining photostability with the rapid knockdown of natural pyrethrin products. Organophosphates used in fly suppression include dibrom (naled) and dichlorvos (vapona or DDVP). The only carbamate registered for fly control is methomyl bait, sold under a variety of brand names. There are no biological pesticides, insect growth regulators, or insect development inhibitors marketed for use by either the general public or vector control agencies.

Chemical use is limited operationally, financially and practically. Available insecticides are being lost for three major reasons (1) insecticide resistance renders them ineffective, (2) the cost of reregistration is not justified by the potential market, so manufacturers drop them, and (3) the general public is less tolerant of "chemicals" (while simultaneously demanding a pest-free environment).

As with any pest management program, the three foundations of filth fly control are sanitation, exclusion, and source reduction. Low-tech strategies including sanitation (cleaning up larval habitat such as animal wastes and spilled garbage), exclusion (screening doors and windows, air doors), and source reduction (eliminating sources from which flies migrate, such as open dumpsters), have had significant impact on fly suppression.

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FLY CONTROL IN AN AGRICULTURAL ECOTONE

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The Chino Valley is located approximately sixty miles east of Los Angeles. The portion of the valley serviced by West Valley MVCD is the home of over 290,000 cows associated with the dairy industry and over 500,000 chickens producing for the egg industry. It is also the home to ever increasing numbers of human residents who purchase homes in subdivisions built in very close proximity to agricultural operations. The people living in this ecotone experience continuing annoyance from high numbers of flies.

The dairy industry and the egg producing industry have been present in the Chino Valley for many decades. Over the past several years the costs of operating dairy and chicken ranches has increased dramatically. To make a profit, agricultural operators have increased animal density to yield greater production. This situation has resulted in greater volumes of manure being present. To lessen water pollution, restrictions on the spreading of manure on open ground have been imposed. The result is that most manure remains on the facility for periodic removal and shipment to other geographical areas. This manure serves as a prime habitat for the production of flies. In addition, the dairy operators stockpile several tons of feed commodities that provide breeding media if left to decompose.

In 1987 the West Valley MVCD initiated an intensive program of fly control. All sources of fly production such as manure and feed were surveyed and their locations mapped. The amount of fly breeding was found to be extremely high, with most locations averaging in excess of 100 larvae per pint of breeding medium. It was quickly determined that manure and feed management practices were marginal at best. While corrals and chicken houses were routinely cleaned, the resulting piled manure was not properly composted to reduce fly production. The perimeter areas of stored feed were not moved systematically, thus allowing the rotting vegetative matter to become a medium for fly production. The only fly control procedures usually employed by the agricultural operators were adulticiding treatments performed when adult fly numbers in and around milk barns and chicken houses became unacceptable. Such treatments temporarily depressed the adult fly population but were of little long term value as the sources of fly production remained.

In an effort to quickly depress the populations of flies, primarily *Musca domestica* and *Fannia* sp., all available fly control chemicals were tested. Chemicals labeled for use as topical applications were found to be ineffective as they did not penetrate the manure or other breeding medium sufficiently. Insecticides registered for use as feed-through fly control agents also were not effective, probably due to the low dosages allowed and the inconsistent applications performed by the agricultural operators. It was further determined that serious insecticide resistance to permethrin and naled (dibrom) existed in the adult fly populations of *M. domestica,* probably caused by the overuse of the two products for many years. The only efficacious control product for adult fly suppression was pyrethrin. However, the method of aerosol application was critical as climatological conditions and insect avoidance mechanisms greatly affected the rate of mortality. In the last year, the citrus based compound called limonene has become available for fly and other insect control in California. This material is very effective when applied topically to manure if the manure is less than about four inches deep. It is also effective in controlling adult flies if applied directly on the flies in a heavy mist such as that produced by a hand-held pressure sprayer. Previously completed tests by researchers from the University of California, Riverside, have shown that fly parasitoids were not very effective in controlling immature flies as introduced parasitoids merely displaced those naturally occurring in this area.

Based upon the preceding information, district fly control technicians embarked on a program of

weekly inspections to educate the agricultural operators regarding proper manure and feed manipulation techniques. Dairy operators were shown the amount of fly breeding present in manure left under fence lines, along feed aprons, and in corrals. Poultry ranchers were shown the maggots inhabiting wet areas in coned manure and in improperly stored dead birds. Both types of operators were also shown the high volumes of immature flies present in piled manure and improperly stored feed. Operators were instructed on how to properly compost manure to reduce moisture and maintain internal pile temperatures high enough to kill immature flies. These cultural methods of control were augmented by ultra low volume ground applications of pyrethrin in and around fly producing sources. To obtain reasonable adult fly mortality, applications were performed in the early morning hours at the point when the adult flies were just beginning to move about. ULV applications made later in the day yielded little or no mortality. Adulticiding applications in neighborhoods were tested and discarded as little mortality was obtained and exposure to the residents was too great.

Currently, the fly control program is in a maintenance phase. Control activities have reduced the fly population levels as assessed by surveillance traps (Figure 1) by over 75 percent, which may be the best obtainable in this situation. These traps are placed in locations where flies are likely to congregate, such as under the eaves of buildings or in trees. The traps are collected weekly and the numbers of flies are counted. At sites close to agricultural operations, it is not uncommon to collect 3000 or more flies (predominantly *Musca, Fannia* and *Mucina).* Traps in residential locations can collect over 1000 flies if fly suppression activities on nearby agricultural sources are below standard. Experience has shown that collecting 700 or more adult flies per week leads to a substantial increase in complaints from residents. Inspections still occur on a weekly or more frequent basis. Each entity inspected receives an inspection report which is followed by a warning notice and an abatement notice if problems persist. The legal abatement process regarding agricultural operations is stipulated by the parameters existing in the California Health and Safety Code, Section 2200. For the Board of Trustees to determine that a public nuisance is present on a given property, each of four parameters

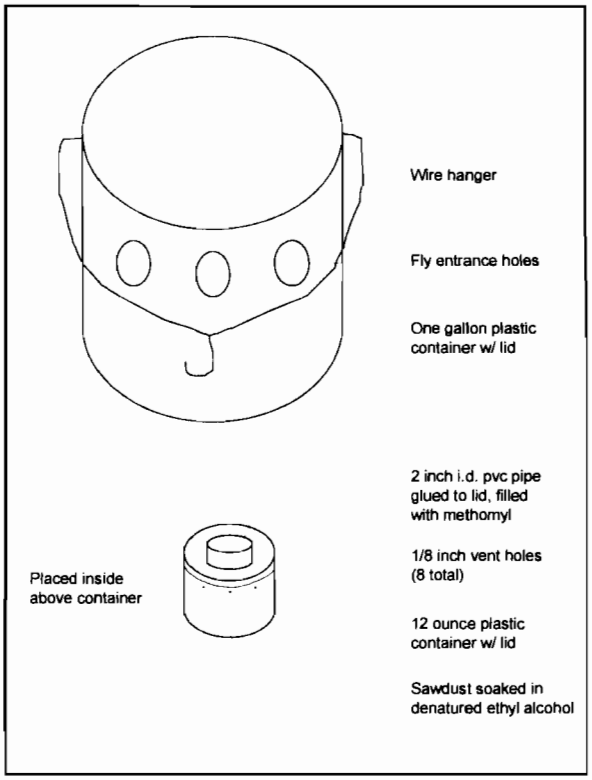

Figure 1. Adult Fly Surveillance Trap.

must be shown to exist: 1) immature and adult flies must be present in numbers considerably in excess of those in the surrounding environment, 2) the problem is associated with the design, layout, and management of the operation, 3) the flies disseminate widely from the property, and 4) the flies cause detrimental effects on the public health and well-being of a majority of the surrounding population. Referring to parameter 1, many times the density of adult flies is greater in the homes across the street from the agricultural operation. With many sources on different nearby properties, it is impossible to determine (parameter 3) where the flies came from unless a particular source is producing flies at a significantly higher rate than the others. Thus, if parameter 1 and/or parameter 3 can't be verified, the legal process is negated as it could not be proven in a Hearing that all of the four parameters exist. Local agencies such as counties and cities are prevented from enacting and enforcing ordinances citing agricultural operations as public nuisances if those operations were present before the conditions around the agricultural operations changed (Civil Code, Sec.

3482.5 & 3482.6). In other words, a city can't initiate an ordinance against agricultural operations if the houses recently built next door now have flies.

Fly control in those areas where housing developments and agricultural operations exist literally side by side is possible, but it is a very difficult and frustrating process. Both the laws of California and the principles of biology dictate that it is not possible to reduce flies in an agricultural ecotone to the level enjoyed by persons living in the typical suburban setting. Unfortunately, such an explanation is not easily accepted by much of the populace who now live next door to thousands of cows and chickens.

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HOST-SEEKING ABUNDANCE OF *Culex tarsalis* IN MICROHABITATS OF THE COACHELLA VALLEY, CALIFORNIA

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ABSTRACT

During year one of a two-year study on the influence of microhabitat on mosquito host-seeking patterns, the physical profile of vegetation closely paralleled *Culex tarsalis* host-seeking abundance as measured by CO_2-baited traps. These data indicated that birds nesting or roosting within elevated vegetation had a greater risk of mosquito contact and arbovirus infection than those utilizing snags over water, sand spits or open fields.

In California, dry ice baited CDC style traps (CO_2 traps) are used to compare the relative abundance of host-seeking female mosquitoes in time and space and to collect specimens for arbovirus isolation attempts. Although traps frequently are positioned along vegetative ecotones, few studies have compared catch size among traps positioned in different microhabitats to determine the influence of landscape features on female hunting patterns.

The objective of the current report was to describe our progress toward determining the influence of microhabitat characteristics on the host-seeking patterns of *Culex tarsalis* in the Coachella Valley of California. These data will help to interpret estimates of relative abundance in surveillance programs as well as to establish the relative risk of mosquito contact among bird species exploiting different habitats as roosting and/or nesting sites.

MATERIALS AND METHODS

Five study areas were positioned along the margin of the Salton Sea from 81st Street to Hayes Street. Four to 6 CO_2 traps were positioned within different microhabitats along each of 3 parallel transects perpendicular to the shore of the Salton Sea. Traps were operated on 3 consecutive days during each experiment (n = 36-54 trap-nights in each experiment).

RESULTS

Experiment 1 (April 97): Habitats sampled were: 1) sandbar, 2) shoreline tamarisk, 3) saltgrass/pickleweed, 4) mesquite clumps, 5) citrus orchard edge, and 6) citrus orchard interior. The profile of the abundance of *Cx. tarsalis* was almost a physical representation of the height and mass of the vegetative habitats. Habitats ranked from lowest to highest catch were sandbar, saltgrass/pickleweed, shoreline Tamarisk, mesquite, citrus interior, and citrus margin; i.e., the edge of the habitat with the highest physical profile had the greatest abundance.

Experiment 2 (June 97): Habitats included: 1) offshore snags (roosting site for cormorants), 2) sand beach, 3) cattail marsh edge, and 4) cattail marsh interior. Similar to Exp. 1, *Cx. tarsalis* abundance at offshore and sand beach habitats was lower than at the high profile cattail habitat; catch at the edge and interior of cattail habitat were not different.

Experiment 3 (July 97): The habitats sampled were: 1) sandbar, 2) cattail marsh, 3) shoreline Tamarisk, 4) pickleweed, 5) citrus edge, and 6) citrus interior. *Cx. tarsalis* abundance again followed the physical profile of the vegetative habitat sampled, with catch in sandbar and saltgrass/pickleweed habitats lowest and catch in citrus edge and interior habitats greatest. Catch in cattail and tamarisk habitats was intermediate and significantly different from other habitats.

Experiment 4 (September 97): Habitats included: 1) off shore snags, 2) sandbar, 3) shoreline, tamarisk/pickleweed, 4) open field (grass and annual plants), 5) Tamarisk edge, and 6) Tamarisk interior. *Culex tarsalis* abundance again appeared to follow the physical profile of the vegetation; however, catch was variable and the only habitat that was significantly different from its nearest neighbor was the off shore snags. The extreme ends of the transect were

different significantly, indicating a trend of increasing abundance from shore to upland tamarisk.

Experiment 5 (October 97): No significant difference was found among rowcrop, citrus, and vineyard habitats, possibly due to the effects of higher humidity and lower temperature at this time of year.

DISCUSSION

Catch in CO_2 traps was influenced strongly by microhabitat as described by vegetative profile. Vegetative ecotones with marked changes in height consistently had the greatest abundance of host-seeking *Cx. tarsalis*. Habitats with low profile (salt grass, pickleweed) or no (sand bars, off shore) vegetation had the lowest catch size, whereas high profile vegetation (Tamarisk, cattails, citrus) consistently had the greatest catch. Other *Culex* species including *erythrothorax* and *quinquefasciatus* exhibited essentially comparable hunting strategies, whereas *Psorophora columbiae* were more abundant in fields and at the edge of vegetation of different height. These data indicated that sampling *Culex* using CO_2 traps would be most efficient when traps are placed along the edges of vegetation with an elevated profile.

Concentration of host-seeking females at elevated vegetation agreed well with the results of our surveys for arbovirus antibodies among birds and for *Culex tarsalis* host selection patterns using precipitin analysis of blood meals. Bird species most frequently infected roost at night in elevated vegetation and included house finches (orchards), Gambel's quail (mesquite clumps), common ground doves (orchards) and redwing blackbirds (cattails). Blood meal analysis similarly indicated that females collected within Tamarisk wind breaks fed most frequently on passerine birds.

Because these studies were confounded over time, there are some questions remaining regarding how weather and photoperiod (as it relates to diapause) affect the pattern of abundance. For example, low temperature and high humidity during October may have reduced the influence of microhabitat on host seeking and dispersal, resulting in comparable catch among sites. During year 2 of this study, we plan to compare the effects of season and density on host-seeking patterns at a single area, extend our database by including additional study areas, and include information on the abundance of blood engorged, resting females as a measure of feeding success.

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VECTOLEX: 1997 OPERATIONAL EXPERIENCES AND FIELD TRIALS

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The characteristics, attributes and mode of action of VectoLex CG were presented in this paper, followed by a summary of data from field trials and operational uses of the material in 1997. VectoLex CG is a granular, biological mosquito larvicide that is based on *Bacillus sphaericus*, a naturally occurring soil bacteria. VectoLex is highly specific in its' action against mosquitoes. It is very effective for the control of *Culex* mosquitoes, but recently has been shown to be quite effective on other species. One of the advantages of VectoLex is that it is very effective in polluted waters, and often provides significant extended residual control. VectoLex also has an excellent environmental profile due to its' low toxicity and specific action against mosquitoes. The mode of action of *Bacillus sphaericus* is very similar to that of *Bacillus thuringiensis israelensis* (BTI) in that the insecticidal components of the material consist of proteins that are non-toxic until digested by mosquito larvae. When VectoLex is applied to the larval habitat, larvae ingest the proteins which are attached to the bacterial spore and encased in a parasporal body. The proteins are broken down into smaller toxic components by enzymes and the high pH that are found in the larval midgut. The proteins then exert their toxic effect upon cells of the midgut epithelium, causing pore enlargement and cell destruction. Fluids from the larval haemolymph then mix with midgut fluids, and the pH of the larval midgut is neutralized. As the larvae die, spores of *B. sphaericus* germinate and the bacteria grow in the larval cadavers. This is believed to contribute to the extended residual that VectoLex often provides. In 1997, Abbott Laboratories obtained considerable information about the field performance of VectoLex CG. This information was obtained both through field trials and close monitoring of operational uses in various settings. VectoLex was applied to the Hemet Wetland in San Jacinto California for the control of *Culex tarsalis* and *erythrothorax*. Following the application, an eighty-fold decrease in *Culex* numbers in CO_2 traps in the wetland was realized. VectoLex CG was tested at 10 lbs per acre in a field trial in Sarasota County, Florida for the control of *Culex nigripalpus*. Greater than 98 percent control was realized for the 36 day sampling period. VectoLex was applied to a salt marsh in St. Tammany Parish, Louisiana in at 10 lbs per acre for the control of *Culex salinarius*. Despite tidal drying and re-flooding of the marsh, no live larvae were found in one of the treated sites for 54 days post treatment, while the control continued to produce. Control ranged from 80 to 100 percent in the other treated sites for 38 days. VectoLex was used in several operational situations in Multnomah County, Oregon in 1997. The material performed well against *Cx. tarsalis* and *pipiens* in a variety of settings. It also appeared to control *Culiseta incidens* when this species was present in the treated habitats. Control for more than 50 days was achieved in one site. Residual control of three to four weeks was typical. The material was tested in septic Ditches in Orange County, Texas for the control of *Culex quinquefasciatus*. It was applied at 15 lbs per acre to a 500 foot section of the ditch. More than 97% control resulted for three weeks. No sampling was done after three weeks, so it is not known if residual control lasted longer than this. VectoLex was also applied in an operational trial to sewage lagoons in the town of Saybrook, CT. The material was applied at rates ranging from 5 to 20 lbs per acre. Greater than 99 percent control of *Culex pipiens* was achieved in all the lagoons for the first 15 days of the test. Control lasted a minimum of three weeks in all the lagoons, and exceeded 91% for seven weeks in several. Finally, VectoLex was tested in catch basins in Champaign and Urbana, Illinois by Joel Siegel and Robert Novak of the Illinois Natural History Survey. This was a comparative study that looked at the residual efficacy of VectoLex as compared to that of Altosid Briquett in the catch basins. VectoLex was applied at 1 gram per basin, and Altosid was applied

at 1 briquett per basin. Both materials were found to deliver approximately 30 days control in the catch basins. In conclusion, VectoLex CG provided extended control of *Culex* mosquitoes in a variety of habitats during the 1997 season. Residual control from three to six weeks was achieved in most situations. Apparent control of *Culiseta incidens* was also noted.

Abbott Laboratories would like to thank everyone who contributed to gathering the information presented in this paper.

LABORATORY AND FIELD SAFETY FOR VECTOR CONTROL AGENCIES

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An increasing number of vector control agencies are developing laboratory facilities for furthering their technical and vector-borne disease surveillance services to agency residents. In the process, those agencies now become responsible for the safety and welfare of their technical staff dedicated to handling infectious materials and/or detecting the presence of vector-borne disease agents such as Hantavirus and plague. The new safety standards not only apply to routine laboratory operations, but also to surveillance activities in the field. With the increased "biological hazard" to technical staff presented by laboratory and field operations, these new activities will necessitate an increased level of safety supported by a higher level of employee awareness, dedication to following established safety protocols and standards (e.g., CDC guidelines), and accepting more personal responsibility. The increased safety is required in leu of the fact that if an accidental exposure occurs and an employee becomes ill from either a willful act of negligence or accident, the consequences of that incident can result in the loss of agency prestige (e.g., perceived professionalism), suspension of laboratory activities, and heavy penalty fines accompanying regulatory and legal actions. This paper briefly outlines some of the hazards presented by operating a vector-borne disease laboratory and associated activities related to field surveillance and handling vectors.

Mandated Worker Safety: The same federal and state statutes that apply to the safe handling and application of pesticides also apply to operating vector-borne disease laboratories and attendance to field surveillance activities. Title 8 (General Industry Safety Orders) of the workers' safety "Hazard Communication Program" entitles vector control technical staff to a safe work place and the "right-to-know" of existing hazards associated with their job requirements. The risks associated with laboratory operations and vector surveillance certainly present clear hazards that require unique programs with explicit language that relates to laboratory and field safety.

Safety Protocols, Training, and Documentation: A well balanced safety program for laboratory and field technical personnel should include the following elements 1) active supervision to facilitate quality control, 2) written safety and operational protocols, 3) verbal instructions, 4) effective use of personal protective equipment (PPE), 5) periodic training and review, and 6) mechanisms to engender personal responsibility and self evaluation.

All safety programs require active supervision to assure that staff are complying with established protocols and minimum safety standards as prescribed by law and agency policy. Lack of active supervision "could be perceived" as justification for management negligence in the event of an accident or act of employee irresponsibility, even though an employee was clearly informed, trained, and signatory to abide by written and verbal safety instructions.

Laboratory Safety Features: The function of a laboratory and attendant activities will dictate the level of isolation and containment required to assure safe handling and protection from exposure to the vectors and vector-borne disease agents being handled. (Fig. 1). The level of laboratory containment, isolation, protection, and handling procedures required for a vector-borne disease agent is stated in a number of guidelines published by the Centers for Disease Control (CDC). The CDC guidelines are considered baseline by both OSHA and Cal OSHA in establishing and enforcing safety minimums. The level (Level 1 through 4 facility) of safety required is further established by the relative pathogenicity (Class 1 through 4) of the agent. For example, among viruses transmitted by mosquitoes, Turlock virus is considered a moderately virulent Class 2 virus while yellow fever virus is highly virulent and accordingly is classified as a Class 4 agent. Therefore, when planning the con-

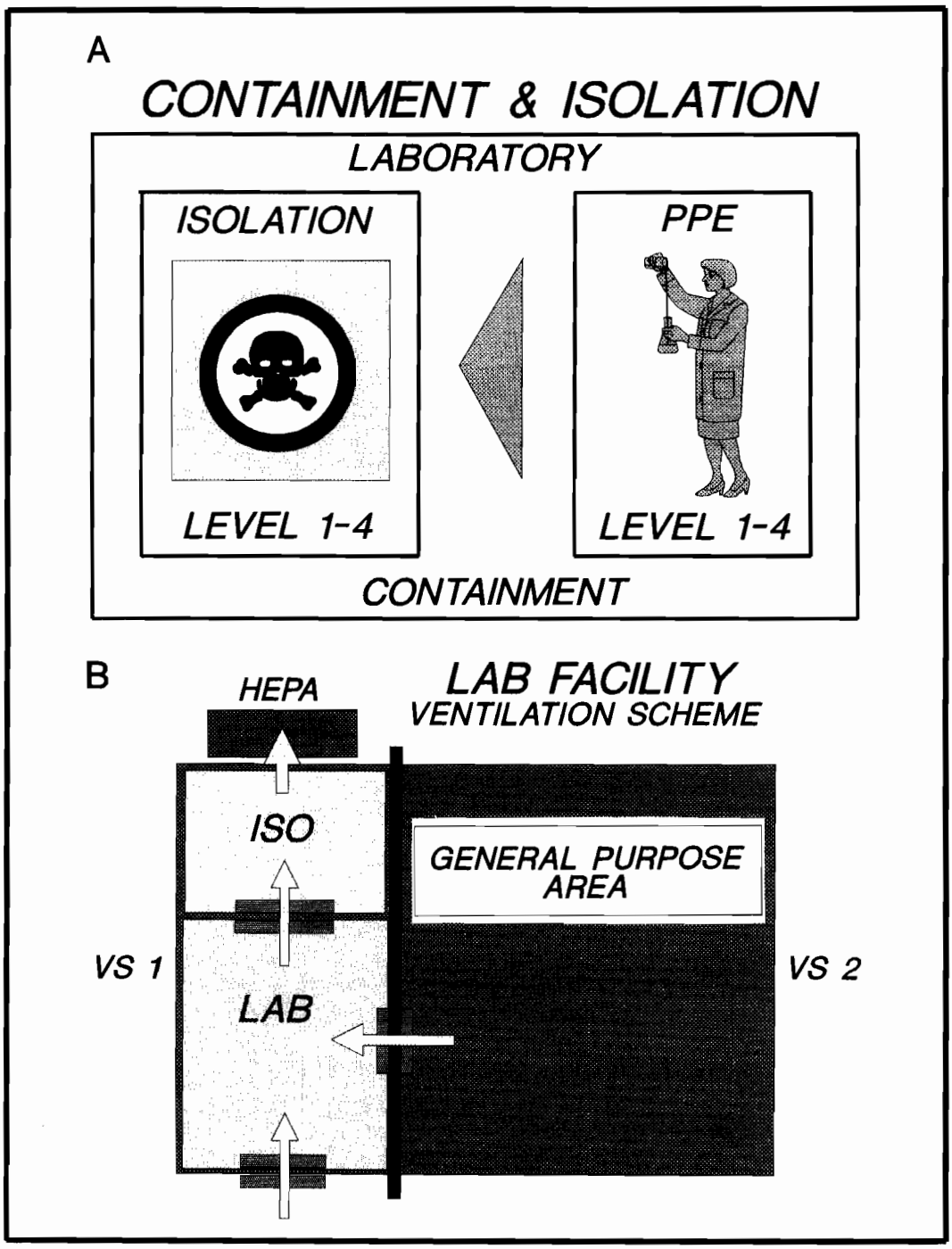

Figure 1. Schematic illustration showing the containment and isolation safety features (A) of a properly constructed vector control laboratory (B). Figure of laboratory facility illustrates direction of air flow (negative pressure) indicated by the arrows, separate ventilation systems (VS 1 and VS 2), and HEPA-type exhaust filter from biological hood and or isolation room.

struction and operation of a laboratory, foresight should be given to what vectors and/or vector-borne diseases will be handled by the facility. If the laboratory is only rated for handling Class I and II, but not III and IV agents, there is insufficient containment to guarantee adequate isolation from human contact. Containment and isolation are provided physically by a combination of air flow requirements (negative pressure gradients), dedicated isolation rooms, biological fume hoods fitted with HEPA-type filtration, glove boxes, and personal protective equipment.

Field Safety and Exposure Control: One of the more frightening aspects of field surveillance activities is the prospect of accidentally contracting a vector-borne disease from exposure to vector bites (e.g., flea infected with plague) and accidental contact (e.g., inhalation of Hantavirus from an infected deer mouse). Avoidance of an accidental exposure can be minimized by proper protection provided by appropriate PPE coupled with proper handling techniques. Guidelines for handling and protection (PPE) minimums are provided by the CDC with oversight from both OSHA and Cal OSHA. As an added safeguard against accidental exposure, field, as well as laboratory staff, should be sero tested periodically for the presence of disease specific (e.g., Lyme Disease or Plague) neutralizing antibodies. If an employee tests sero positive, then it is likely that an accidental exposure has occurred, which also indicates that exposure control procedures failed and should be reevaluated immediately.

Transporting Vectors: If a vector surveillance program includes the routine transport of vectors (e.g., rodents) back to the laboratory for processing, then an additional tier of vehicle isolation and containment safety is required to assure that accidental exposure does not occur, even in the event of a vehicular accident. Any transportation of animals back to the laboratory or nearby processing facility should be done in a manner that physically separates the air spaces of the driver/passenger compartment and animal containment compartment (Fig. 2). Also, placing either trapped or caged animal in an air tight insulated container that further limits the air space of the animals to confined space. Sufficient air and cool temperatures should be provided to facilitate the humane treatment of all confined animals during transport.

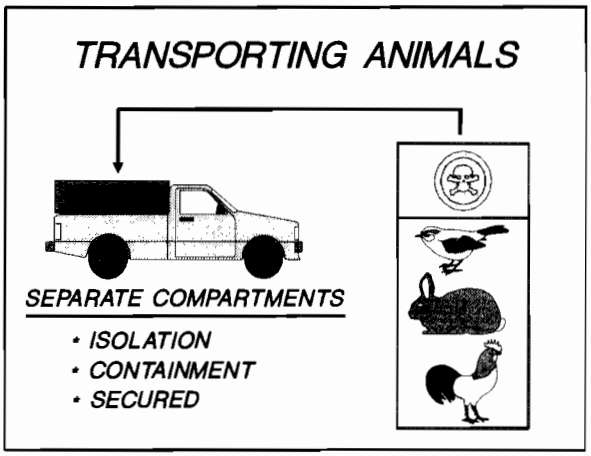

Figure 2. Illustration showing the proper separation of driver/passengers and potentially infected vectors for vehicular transport to either a laboratory or processing facility.

SUMMARY AND CONCLUSIONS

The unique role of vector control agencies and their expanding role with developing laboratory facilities and field surveillance efforts has by default extended their safety responsibility in protecting their technical staffs from exposure to vectors and vector-borne diseases. Within reason, all it takes is a single negligent or irresponsible act, or unforseen accident, to destroy the credibility of a vector control agency. For those reasons alone, safety in vector control laboratory and field operations is designed for one explicit purpose, to adequately protect the vector control employee and public from being harmed by vectors and the diseases they carry.

VISITING SCIENTIST PROGRAMS: A NEW ROLE FOR VECTOR CONTROL AGENCIES

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The increasing role of vector control agency participation in vector surveillance, control, and education now presents a unique opportunity to extend this operational reality to a new level of international cooperation in vector research and awareness. Many developing countries with well educated and experienced vector scientists currently are in a position to extend their expertise in a cooperative effort to facilitate "global" uniformity in vector research and management. The impetus for this inevitability will ultimately result in a working collaboration that is necessary in today's reality of commingling human populations that bring with them both communicable and vector-borne diseases. Therefore, the benefit of reciprocal scientific arrangements will produce 1) uniformity in research efforts, 2) development of more effective vector management practices, and 3) ultimately the exchange of disease-free populations. The role vector control agencies play in this new paradigm is important because most agencies have qualified staff that can assist with the process of providing practical expertise in both vector research and management that can be augmented with assistance from state and local public health agencies, and the University of California.

The Orange County Vector Control District's perception of this potential new role for vector control agencies has now become a working reality. Beginning in 1995, Dr. Webb initiated contact (via Dr. C.Y. Chow) with vector-borne disease scientists at the Guangxi Institute of Parasitic Diseases located in Nanning, Guangxi Province, Peoples Republic of China. Following months of correspondence, a formal visit was arranged for the Director of the Institute, Dr. Wang Shusheng, and Dr. Neng Wu to meet with District staff in Orange County and establish the guidelines for exchanging scientific staff. The outcome of that meeting resulted in a cooperative agreement with 1) the senior author traveling to Guangxi, PRC, to visit areas of active Lyme and mosquito-borne (malaria and Japanese Encephalitis) disease transmission and 2) arranging the eventual visit of Dr. Tan Yi to study tick-borne and other vector-borne diseases in southern California during the summer of 1998.

The senior author visited Guangxi, PRC, in September of 1997 to present the exchange program to selected staff of the Institute in Nanning, and to field staff at antiepidemic centers (equivalent to county medical centers) located in Ziyuan and Daxin. Presentations to Institute and field staff included 1) establishing equitable liaison between the United States and Peoples Republic of China, 2) commitment to provide our technology in development of state-of-the-art disease detection and surveillance presumably to be implemented in Guangxi, and 3) foster China's emerging role as a leader in vector-borne disease surveillance and management. The visit further solidified future visits and exchanges of scientific technologies to the benefit of both participating agencies and their respective countries.

Dr. Tan Yi will be visiting the Orange County Vector Control District from July through mid-September of 1998. During his stay at the District, Dr. Yi will have an opportunity to observe routine District activities and to take an active role in District field surveillance programs plus visit other vector control agencies in southern California and the Central Valley. As part of the mutual exchange package, Dr. Alan G. Barbour has agreed to provide PCR technology available in his laboratory at the University of California, Irvine, to Dr. Yi for testing alcohol preserved ticks collected from Lyme active foci in northern Guangxi.

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SURVEILLANCE FOR MOSQUITO-BORNE ENCEPHALITIS VIRUS ACTIVITY AND HUMAN DISEASE IN CALIFORNIA, 1997

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The California Mosquito-Borne Encephalitis Surveillance Program is a cooperative effort conducted by the California Department of Health Services' Division of Communicable Disease Control, the Arbovirus Research Unit of the University of California at Davis, the Mosquito and Vector Control Association of California, local mosquito and vector control agencies, local health departments, physicians, and veterinarians, and other interested parties. The multifaceted program includes: 1) mosquito population monitoring and testing for St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) virus infection, 2) serological monitoring of sentinel chickens in areas of California with historical evidence of encephalitis virus activity, 3) testing of domestic animal species that show clinical signs of possible SLE or WEE infection, and 4) serological testing of patients presenting symptoms of viral meningitis or encephalitis. The 1997 surveillance program began in April with the deployment of sentinel chicken flocks and the beginning of mosquito collection data for the Adult Mosquito Occurrence Report. On May 9, the first of 29 weekly bulletins and adult mosquito abundance reports was distributed to all surveillance program participants. Positive serology and mosquito pool results were communicated immediately to submitting agencies.

Human Disease Surveillance: In 1997, 198 human serum and/or cerebrospinal fluid specimens from patients presenting symptoms of viral meningitis or encephalitis were tested by the Department's Viral and Rickettsial Disease Laboratory (VRDL) for antibodies to SLE and WEE viruses. In addition, several specimens were referred to the Centers for Disease Control and Prevention's (CDC) Arbovirus Disease Laboratory by the Infectious Diseases Society of America emerging infections network. Neither elevated IgM antibody nor a four-fold rise in total antibody between paired sera was observed in specimens from any of the suspect patients whose specimens were tested at VRDL. However, of the specimens tested at CDC, cerebrospinal spinal fluid from one patient from Pomona (Los Angeles County) was positive for SLE IgM antibody by IFA and neutralization tests. The patient, an 83 year-old female, developed a stiff neck and drowsiness on October 17, became comatose, and died on November 5. The patient reportedly had frequent evening exposure to mosquitoes.

Equine Surveillance: Serum and brain tissue specimens from three horses displaying neurological signs were submitted by practicing California veterinarians for arboviral testing at VRDL in 1997. Autopsy specimens from a four-month old foal that expired on July 21 were positive for WEE via serology and antigen testing. The foal was from a Thoroughbred broodmare ranch near Stevinson, Merced County. In addition, the United States Department of Agriculture / Animal and Plant Health Inspection Service (USDA/APHIS) Veterinary Diagnostic Laboratory in Ames, Iowa reported positive WEE samples from three horses from Kern County (July), and Shasta and Modoc Counties (August).

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Table 1. Mosquitoes tested by VRDL for WEE, SLE, and CE viruses in 1997, by submitting agency and mosquito species.

County*	Agency	*Cx. tarsalis*		*Cx. pipiens/ Cx. quinquefasciatus*		*Cx. stigmatosoma*		*Ae. melanimon*		Total	
		pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.
Butte	BUCO	1	30	2	75	0	0	0	0	**3**	**105**
Contra Costa	CNTR	108	5400	0	0	0	0	0	0	**108**	**5400**
Fresno	CNSL	6	271	0	0	0	0	0	0	**6**	**271**
Fresno	FRNO	10	363	0	0	0	0	0	0	**10**	**363**
Glenn	GLEN	31	1550	0	0	0	0	0	0	**31**	**1550**
Jackson/OR	JCVC	65	3135	9	450	0	0	0	0	**74**	**3585**
Kern	KERN	168	7481	0	0	0	0	32	1326	**200**	**8807**
Kings	KNGS	32	1579	0	0	0	0	1	50	**33**	**1629**
Lake	LAKE	138	6704	0	0	1	44	7	285	**146**	**7033**
Los Angeles	GRLA	9	449	3	136	3	107	0	0	**15**	**692**
Los Angeles	LACW	151	6555	126	5207	30	701	0	0	**307**	**12463**
Los Angeles	LONG	145	5080	104	4015	0	0	0	0	**249**	**9095**
Los Angeles	SGVA	5	226	5	195	2	70	0	0	**12**	**491**
Madera	MADR	1	50	6	300	0	0	0	0	**7**	**350**
Marin	MARN	3	10	0	0	5	181	0	0	**8**	**191**
Orange	ORCO	18	354	101	2444	0	0	0	0	**119**	**2798**
Placer	PLCR	21	860	0	0	0	0	0	0	**21**	**860**
Riverside	NWST	15	448	126	5927	4	116	0	0	**145**	**6491**
Sacramento/Yolo	SAYO	408	18221	0	0	0	0	19	767	**427**	**18988**
San Bernardino	SANB	10	182	11	410	13	460	0	0	**34**	**1052**
San Diego	SAND	80	4000	0	0	0	0	0	0	**80**	**4000**
San Joaquin	SJCM	40	1965	0	0	0	0	0	0	**40**	**1965**
Santa Barbara	GLVY	3	83	1	30	0	0	0	0	**4**	**113**
Shasta	SHAS	18	753	5	262	0	0	0	0	**23**	**1015**
Stanislaus	EAST	10	250	0	0	0	0	0	0	**10**	**250**
Stanislaus	TRLK	30	1500	4	200	1	50	0	0	**35**	**1750**
Sutter/Yuba	SUYA	230	10293	0	0	0	0	16	724	**246**	**11017**
Tulare	DLTA	27	1077	0	0	0	0	0	0	**27**	**1077**
Ventura	VENT	14	685	0	0	0	0	0	0	**14**	**685**
	Total	**1797**	**79554**	**503**	**19651**	**59**	**1729**	**75**	**3152**	**2434**	**104086**

* Bold font indicates counties with WEE isolation from *Culex tarsalis*

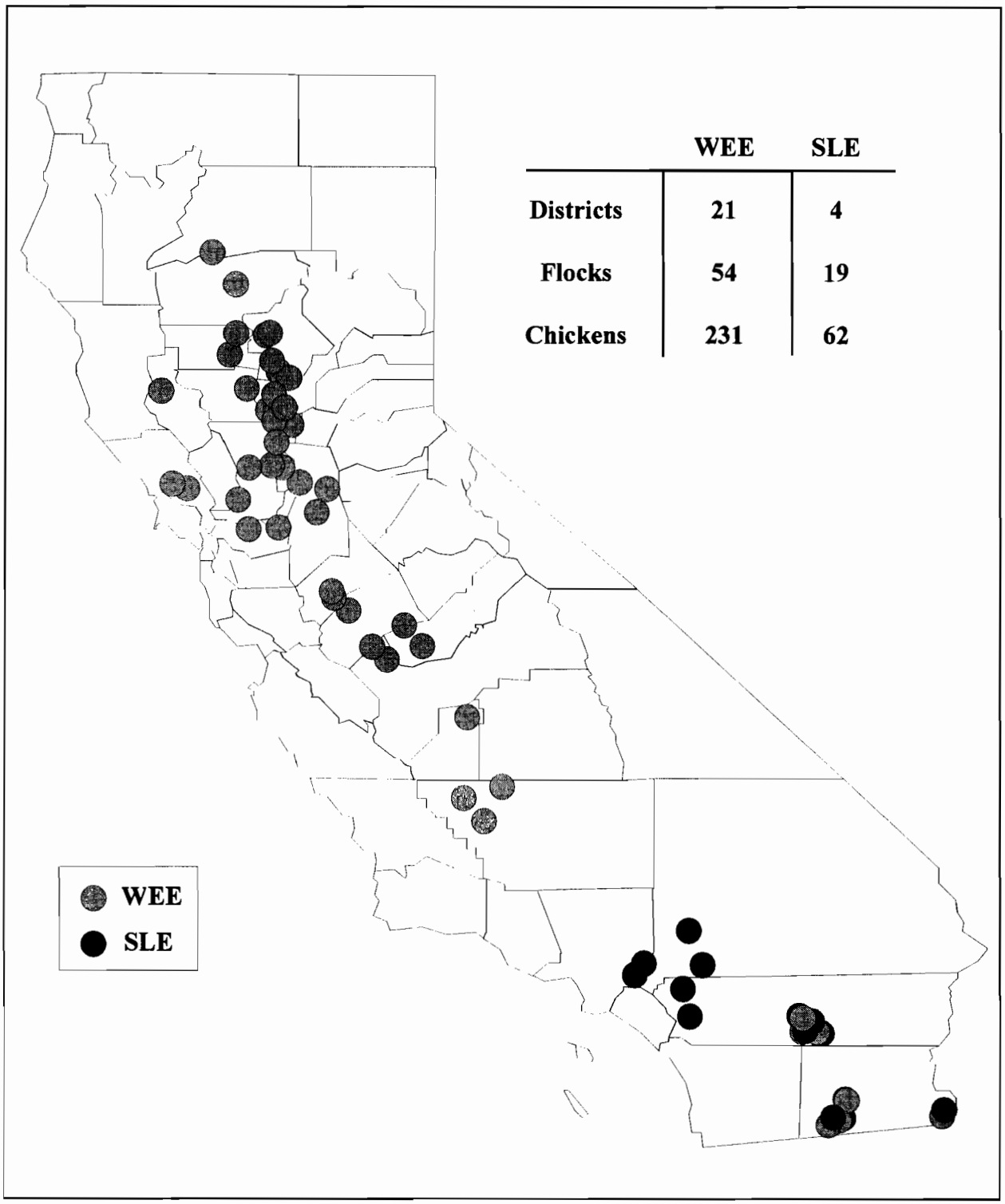

Figure 1. Sentinel chicken flocks with at least one seroconversion to St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus, California, 1997.

Mosquito Testing: Twenty-nine local mosquito control agencies submitted for testing a total of 104,086 mosquitoes (2,434 pools) in 19 97 (Table 1). Mosquitoes were pooled and tested for arboviruses by plaque assay using Vero cell culture. Of these, 35 pools were positive for WEE and none for SLE. Positive *Culex tarsalis* pools were collected between July 4 and September 12 in Glenn (2), Lake (4), Placer (3), Sacramento (2), Stanislaus (2), Sutter (14), and Yolo (8) Counties. An additional 1,302 pools of mosquitoes collected from Kern and Riverside counties were tested for infection by the Arbovirus Research Unit using a similar plaque assay; a single pool of *Cx. tarsalis* from Coachella Valley was positive for WEE.

Chicken Sero-surveillance: In 1997, a total of 209 sentinel chicken flocks was maintained and bled biweekly by 52 local mosquito and vector control agencies. Fourteen of these flocks were involved in arbovirus research projects conducted by the Arbovirus Research Unit in Riverside and Imperial counties. Over 20,027 chicken sera were tested for WEE and SLE. A total of 226 chickens seroconverted to WEE (Table 2) and 62 seroconverted to SLE (Table 3). The first seroconversions to WEE were detected in sera collected from Merced County during the week of June 15, and to SLE in sera collected from San Bernardino County during the week of July 6. Locations of chicken flocks that contained one or more positive chickens in 1997 are shown in Figure 1. SLE activity was confined to southern California, primarily in the irrigated agricultural and salt marsh habitats of Imperial and Riverside Counties. WEE activity was found in Imperial and Riverside Counties and throughout the Central Valley from Kern to Shasta County.

More WEE activity was detected in 1997 than in 1996; 54 flocks with 231 birds seroconverted in 1997 versus 50 flocks with 195 birds in 1996. SLE activity in 1997 was greater than in 1996, but still less than the activity recorded during 1991 - 1995. WEE and SLE activity detected by the encephalitis virus surveillance program from 1991-1997 is summarized in Figures 2 and 3.

ACKNOWLEDGEMENTS

We are grateful to staff members of the Viral and Rickettsial Disease Laboratory and the Disease Investigation and Surveillance Branch, California Department of Health Services; the Arbovirus Research Unit, University of California at Davis; participating local mosquito and vector control agencies; local health departments; the Department of Food and Agriculture, Animal Health Branch; and physicians and veterinarians who submitted specimens from suspect clinical cases.

Special thanks to the Mosquito and Vector Control Association of California and other participating agencies for financial support of laboratory testing.

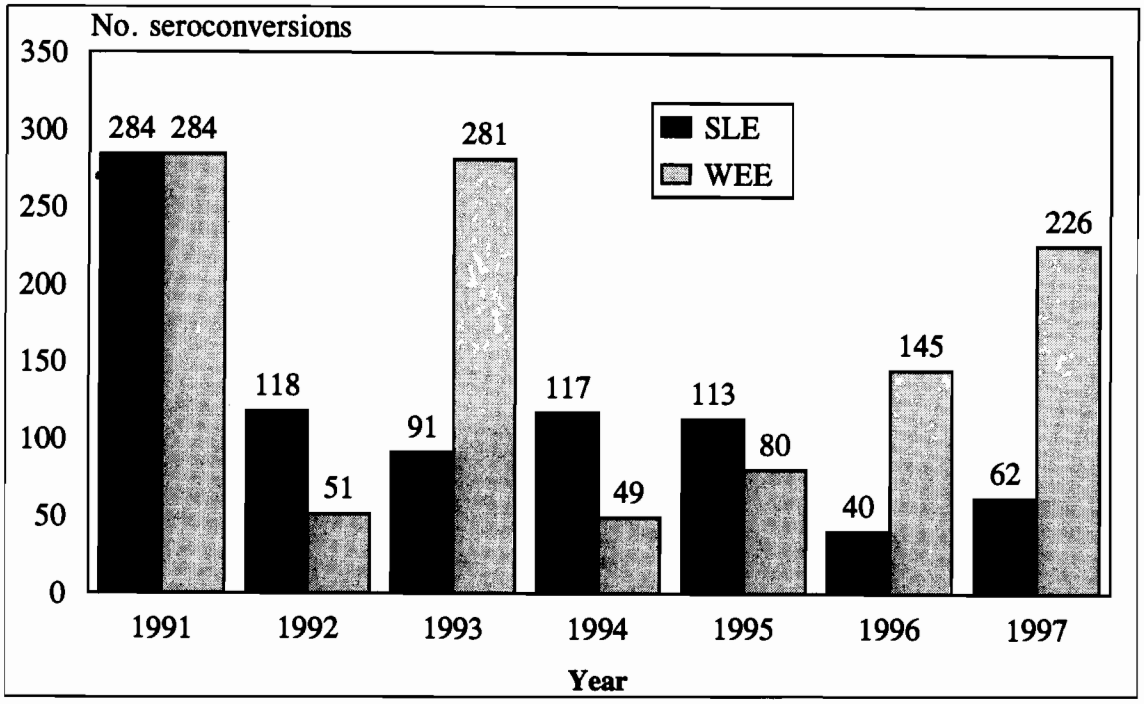

Figure 2. Seroconversions to St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus in sentinel chickens, 1991-1997.

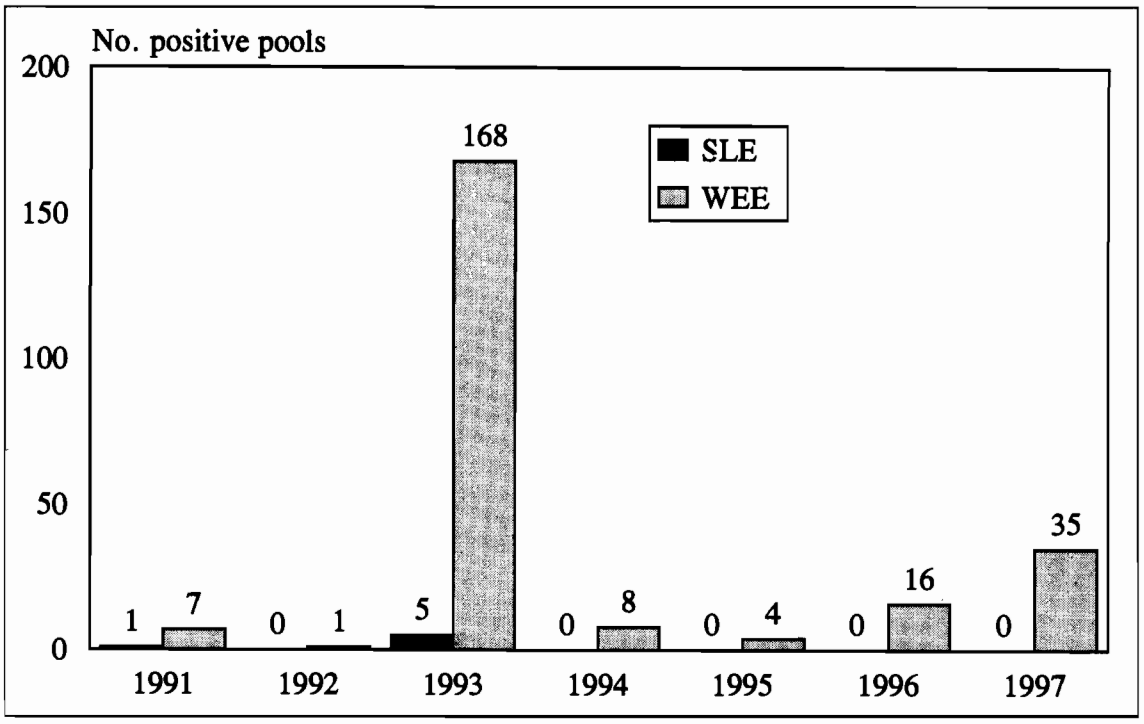

Figure 3. St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus activity in pooled *Culex tarsalis*, 1991-1997.

Table 2. Chicken Seroconversions to WEE by Location and Biweekly Sampling Date, 1997

County	Location	City	6/28	7/12	7/26	8/09	8/23	9/06	9/20	10/04	10/18	11/1	11/15	Total
Butte	Chico	Chico	0	0	0	0	1	0	0	0	0	0	0	1
Butte	Gray Lodge	Gridley	0	0	4	2	3	0	0	0	0	0	0	9
Butte	Honcut Road	Honcut	0	0	3	3	5	0	0	0	0	0	0	11
Butte	M & T Ranch	Chico	0	1	1	1	3	2	1	0	0	0	0	9
Butte	Paiva Ranch	Chico	0	0	3	0	2	1	0	0	0	0	0	6
Butte	Thebach Ranch	Biggs	0	0	2	3	2	0	2	0	0	0	0	9
Churchill, NV	Tarzyn	Fallon	0	0	0	0	0	1	0	0	0	0	0	1
Colusa	Grussenmeyer R.	Colusa	0	0	1	0	1	0	0	0	0	0	0	2
Contra Costa	Holland Tract M.	Knightsen	0	0	0	0	1	0	0	0	0	0	0	1
Contra Costa	Rhone Poulenc	Martinez	0	0	0	1	0	0	0	0	0	0	0	1
Fresno	Firebaugh High	Firebaugh	0	3	0	2	1	0	0	0	0	0	0	6
Glenn	Orland	Orland	0	2	2	4	0	0	0	0	0	0	0	8
Glenn	Willows	Willows	0	2	0	0	3	0	0	0	0	0	0	5
Imperial	Bard	Bard	0	0	6	3	0	0	0	0	0	0	0	9
Imperial	Cady Road	Brawley	0	0	0	0	1	3	0	0	0	0	0	4
Imperial	Campbell	Seeley	0	0	0	0	1	1	0	1	0	0	0	3
Imperial	Nichols	El Centro	0	0	0	6	2	0	1	1	0	0	0	10
Kern	Delano MAD	Delano	0	0	0	0	0	0	0	1	0	0	0	1
Kern	Kern NWR	Lost Hills	0	0	0	0	0	0	1	0	0	0	0	1
Kern	Teviston	Delano	0	0	0	0	0	0	0	1	0	0	0	1
Kern	Tracy Ranch	Buttonwillow	0	0	0	0	0	0	0	1	0	0	0	1
Kings	KMAD Facility	Hanford	0	0	0	2	0	0	0	0	1	0	0	3
Lake	VCD Fish Ponds	Upper Lake	0	0	1	2	2	1	0	1	0	0	0	7
Madera	Custom Rasin	Madera	0	0	0	1	1	0	0	0	0	0	0	2
Madera	Riverview Ranches	Chowchilla	0	0	0	0	1	0	0	0	0	0	0	1
Marin/Sonoma	Barcaglia Rd.	Sebastopol	0	0	0	0	0	1	0	0	0	0	0	1
Marin/Sonoma	Concord Ave.	Santa Rosa	0	0	0	2	0	0	0	0	0	0	0	2
Merced	Koda Farms	Dos Palos	0	0	3	1	1	1	0	0	0	0	0	6
Merced	Newman Gun	Gustine	2	0	0	0	0	1	0	1	0	0	0	4
Riverside	Adohr Farms	Mecca	0	0	0	0	1	1	0	0	0	0	0	2
Riverside	Desert	North Shore	0	0	0	0	1	1	0	0	0	0	0	2
Riverside	Gordon	Mecca	0	0	0	1	2	1	0	0	0	0	0	4
Riverside	Shore	North Shore	0	0	0	0	1	0	0	0	0	0	0	1

Table 2. (Cont.) Chicken Seroconversions to WEE by Location and Biweekly Sampling Date, 1997

County	Location	City	6/28	7/12	7/26	8/09	8/23	9/06	9/20	10/04	10/18	11/1	11/15	Total
Riverside	Thermal	Thermal	0	0	0	0	0	1	0	0	0	0	0	1
Sac/Yolo	Collins	Freeport	0	0	0	0	2	0	0	0	0	0	0	2
Sac/Yolo	G. Whitney	Hood	0	0	0	4	2	0	2	0	0	0	0	8
Sac/Yolo	Herald	Herald	0	0	0	3	1	0	0	0	0	0	0	4
Sac/Yolo	Knights Landing	Knights Landing	0	0	0	0	0	1	0	0	0	0	0	1
Sac/Yolo	W. Sacramento	W. Sacramento	0	0	0	1	0	0	0	0	0	0	0	1
Sac/Yolo	Winters	Winters	0	0	0	0	1	0	1	0	0	0	0	2
San Joaquin	White Slough	Lodi	0	0	0	1	0	1	0	0	0	0	0	2
Shasta	Shasta MVCD	Anderson	0	0	1	1	1	1	0	0	0	0	0	4
Solano	FP Smith Equip	Cordelia	0	0	1	2	2	1	2	0	0	0	0	8
Stanislaus	Modesto Sewer	Patterson	0	1	0	1	0	0	0	0	0	0	0	2
Stanislaus	Victoria's Diary	Crow's Landing	0	2	3	2	1	1	0	0	0	0	0	9
Sutter/Yuba	Barker	Rio Oso	0	0	2	4	1	0	0	0	0	0	0	7
Sutter/Yuba	Dean Ranch	Sutter	0	6	3	0	0	0	0	0	0	0	0	9
Sutter/Yuba	Ledbetter	Marysville	0	0	2	1	0	0	0	0	0	0	0	3
Sutter/Yuba	Meridian	Meridian	0	1	1	2	2	0	0	0	0	0	0	6
Sutter/Yuba	Robbins	Robbins	0	0	3	1	2	0	0	0	0	0	0	6
Sutter/Yuba	Sheppard	Live Oak	0	0	1	0	1	0	0	0	0	0	0	2
Sutter/Yuba	Sr. Citizen Center	Olivehurst	0	0	0	6	1	1	0	0	0	0	0	8
Tehama	Corning	Corning	0	0	0	2	0	0	0	0	0	0	0	2
Tehama	Red Bluff Shop	Red Bluff	0	0	0	5	0	0	0	0	0	0	0	5
		WEE Totals	2	18	43	72	56	22	10	7	1	0	0	226

Table 3. Chicken Seroconversions to SLE by Location and Biweekly Sampling Date, 1997

County	Location	City	6/28	7/12	7/26	8/09	8/23	9/06	9/20	10/04	10/18	11/1	11/15	Total
Imperial	Bard	Bard	0	0	0	1	1	2	2	3	0	0	0	9
Imperial	Cady Road	Brawley	0	0	0	1	1	0	1	2	1	0	0	6
Imperial	Campbell	Seeley	0	0	0	0	0	0	2	0	0	1	0	3
Imperial	Nichols	El Centro	0	0	0	1	0	0	0	2	2	1	0	6
Los Angeles	Bernard Bio Stat.	Claremont	0	0	0	0	0	0	0	0	1	0	0	1
Los Angeles	Gartel Drive	Walnut	0	0	0	0	0	0	0	1	0	0	1	2
Los Angeles	Monterey Park	Monterey Park	0	0	0	0	0	0	0	0	0	0	1	1
Riverside	Adohr Farms	Mecca	0	0	0	0	0	1	0	0	0	0	0	1
Riverside	Desert	North Shore	0	0	1	3	1	0	0	0	0	0	0	5
Riverside	Gordon	Mecca	0	0	0	1	0	0	0	1	0	5	0	7
Riverside	Jessup	Valerie	0	0	0	0	0	1	0	0	0	0	0	1
Riverside	Lake Elsinore	Lake Elsinore	0	0	0	0	0	0	0	1	0	0	0	1
Riverside	Mecca	Mecca	0	0	0	0	0	1	0	1	1	2	0	5
Riverside	Rancho Jurupa Pk.	Rubidoux	0	0	0	0	0	0	1	1	0	0	0	2
Riverside	Salton Sea State Pk.	North Shore	0	0	0	1	0	0	0	0	0	0	0	1
Riverside	Shore	North Shore	0	0	0	0	0	0	0	0	1	1	0	2
Riverside	Thermal	Thermal	0	0	0	0	2	1	1	2	0	1	0	7
San Bernardino	Treatment Plant	Redlands	0	0	0	0	1	0	0	0	0	0	0	1
San Bernardino	Wildwood Park	San Bernardino	0	1	0	0	0	0	0	0	0	0	0	1
		SLE Totals	0	1	1	8	6	6	7	14	6	11	2	62

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MOLECULAR EPIDEMIOLOGY OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS IN CALIFORNIA

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Western equine encephalomyelitis virus (WEE) is transmitted enzootically in western North America during summer between wild birds and the primary mosquito vector, *Cx. tarsalis*. In California, a secondary cycle involving *Ae. melanimon* and jack rabbits occurs in the Central Valley in late summer. Humans and horses become infected tangentially. A major unanswered question regarding the epidemiology of WEE is how the virus is maintained between periods of horizontal transmission. Phylogenetic analyses can shed light on mechanisms of viral recrudescence, because relatedness of viral isolates can be compared from different locations in the same year and the same location in different years. The relatedness of isolates from mammals and birds captured in the winter to strains isolated in the summer, as well as an analysis of isolates from alternate mosquito vectors, can help clarify viral movement or persistence and genomic stability of WEE in California. The goal of this project was to conduct an in depth analysis of WEE isolates from California, and this report presents results from the first year of study.

MATERIALS AND METHODS

Virus strains: Phylogenetic analysis was conducted on 31 strains of WEE isolated from 1938 to 1996 predominantly from *Culex tarsalis* Coquillett from geographically different areas of California, and the prototype Fleming strain isolated in Fresno in 1938 from man. Strains were selected from the Imperial and Coachella Valleys and the Colorado River valley in southern California; from the Central Valley, including the San Joaquin and Sacramento Valleys, as well as Lake and Shasta Counties; and from coastal areas, including Los Angeles, San Luis Obispo and Contra Costa counties. All strains were passed once in Vero cell culture, reverse transcribed using random hexanucleotides, and amplified with primers specific for the E2 region of the WEE genome.

Sequencing and phylogenetic analysis: Direct sequencing of the PCR product was carried out on an ABI Automatic Sequencher using dye terminator sequencing. Sequences were aligned using Sequencher (Gene Codes Corp, Ann Arbor, MI), Edit Seq and Megalign (Lasergene DNASTAR Inc., Madison, WI) programs. Phylogenetic analysis was conducted using bootstrap analysis followed by the DNAdist, Neighbor, Majority-rule and strict consensus tree programs of Phylip. Sindbis and eastern equine encephalitis viruses were used as the outgroups in the phylogenetic analysis.

RESULTS

Initial phylogenetic analyses yielded three lineages (Fig.1): A) isolates from 1938-1953 from the San Joaquin Valley and Washington, including 3 isolates from *Aedes dorsalis* collected in Morro Bay, B) Central Valley isolates from 1993-1996, including a 1991 isolate from Los Angeles county, and C) southern California isolates from 1978 to 1993, including a Central Valley isolate from 1978.

DISCUSSION

Since 1991, WEE from the Central Valley appears to be evolving independently from the southern California strains, suggesting lack of viral movement between the two geographic areas which are separated by the Tehachapi and San Bernadino mountains. However, the close genetic relatedness between the 1978 San Joaquin Valley isolate and the southern California strains indicates that at one time virus did circulate freely between the two areas. The significance of the homogeneity in nucleotide sequence of the 3 Morro Bay isolates and the 1953

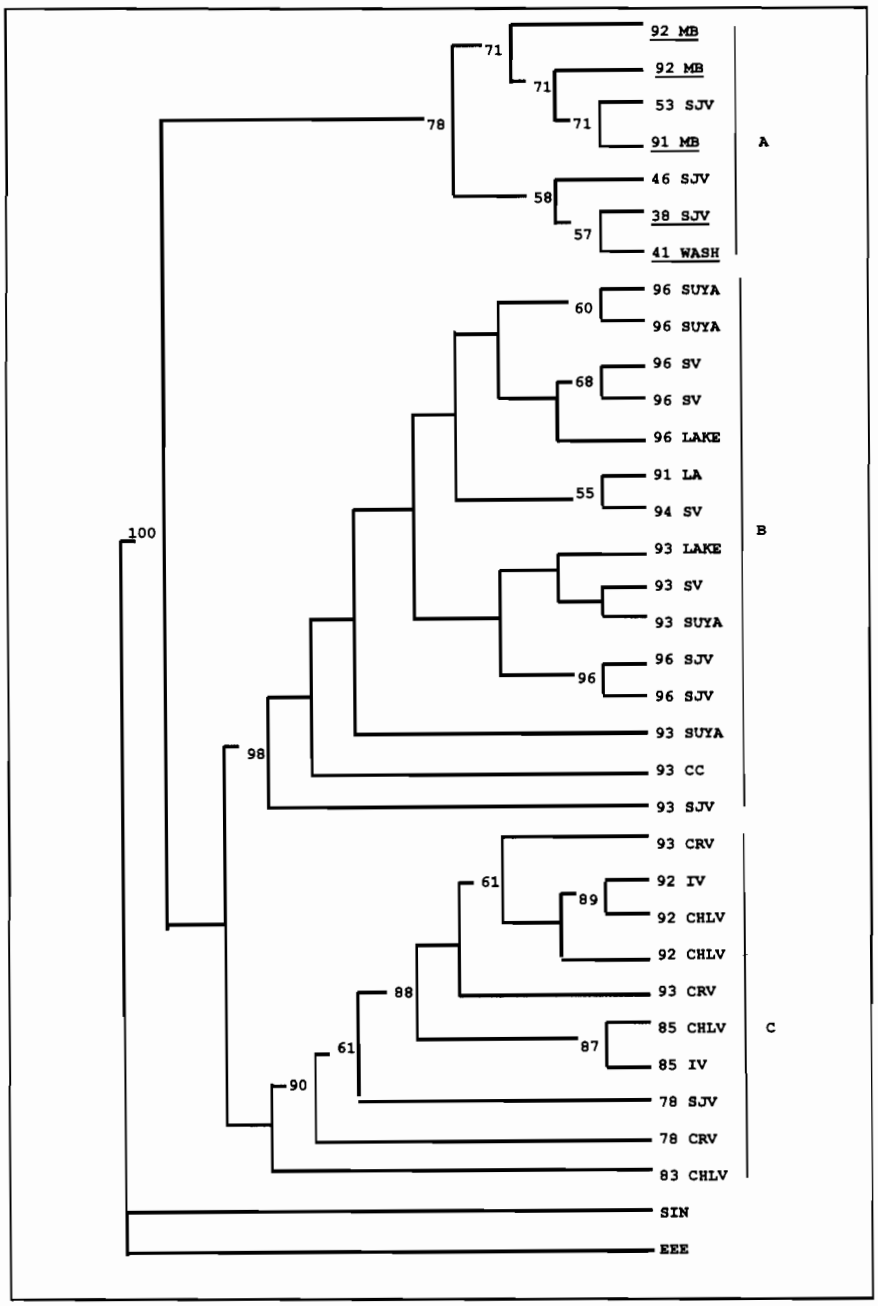

Figure 1. Phylogenetic relationships of 31 strains of western equine encephalomyelitis virus isolated in California (with the exception of 1 isolate from Washington), as determined by the nucleotide sequence (map distance 1085-2415) within the E2 region. The tree was constructed by the consensus tree program following SEQBOOT, DNADIST and neighbor-joining analyses, with Sindbis and eastern equine encephalitis viruses as the outgroups. Numbers at the nodes indicate bootstrap confidence values >50 (100 replicates) for the groups composed of viruses to the right of the node. Groups A, B, C represent 3 major lineages. Virus strains are designated by year of isolation and location. All strains were isolated from *Culex tarsalis* except for those which are underlined. MB, Morro Bay; SJV, San Joaquin Valley; Wash, Washington; SUYA, Sutter-Yuba; SV, Sacramento Valley; LA, Los Angeles County; CC, Contra Costa County; IV, Imperial Valley; CHLV, Coachella Valley; CRV, Colorado River Valley.

San Joaquin Valley isolate remains unclear. One possible explanation for this similarity is that virus maintained vertically is not subjected as often to pressure from circulating antibody in vertebrate hosts allowing the genome to remain stable over time. Laboratory contamination seems unlikely after close examination of laboratory procedures at the time the Morro Bay viruses were isolated (Kramer, et al. 1998. In Press).

Sequence analysis is underway on viral strains which differed in mouse virulence, strains which were isolated from an alternate mosquito vector in the WEE transmission cycle, i.e., *Aedes melanimon* Dyar, and strains which were isolated during the winter from mammals and birds, to study variation stemming from factors other than geographic separation.

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A MODEL SURVEILLANCE PROGRAM FOR VECTOR-BORNE DISEASES IN CALIFORNIA: 1997-1998

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ABSTRACT

The results of the first year's study of a multi-year project to modernize the California Vector-borne Disease Surveillance Program are summarized. The project used California data on arboviruses as a model for the entire system. Results are discussed under 5 primary areas of study: (1) human viral infections, (2) enzootic viral activity, (3) mosquito abundance, (4) analysis, prediction and reporting and (5) weather and water.

On July 1, 1997, a cooperative research program was begun to develop a model surveillance program for vector-borne diseases in California. The purpose of the research was to study new approaches to various components of surveillance eventually leading to their incorporation into the California Vector-borne Disease Surveillance Program. The research was conducted under the direction of the UC Mosquito Research Program and the UC Davis Center for Vector-borne Disease Research in cooperation with the California Department of Health Services, the Mosquito and Vector Control Association of California and 10 individual mosquito and vector control districts.

Background

The California Vector-borne Disease Surveillance Program is one of the most comprehensive and well-supported disease surveillance programs in the world. A key component of this program is surveillance for mosquito-borne arboviruses, especially western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE). A description of this program may be found in Walsh (1987), with updated recommendations by Reisen (1995). Research leading to the arbovirus surveillance aspects of the program was described by Reeves et al. (1990), and the program was discussed in terms of its value in preventing and controlling arbovirus disease in humans by Eldridge (1987). There can be little question of its value in protecting California citizens from arboviral diseases. However, this program, which is a collaborative effort of the California Department of Health Services, the University of California and the Mosquito and Vector Control Association of California, is overdue for a comprehensive review because: (1) marked changes in human population density, agricultural and irrigation methods, and wetlands availability have changed the ecology of virus endemic areas; (2) new methods of mosquito sampling, laboratory testing, data manipulation, and reporting are available; (3) passive medical and veterinary disease case detection has become insensitive due to major changes in medical care delivery systems; and (4) extensive vaccination of equines has reduced their value as sentinels.

Individual Components of the Research

The model surveillance research was designed to include five basic components: (1) human infections, (2) enzootic viral activity, (3) mosquito abundance, (4) analysis, prediction and reporting and (5) weather and water. Research conducted during the latter half of 1997 is summarized below under these subject area headings. The many individuals from the University of California, the California Department of Health Services (DHS), the Mosquito and Vector Control Association of California (MVCAC)

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and individual mosquito and vector control agencies who participated in this research are listed at the conclusion of this report.

Human infections: One of the most perplexing aspects of WEE and SLE activity over the past 25 years has been the disparity between enzootic viral activity as estimated by sentinel chickens and virus isolations from mosquitoes, and the detection and confirmation of human cases of disease (Table 1). There have been many explanations offered for this. Apparently, reduced virulence of modern WEE (Hardy et al. 1997) and SLE (Kramer et al. 1997) viral strains is not an important factor. Gahlinger et al. (1986) suggested that reduced exposure of California citizens to vector mosquitoes has played an important role in the gradual reduction of human disease over the past decades. Alternatively, apparent infection is not being recognized clinically and proper specimens are not being taken for laboratory confirmation. For example, only 50 sera were submitted through week 39 of the 1996 surveillance year, in spite of reports of over 1,300 cases of encephalitis (hospital discharge data from Dr. Carol Glaser) and over 1,300 cases of aseptic meningitis for California for 1994 (CDC 1994). Therefore, to establish morbidity rates for arboviral disease in California, it will be necessary to employ active case surveillance to determine the etiology of human infections, and to make correlations with other components of the surveillance system.

Table 1. Number of confirmed human cases of WEE and SLE, California.

Decade	WEE	SLE
1945-1954	613	329
1955-1964	88	141
1965-1974	46	40
1975-1984	2	40
1985-1994	2	45

In 1997, arrangements were made to increase greatly the number of human samples tested for evidence of arboviral infection, especially from individuals who had experienced central nervous system impairment. This effort has been aided greatly by the addition of Dr. Carol Glaser, a pediatrician, to the staff of the Viral and Rickettsial Disease Laboratory (VRDL), DHS. Dr. Glaser presented hospital discharge data from California for the year 1995 showing that over 1,300 cases had been diagnosed as encephalitis of one kind or another. She also has obtained commitments from Kaiser Health Services to receive samples from that system. Further arrangements have been made for the University of California Davis Center for Vector-borne Disease Research (CVBDR) to test samples submitted to VRDL that have tested negative to WEE and SLE for evidence of infection by other arboviruses.

Enzootic virus activity: Because demographic and land use patterns in California have changed during the past 30 years, a comparative re-examination of virus infection in wild birds, sentinel chickens and mosquitoes as surveillance tools is merited. Estimates of enzootic virus activity in California currently are based on detection of antibodies in sentinel chickens using an indirect ELISA test (Reisen et al. 1996) and on isolation of virus from pools of female *Culex tarsalis* mosquitoes by plaque assay using Vero cell culture. There is considerable historical information indicating that several indicators of viral activity (sentinel chicken seroconversions, virus-positive mosquito pools, and equine cases) occur prior to human infections (Reeves 1990).

There are two surveillance goals for investigation of WEE and SLE enzootic virus transmission. First, detection of antibody in sentinel chickens, wild birds and virus in mosquitoes is used as an early warning system to indicate relative risk for virus transmission to people. When levels of transmission exceed a critical level (threshold) emergency control measures should be initiated (Eldridge 1987, Reeves 1990). However, after more than 50 years of research in California, the nature of the relationship between fluctuations in enzootic virus transmission and infections in humans remains unclear.

The second surveillance goal is to compare the sensitivity of enzootic virus transmission using seroprevalence rates in wild birds, serconversion rates in sentinel chickens, and infection rates in mosquito vectors to document the intensity of local virus transmission. This second aim does not attempt to establish transmission thresholds, but rather it determines which system is most sensitive in determining that virus is being actively transmitted.

There is a need to evaluate the role of mosquito vectors other than *Cx. tarsalis* in WEE and SLE transmission within the context of surveillance. Species such as *Cx. quinquefasciatus, Cx. stigmatosoma* (Magy et al. 1976, Reisen et al. 1992a, 1992b) and *Aedes melanimon* (Hardy 1987) are involved in the ecology of SLE and WEE, respectively. Addi-

tionally, there are mosquito-borne viruses other than WEE and SLE that are present in California and are potential human pathogens. These viruses, such as Jamestown Canyon virus, have vectors other than *Cx. tarsalis*.

During 1997, progress was made in studies that were carried out in both northern and southern California. These studies included the development of a new sensitive detection system for viral antibodies in wild birds and the comparison of methods of detection of enzootic viral activity for surveillance.

A new immunoassay to detect antibodies to arboviruses in the blood of wild birds: In 1997, a new indirect enzyme immunoassay (EIA) was developed that allows the detection of antibody against any arbovirus antigen in the blood of any wild bird species. In conjunction with Bethyl Laboratories, Montgomery, Texas, a broadly reactive, polyvalent antibody was developed by immunizing laboratory rabbits with serum proteins from single species representatives of the orders Anseriformes (Muscovy duck), Columbiformes (ringed turtle doves), Galliformes (chicken), and Passeriformes (white-crowned sparrows). The resulting antibody was conjugated with horse radish peroxidase and then evaluated for broad reactivity in a sandwich EIA. Sera from 13 of 16 bird species in 7 of 10 orders cross-reacted with the antibody.

An indirect EIA then was developed to detect anti-viral antibodies in wild birds. Accuracy, specificity and sensitivity of this EIA were evaluated against the standard plaque reduction neutralization test (PRNT) in house finches, mourning doves and house sparrows infected experimentally with either WEE or SLE. By day 22 post-infection, all birds produced antibody that gave a strong positive reaction in both the new EIA and the PRNT. To compare these assays under field conditions, 898 sera from 70 bird species collected in Sacramento and Riverside counties were tested by both the new EIA and the standard PRNT. Overall, 9 sera from 5 bird species and 5 sera from 2 species were positive for WEE and SLE by PRNT, respectively. When tested by EIA, there were no false negatives for WEE, but 2 quail positives for SLE by PRNT remained negative by EIA – even after retesting. Three birds positive for WEE by EIA were negative by PRNT, indicating the importance of using a confirmatory test. In general, the EIA seemed useful for screening wild bird sera for presumptive positives. Importance of PRNT confirmation of EIA results was indicated clearly during subsequent studies in Coachella Valley and Kern County. Including some data that were collected in 1996, 10,077 sera from 124 bird species were tested, of which 136 and 69 were positive by EIA for antibodies to WEE and SLE, respectively. However, only 47 (35%) and 14 (20%) of these presumptive positives could be confirmed by PRNT. The new EIA has become an important tool in studies comparing the sensitivity of wild bird seroprevalence, sentinel chicken seroconversions and mosquito infection in detecting enzootic virus activity.

Comparative ability of wild bird seroprevalence, sentinel chicken seroconversions and mosquito infections to detect enzootic activity: On-going new research compared three systems to detect enzootic viral activity in replicated marsh habitats along the north shore of the Salton Sea in the Coachella Valley, in riparian, agricultural and marsh habitats in the southern San Joaquin Valley and southern Sacramento Valley. Wild birds were captured in mist nets and ground and modified crow traps baited with millet biweekly in the Coachella, San Joaquin and Sacramento Valleys. Birds were banded, bled and released. Sera were screened for WEE and SLE antibody by EIA, with all positives confirmed by PRNT. One or more flocks of 10 sentinel chickens were positioned within 1.6 km of each bird collection site and bled biweekly. Blood samples were screened by EIA and positives confirmed by indirect immunofluorescent assay (IFA). Host-seeking mosquitoes were collected by 2-5 dry ice-baited CDC traps operated biweekly at fixed sites near each study area. Mosquitoes were identified to species, and pools of 50 *Cx. tarsalis* were shipped to the Arbovirus Research Laboratory in Davis where they were assayed for virus by plaque assay in Vero cells.

WEE was active at all three sites, being detected by positive wild bird sera, seroconversions by sentinel chickens, and isolation of virus from mosquitoes (Table 2). In 1997, virus was detected in Coachella Valley by all three systems. In the San Joaquin Valley, serologically positive wild birds (79% of which were house finches) were collected from March through November, whereas single sentinel seroconversions occurred during September and October. In the Sacramento Valley, all three systems detected virus activity concurrently during July and August 1997.

SLE was active in the Coachella Valley during 1997, but was not isolated from mosquitoes.

Table 2. Enzootic virus activity detected by 3 methods at 3 study areas, 1997.

	N	WEE	SLE
Coachella Valley			
Mosquito pools	978	1	0
Seroconversions	10	0	29
Wild bird sera	4,340	17	9
Kern County			
Mosquito pools	261	0	0
Seroconversions	10	2	0
Wild bird sera	3,431	33	0
Sacramento/Yolo Counties			
Mosquito pools	28	1	0
Seroconversions	3	9	0
Wild bird sera	713	8	1

N = Number of mosquito pools, number of sentinel chicken flocks or number of wild bird serum samples
Mosquito pools = number positive pools
Seroconversions = number of individual chickens seroconverting (positives replaced in Coachella Valley)
Wild bird sera = number of sera positive by both EIA and PRNT (includes new and recaptured birds)

Serologically positive birds were collected before sentinel chickens seroconverted. A single after-hatching-year song sparrow from Stone Lake Refuge in Sacramento Valley was negative for antibody on April 17, but had converted to SLE positive when recaptured June 26. Song sparrows are resident species, and therefore, this conversion indicates possible local enzootic SLE transmission. This finding requires confirmation, because SLE has not been detected by sentinel chickens, or by testing mosquito pools since 1973 in the Sacramento Valley and since 1989 in the San Joaquin Valley.

Careful examination of the species of birds positive for antibodies provided some indication of the time and place of transmission. Winter residents and spring/fall migrants rarely were infected (Table 3), indicating permanent residents may be important in virus persistence. In the Coachella Valley, positive birds were cattail residents or transients (e.g., red-winged black bird), upland brush residents (e.g., Gambel's quail), and peridomestic/orchard residents (e.g., house finch). In the San Joaquin Valley, most positives were house finches collected in a peridomestic setting associated with cotton agriculture. House finches also were important in riparian habitat in the San Joaquin and Sacramento valleys.

Additional research is needed to extend our data comparing the three surveillance systems over both time and space. Delineating key wild bird species and then focusing collections on these species may improve the sensitivity of using seroprevalence data as well as reducing the sample sizes required for monitoring purposes. In addition, planned studies will evaluate the susceptibility, viremia and antibody response of key bird species to sympatric strains of WEE and SLE under experimental conditions. These data are necessary before seroprevalence data can be completely evaluated.

Mosquito abundance: Many previous studies have attempted to correlate adult mosquito activity, as estimated from NJ Light Trap data, with human arbovirus disease (Longshore et al. 1960, Olson et al. 1979, Reeves 1968). Recent evidence of high enzootic arbovirus activity in the Sacramento Valley in 1993 and 1994, and in southeastern California without confirmed human cases indicates that a re-evaluation of this subject is needed. Historically, NJ light traps have been used to monitor changes in *Cx. tarsalis* population sizes; however, the use of security lighting has increased background illumination that competes with the light from the traps for the attraction for mosquitoes. This has reduced trap effectiveness, especially in suburban habitats and has led to many mosquito control agencies supplementing NJ light trap surveillance with CDC miniature traps baited with dry ice (Webb et al. 1989). It is critical to integrate data from NJ lights traps and CO_2 traps and to relate them to other surveillance data. There have been many studies comparing trap rates of New Jersey light traps and CO_2 traps, but only the studies of Milby et al. (1978, 1989) have produced conversion ratios for the two types of traps. These studies raise several important questions, such as the drop-off in efficiency of NJ traps in areas where urban glow interferes with attractiveness and where mosquito abundance increases. There is also the question of the standardization of CO_2 flow rates and trap deployment strategies if CDC traps are adopted. In addition, many entomological factors relating to transmission suggested by Reeves (1967) (e.g., age structure, autogeny, susceptibility to infection, host selection) are related to enzootic virus activity levels and perhaps the risk of human infection, and should be monitored.

The purpose of the research carried out in 1997 was to compare the effectiveness of two of these alternative sampling methods with the standard NJ light trap in 4 biomes in California.

NJ light, dry ice-baited CDC or EVS (encephalitis virus surveillance), and gravid female traps were operated weekly from July through October 1997 at

multiple habitats representative of the Coachella, San Joaquin and Sacramento valleys and the Los Angeles basin. Walk-in red boxes and small red resting cans also were operated at selected sites. Habitat types included urban and suburban premises, urban parks, riparian corridors, managed marshland, irrigated agriculture, and golf courses.

NJ light traps effectively sampled *Anopheles freeborni, Culex tarsalis, Psorophora columbiae* and several *Aedes* when abundance was high in rural areas with minimal competitive illumination. Dry ice-baited EVS or CDC style traps provided an alternative to NJ light traps, collecting significantly more females of most species at most localities regardless of background illumination. The Cummings modification of the CDC gravid female trap baited with a bulrush (*Schoenoplectus californicus*) infusion was the best method for collecting *Cx. pipiens* complex females in most habitats (Walton and Workman 1998). Walk-in red boxes and small red resting cans collected comparatively few mosquitoes. In the Los Angeles Basin, gravid traps baited with bulrush infusion collected, on average, 4.5 times more *Cx. quinquefasciatus* females than traps baited with the standard alfalfa, lactalbumin and yeast infusion (Reiter 1986). NJ light and dry ice-baited traps collected mostly (>90%) empty females (i.e., not blood-fed or gravid) of all species and >60% empty females were collected resting, whereas gravid traps collected >50% gravid females as well as blood-fed and empty females and males. The bulrush infusion in combination with the Cummings trap design seemed to provide resting as well as oviposition site cues, attracting males as well as empty and blood-fed females. In general, abundance measured by NJ light, dry ice-baited and gravid traps was not correlated well over time and space, because of significant interactions among catch size and habitat type. We conclude that mosquito surveillance programs in California should include the systematic operation of dry ice-baited and gravid female traps to improve surveillance sensitivity for selected species in appropriate habitats.

Analysis, prediction and reporting: There has been a virtual revolution in the application of computers to research and other activities. Today, data of various kinds are available almost instantly via telecommunications networks such as the World Wide Web. For arbovirus surveillance, we proposed establishing a computerized network employing graphical interfaces and databases to report, analyze and summarize surveillance data. The system was intended to be consistent with systems under development by the Centers for Disease Control and Prevention, so that California data may be incorporated into a nationwide system.

A website (http://mosqnet.ucdavis.edu) became operational on July 1, 1997, and a surveillance component was incorporated. For this first year, only sentinel chicken seroconversions were included as part of the website. To the user, the information was presented in the form of a map of California with various colored dots representing individual flocks of sentinel chickens (Fig. 1). White dots showed the location of flocks that had had no chickens seroconverting. Blue dots indicated new seroconversions to WEE, red dots to SLE. Previous conversions were depicted as pale blue and pale red, respectively. Ten individual maps were also available that roughly covered the areas served by the 10 cooperating mosquito and vector control districts. Each week the maps were updated upon the issuance of the weekly surveillance report from the State Department of Health Services.

The website was based on a database written in Microsoft Access, with a programmed form interface used to produce data in a form readable by MapInfo version 4.5. Updated maps were moved to HTML files for posting on the website. Initial postings took about 2 hours of effort, but with automation provided by programming of both Access and MapInfo, the time required for posting was reduced to about 20 minutes.

Weather and water: In spite of the availability of weather data, both current and historical, from both state and federal agencies, the present surveillance program does not incorporate these data into information that is furnished to mosquito abatement agencies in weekly surveillance bulletins. Mosquito abundance data are furnished, but temperature and precipitation data, which might be used to predict future trends in mosquito abundance data, are not.

Another factor which is related to the amount of standing water available for mosquito breeding, but which is difficult to correlate with precipitation data, is the amount of water used for irrigation and on wildlife refuges. Not only is the amount of water important, but also the pattern and cost of its use. Management patterns on crops such as rice, cotton and pastures, as well as on wildlife refuges, should be studied in the context of seasonal mosquito abundance.

Table 3. Bird species positive for antibodies against WEE and SLE viruses in California, 1996-1997.

Bird species	Coachella			Kern		Sacramento/Yolo		
	N	WEE	SLE	N	WEE	N	WEE	SLE
Abert's Towhee	45	2						
American Goldfinch						26	1	
American Robin				39	2			
Bewick's Wren						9	1	
Blackheaded Grossbeak	8	1						
Bullock's Oriole				119	2			
California Thrasher				19	2			
Common Ground Dove	178	5	4					
Common Yellow	113	1						
Gambel's Quail	597	2	2					
House Finch	257	5	1	890	26	47	4	
House Sparrow	367		1					
Least Bittern	27	1						
Marsh Wren	101		1					
Oak Titmouse						3	1	
Song Sparrow						34	1	1
Warbling Vireo				4	1			
Totals		17	9		33		8	1

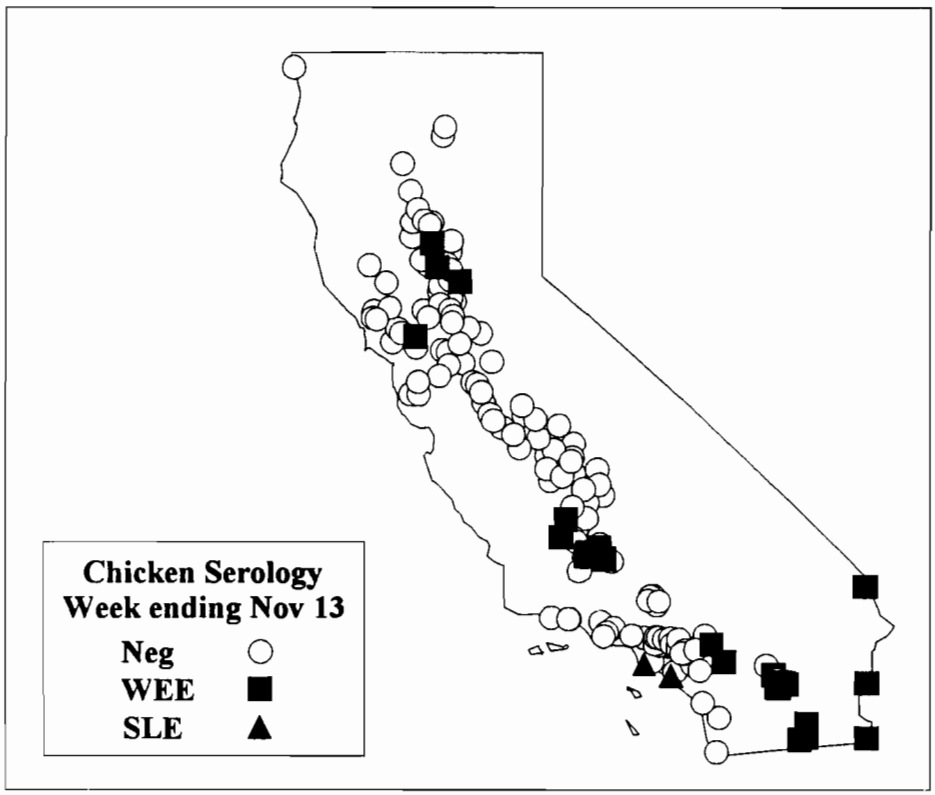

Figure 1. Surveillance map of California during 1997.

This component was considered to have a lower priority than the other components, and no research was conducted in this area during 1997.

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HABITAT AND SEASONAL ABUNDANCE OF TICKS IN URBAN RECREATION AREAS OF ALAMEDA COUNTY, CALIFORNIA, 1992-1996

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ABSTRACT

Surveillance for anthropophagous ticks was conducted at six urban regional parks in Alameda County between 1992 and 1996. Adult ticks were collected biweekly by hand removal from vegetation and flagging. Nymphs and larvae were collected by flagging in areas of oak tree leaf litter. A total of 6,044 adult and immature ticks of three human-biting species were found in the six regional parks: *Ixodes pacificus* =3,785 (62.6%), *Dermacentor occidentalis* =2,219 (36.7%), and *Dermacentor variabilis* = 40 (0.7%). Adult ticks were most prevalent along trails within oak or bay woodland and with mixed grassland and shrub understory areas. Adult ticks were less frequent along trails in redwood, evergreen, or eucalyptus forests. Seasonal variability of immature *I. pacificus* was observed at only one site. Two pools of 10 females collected from Roberts Regional Park, among 1060 adult *I. pacificus* submitted, tested positive for *Borrelia burgdorferi* by indirect fluorescent antibody (IFA). Three hundred and two adult and nymphal ticks were also submitted for an *Ehrlichia* study. One pool of 10 females and two pools of 5 males of adult *I. pacificus* collected from Lake Chabot Regional Park were tested positive by polymerase chain reaction (PCR) for *Ehrlichia equi/phagocytophila*.

The East Bay Regional Park District encompassing over 70,000 acres in urban Alameda and Contra Costa Counties, consists of 53 public parks and 20 regional trails (Lane 1996). These parklands are heavily used by the public not only for recreational purposes but also for educational activities, wildlife studies, and endangered species and wild land preservation. Little is known about the tick population and the diseases carried by the ticks in the regional parks of Alameda County.

In California, the Western black-legged tick, *I. pacificus* is a known vector for Lyme disease (Burgdorfer et al. 1985). Spirochete isolations were documented from Tilden Regional Park (Lane 1996), Briones Reservoir (Lane and Lavoie 1988) and Kennedy Grove Regional Park (Kramer and Beesley 1993) in Contra Costa County. Ehrlichiosis is a tick-borne disease caused by rickettsia that parasitizes leucocytes of humans and animals (Dumler and Bakken 1995). *Ehrlichia chaffeensis* is the pathogen of Human Monocytic Ehrlichiosis (HME) first recognized in Arkansas in 1986 (Maeda et al. 1987; Anderson et al 1991; Dawson et al. 1991). The first confirmed human case of HME in California was from Marin County (Vugia et al. 1996) in 1994. Human Granulocytic Ehrlichiosis (HGE) is a newly described tick-borne disease occurring in the upper Midwestern United States (Bakken et al. 1994; Chen et al. 1994). The first two confirmed California HGE cases appeared from Santa Cruz County in 1995 (Vugia and Kramer 1996).

The objectives of this study were: (1) to determine the temporal distribution of different tick species; (2) to identify the ecological habitat associated with different tick species; (3) to describe the habitat for adult and immature stages of *I. pacificus*; (4) to determine the infection rate of *Borrelia burgdorferi* in the Western black-legged tick, *Ixodes pacificus*

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Cooley and Kohls; and (5) to test the ticks for *Ehrlichia*.

MATERIALS AND METHODS

Twelve highly used public trails in six regional parks were selected for the adult tick population study. A linear transact of a total of 400 meters in each park was established as the study site for sampling adult ticks (Table 2). Adult ticks were collected by hand removal of the questing ticks from the vegetation, as well as, using a one meter square flannel cloth to flag the vegetation along both sides of each transact every two weeks from November to August. An oak-woodland habitat with dense leaf litter area in each park was selected as an immature tick study site. Immature nymphs and larvae were collected by using the flag method over the oak tree leaf litter biweekly from April to August. Collected ticks were identified to species, sexed and the total number recorded.

Many of the trails in the regional parks are extensive and may traverse several broad classifications of vegetative types. With the assistance of a regional park biologist, all the study sites were marked and each vegetation community was identified by using the U.C. modified Holland classification system (Sawyer and Keeler-Wolf 1995) for identifying California vegetation. Data were studied and analyzed to ascertain the differences in tick numbers for each vegetative type.

In collaboration with Dr. R. S. Lane of University of California, Berkeley, pooled adult ticks were tested for the presence of *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt and Brenner by indirect fluorescent antibody (IFA). With the assistance of the U. S. Army Center for Health Promotion and Preventive Medicine of Garrison-Fitzsimons, Aurora, Colorado, pooled and single adult and nymphal ticks of *I. pacificus*, *D. occidentalis* and *D. variabilis* were submitted and tested by polymerase chain reaction (PCR) for both *E. chaffeensis* (HME) and *E. equi/phagocytophila* (HGE).

RESULTS

A total of 6,044 adult and immature ticks were collected during the study period from the winter of 1992 to the summer of 1996 (Table 1). The most abundant tick species found in all six parks was *Ixodes pacificus* (3785/6044, 62.6%), followed by the Pacific coast tick, *Dermacentor occidentalis* Marx (2219/6044, 36.7%), and the American dog tick, *Dermacentor variabilis* (Say) (40/6044, 0.7%).

Table 1. Distribution of tick species collected from six regional parks, Alameda County, California, 1992-1996.

	I. pacificus Total =3785 (62.6%)				***D. occidentalis*** Total =2219 (36.7%)				***D. variabilis*** Total=40(0.7%)		
	female	male	nymph	larva	female	male	nymph	larva	female	male	Total
Anthony Chabot	50	61	1	7	170	215	3	2	4	2	515
De Valle	127	59			26	30					242
Lake Chabot	614	507	18	107	471	454			14	7	2192
Redwood	145	145	305	769	120	132	17	14			1647
Roberts	178	212	28	258	218	227	1	2	5	8	1137
Sunol	98	94	1	1		65	52				311
Total	1212	1078	353	1142	1005	1123	73	18	23	17	6044

Table 2. Plant communities and tick prevalence at trails located in six regional parks, Alameda County, California, 1992-1996.

Anthony Chabot Regional Park		Presence (+) or absence (-) of Ticks		
		I. pacificus	*D. occidentalis*	*D. variabilis*
Bird (100 meters)	oak and bay woodland	+ (medium)	-	-
Brandon (200 meters)	non native grassland and mixed shrub land	+ (low)	+ (high)	+ (low)
Family Campground (100 meters)	eucalyptus forest	-	-	-
Del Valle Regional Park				
Sailor Camp (400 meters)	oak, bay, and digger pine woodland	+ (medium)	+ (medium)	-
Lake Chabot Regional Park				
East Shore (400 meters)	oak, bay, and digger pine woodland	+ (high)	+ (high)	+ (low)
Redwood Regional Park				
Cannon (200 meters)	coyote brush and poison oak shrub land	+ (medium)	+ (medium)	-
Lupine (200 meters)	redwood forest	+ (medium)	+ (medium)	-
Roberts Regional Park				
Diablo Vista (100 meters)	evergreen forest	+ (low)	-	-
Graham (200 meters)	oak and bay woodland (95%); coyote brush and poison oak(5%)	+ (high)	+ (high)	+ (low)
Manzanita (100 meters)	shrub land	+ (high)	+ (high)	+ (low)
Sunol Regional Park				
Lower Maguire Peak (200 meters)	oak woodland interspersed with non-native grassland	+ (medium)	+ (medium)	-
Maguire Peak (200 meters)	oak woodland interspersed with non-native grassland	+ (medium)	+ (medium)	-

Anthony Chabot Regional Park: Adult *I. pacificus* numbers were significantly less abundant than *D. occidentalis* (Fig. 1). The three trails selected for the study sites were extremely variable. Bird Trail has a thick canopy of oak and bay trees (Table 2). The understory plants are shade tolerant shrubs such as California blackberries, ferns and poison oak. *I. pacificus* was the predominant tick collected on this trail. Brandon Trail is nonnative grassland with mixed shrub, very open and no canopy covers the trail. The adult ticks collected here were predominately *D. occidentalis*. Only 1 nymph and 7 larvae of *I. pacificus*, 3 nymphs and 2 larvae of *D. occidentalis* were collected from oak tree leaf litter near Brandon Trail (Table 1). Adult *I. pacificus* were collected from February to May. *D. occidentalis*, numbers peaked from March to May (Fig. 1). Family campground is located in the eucalyptus forest where no ticks were found during the two-year study period of 1992 and 1993.

Del Valle Regional Park: Sailor Camp Trail is an oak, bay and digger pine woodland (Table 2). The study site is dense with tall digger pine trees on a steep slope. The understory is composed of annual

grassland vegetation. From January to March of 1996, the ticks collected at this site were adult *I. pacificus* (Fig. 2). Fifty-six adult of *D. occidentalis* were collected in April and May of 1996. No immature ticks of any species were found in the oak tree leaf litter at this site.

Lake Chabot Regional Park: East Shore Trail is the most popular trail around the lake. It is an oak, bay and digger pine woodland (Table 2). The study site is heavily covered with a widely spaced oak tree canopy. The understory is grassland with scattered shrubs. There were 2,192 ticks of three different species collected from the study site in 1994 and 1996 (Table 1), including 614 females and 507 male *I. pacificus*. In both 1994 and 1996, nymphs and larvae of *I. pacificus* were collected in the month of June only (Fig. 3). Adults of *D. occidentalis* were found from March to June (Fig. 4). Unusually high numbers of *D. occidentalis*, 330 females and 324 males, were collected in June of 1996.

Redwood Regional Park: Two trails were selected for tick sampling. Cannon Trail is a coyote brush and poison oak shrub land shaded with mixed conifer hardwood trees (Table 2). Lupine Trail is in a redwood forest. The area selected for the study is a mixed redwood and oak woodland with understory composed of poison oak, coyote brush, blackberries, and fescue. Adult ticks of *I. pacificus* were scarce from both trails in 1993 (Fig. 5). In 1996, adult ticks of *I. pacificus* were abundant from January to May. The oak tree leaf litter selected for a study site near Lupine Trail, was located on a gentle slope covered with dried oak tree leaves. The population of immature ticks of *I. pacificus* was unprecedented in 1993 from April to July. A total of 272 nymphs and 673 larvae were collected (Fig. 5). We repeated the field study in 1996, and only 33 nymphs and 96 larvae were found in May, June and July. *D. occidentalis* showed the same pattern from both trails in significantly less numbers (Fig. 6).

Roberts Regional Park: Three trails were studied. Diablo Vista is an evergreen forest where very few adult ticks of *I. pacificus* (5 females, 3 males) were found. Graham Trail is an oak and bay woodland, with an understory composed of coyote brush and poison oak. This is a slightly sloped hillside trail. Ticks were collected in clusters. When the trail is shaded with oaks or situated on a west facing slope, the predominate adult tick species was *I. pacificus*. When the trail is in an open woodland or in a more sun lit area, the most abundant adult tick species was *D. occidentalis*. Manzanita Trail is a shrub land consisting mostly of manzanita and chamise. Along most parts of the trail, shrubs are scattered under sparse trees. There are also areas covered with a thick carpet of leaf litter under dense oaks, where most of the immature ticks were collected (Fig. 7 and Fig. 8).

Sunol Regional Park: The two trails chosen are much more secluded than other trails we have studied. Unlike the other sites chosen in this study, they are not heavily visited by the public. Both Lower Maguire Peak Trail and Maguire Peak Trail are oak woodland interspersed with non-native grassland. The adult ticks on the trails were not prevalent except at the end of Maguire Peak Trail adjacent to a seasonal creek (Table 1 and Fig. 9).

To assess the impact of tick removal from the study sites in relation to the measured tick abundance, a two-year population study was conducted in four regional parks, Anthony Chabot, Lake Chabot, Redwood, and Roberts. The data from four parks indicated that the number of adult *I. pacificus* and *D. occidentalis* had no measurable impact on the number of future adult tick population (Fig. 1, Fig. 3, Fig. 4, Fig. 5, Fig. 6, Fig. 7, and Fig. 8). The adults collected in the second year at Anthony Chabot Park, or two years after at Lake Chabot, Redwood, and Roberts were actually higher than the first year.

Of the 1060 pooled adult *Ixodes pacificus* (10 ticks per pool) tested by Dr. R. S. Lane, UC Berkeley for *Borrelia burgdorferi*, two pools of 10 females collected from Roberts Regional Park were positive for the presence of *B. burgdorferi*, using indirect fluorescent antibody (IFA) assay (Barbour et al. 1983). The infection rate of Lyme *Borrelia* range from 0.19% to 1.9% (2/1060 > <20/1060). Three hundred and two adult and nymphal ticks were tested for *Ehrlichia equi/phagocytophila* (HGE) and *Ehrlichia chaffeensis* (HME) at the U. S. Army Center for Health Promotion and Preventive Medicine of Garriason-Fitzsimons. One pool of 10 females and two pools of 5 males of adult *Ixodes pacificus* collected from Lake Chabot Regional Park were positive for *Ehrlichia equi/phagocytophila* by using a nested PCR technique (Barlough et al. 1996).

DISCUSSION

Prior to the selection of study sites, surveillance was conducted in different parks to search for suitable tick habitat. The abundance of the ticks captured

by dragging the one-meter square flannel flag over vegetation varied in each habitat. The general habitats of the six regional parks include chaparral, eucalyptus forest, grassland, oak woodland, redwood forest, valley foothill riparian and urban vegetation. Adult ticks were most abundant in chaparral, oak woodland, and valley foothill riparian; less abundant in redwood forest and grassland; and almost non-existent in eucalyptus forest and urban vegetation.

Each regional park has a very well groomed and landscaped urban vegetated area. The structure of each landscaped urban vegetation in the six regional parks varies, but commonly can be described as five types: tree grove, street strip, shade tree/lawn, lawn, and shrub cover (Mayer and Laudenslayer 1988). Most tree groves are composed of eucalyptus, Monterey cypress and Monterey pine. Lawns are the most uniform vegetation in the park. A variety of grass species are planted, commonly invaded by Bermuda grass, crab grass as well as broadleaf weeds. All lawns are routinely maintained and mowed at a uniform height. No ticks were found in either of these two habitats.

Shrub cover is more limited in the parks than other structural types. Hedges of shrubs are planted around the edges of open lawns. Occasionally, adult ticks were found between the two ecotones. This aggregation pattern was observed also in an earlier study (Lane et al. 1985).

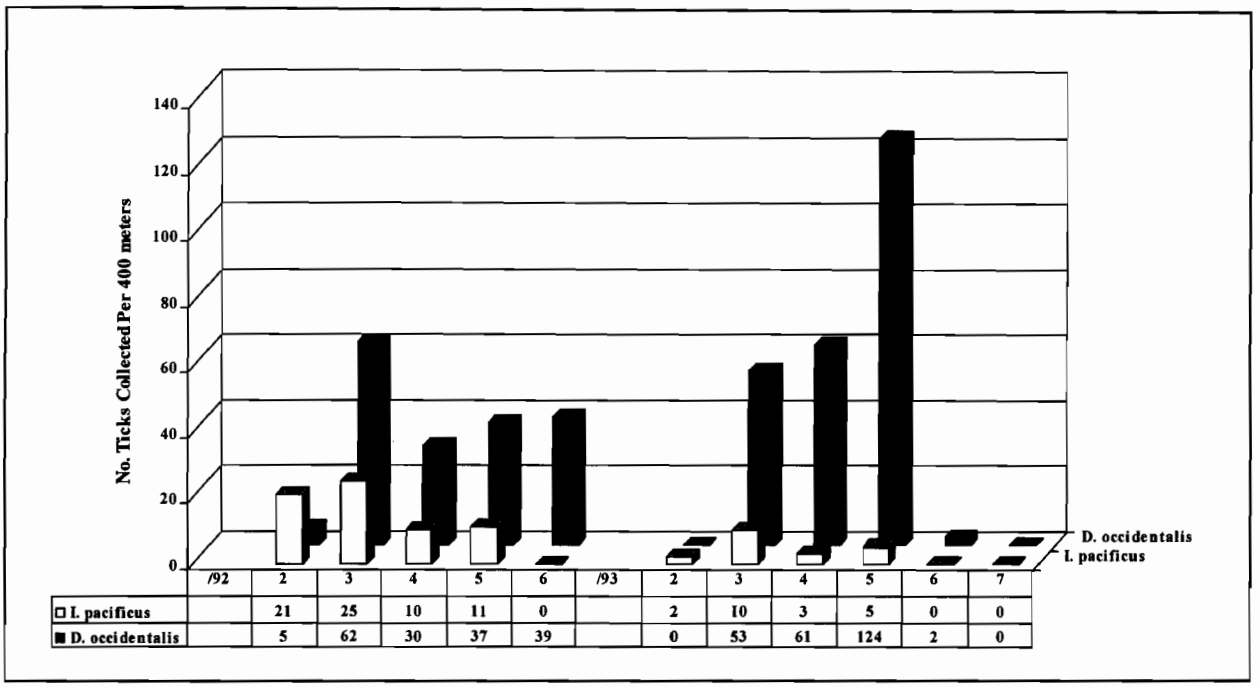
The risk of the public coming in contact with the ticks in the urban vegetation is generally very low. The exception in this area, however, are resting or picnicking under shaded oak trees where the immature ticks are most prevalent or contact with the shrub cover hedges where adult ticks might be encountered.

Seasonal abundance of adult *I. pacificus* ticks varied at each regional park. There were significantly higher numbers in February and March at most of the regional parks in Alameda County in this study, compared to the study at Kennedy Grove Regional Park in Contra Costa County (Kramer and Beesley 1993), where the adult population peaked in January. For *D. occidentalis* adults, the seasonal abundance occurred from March to June. Only a few adult ticks of *D. variabilis* (23 females and 17 males) were collected in May, June and July at Anthony Chabot, Lake Chabot and Roberts Regional Parks (Table 1).

During this five-year study, low numbers of immature ticks were removed from oak tree leaf litter habitats. The only site with enough information to indicate a seasonal distribution pattern was at Redwood Regional Park. Nymphs and larvae of *I. pacificus* were prevalent from April to August. May was the peak month for immature ticks (Fig. 5). In 1993 we collected 945 nymphs and larvae of *I. pacificus*. However, in 1996 the same oak tree litter, with repeated sampling, yielded only 33 nymphs and 96 larvae. Very few immature *D. occidentalis* were collected through the course of the study. This is most likely because little flagging of the leaf litter sites was done during the summer months and early fall. Throughout the five-year study, it was noticed that with two seemingly identical oak trees situated side by side, one might find many nymphs and larvae under one, and none from the other. Small rodent droppings have been observed in leaf litter where higher numbers of immature ticks were collected. Since birds, rodents and lizards are the major animals believed to disburse the nymphal and larval ticks (Furman and Loomis 1984), their preference of resting or congregating under one particular oak tree canopy may explain this clustered distribution pattern.

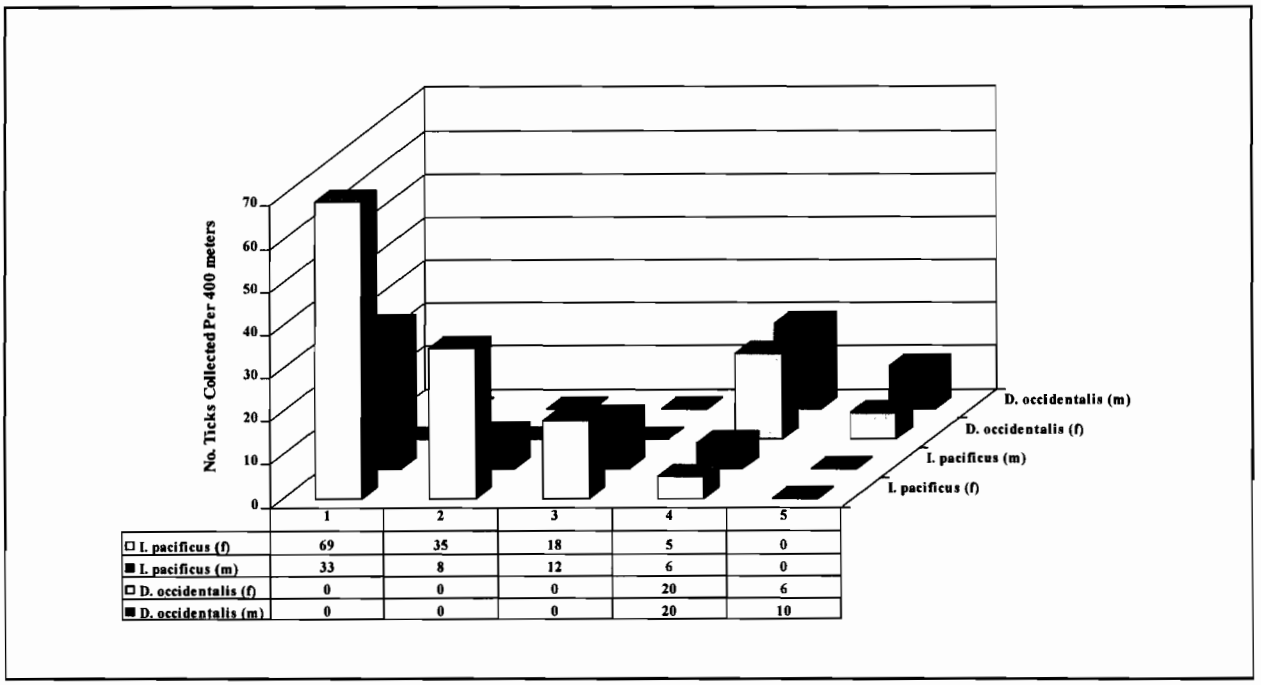
There is an oak tree adjacent to the paved street strip in the Redwood Regional Park where nymphs and larvae of *I. pacificus* were collected from April to July of 1993. During the biweekly sampling, each collection consisted of at least 20 immature ticks from this less than 7-foot diameter oak canopy leaf litter area. During the early months of 1996, major construction of new sewer pipelines were installed right next to the oak tree. When construction was completed, immature ticks could no longer be found or collected from the leaf litter under the tree. Construction activities had greatly disturbed the area and habitat. Host animals carrying the ticks were apparently denied access to and the use of the leaf litter by the construction activities and a newly created ditch near the base of the tree.

At East Shore Trail of Lake Chabot Regional Park and Brandon Trail of Anthony Chabot Regional Park, trail maintenance was done routinely. Cutting weeds and tall grass, and physically widening the road by removing the mud did not reduce tick numbers. Adult ticks were also found in the higher grassy area; instead of collecting ticks by flagging the vegetation at knee high level, ticks were recovered by flagging vegetation at shoulder height. Among the 12 trails studied, adult ticks were found in higher numbers in heavily used trails (East Shore Trail of Lake



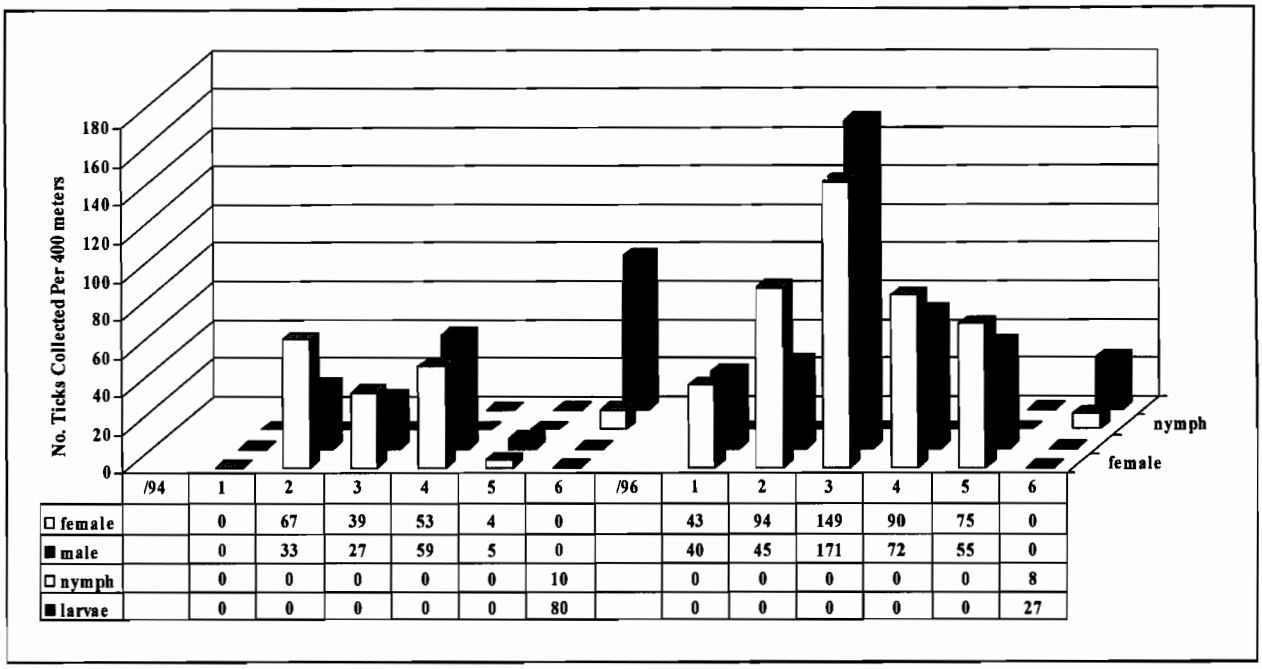
	/92	2	3	4	5	6	/93	2	3	4	5	6	7
I. pacificus		21	25	10	11	0		2	10	3	5	0	0
D. occidentalis		5	62	30	37	39		0	53	61	124	2	0

Figure 1. Seasonal distribution and abundance of *I. pacificus* and *D. occidentalis* in Anthony Chabot Regional Park, 1992-1993.



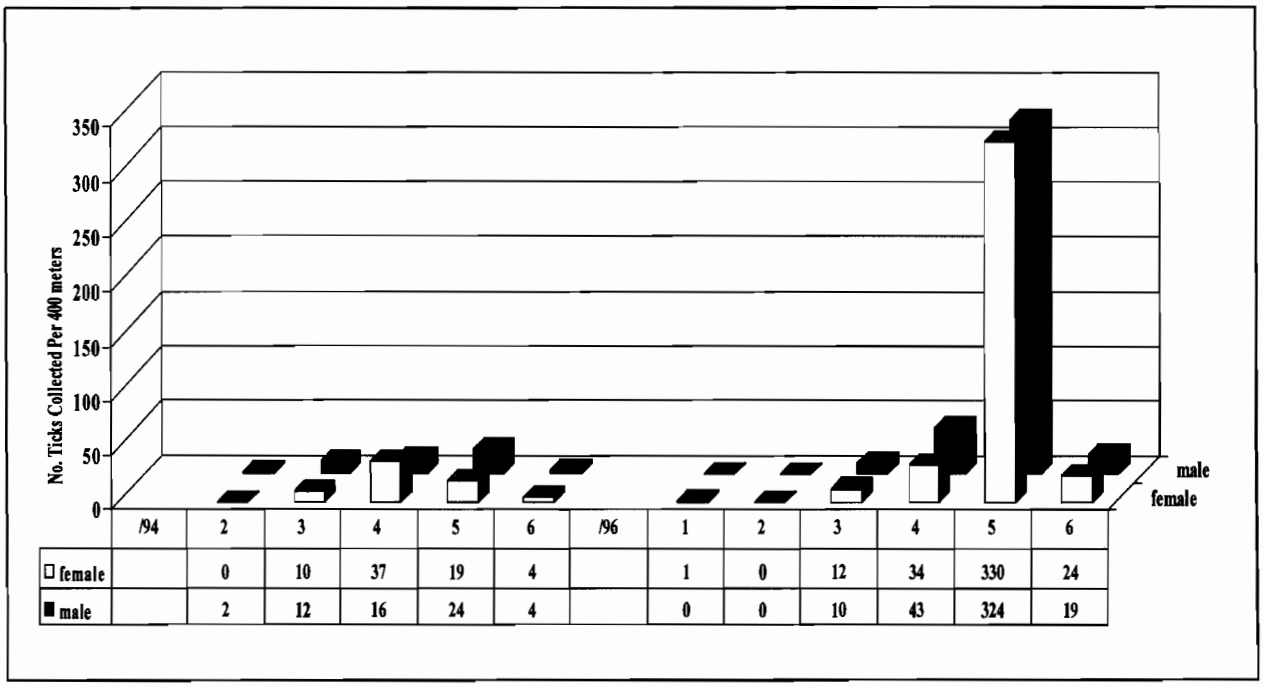
	1	2	3	4	5
I. pacificus (f)	69	35	18	5	0
I. pacificus (m)	33	8	12	6	0
D. occidentalis (f)	0	0	0	20	6
D. occidentalis (m)	0	0	0	20	10

Figure 2. Seasonal distribution and abundance of *I. pacificus* and *D. occidentalis* in De Valle Regional Park, 1996.



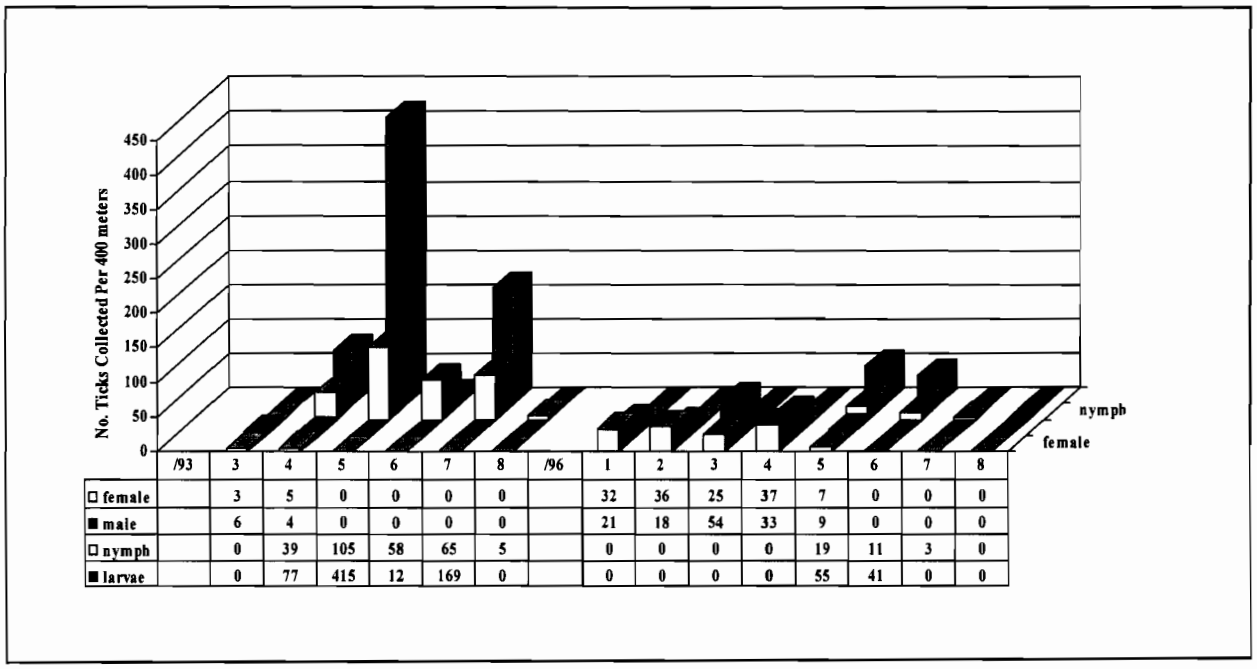
	/94	1	2	3	4	5	6	/96	1	2	3	4	5	6
female		0	67	39	53	4	0		43	94	149	90	75	0
male		0	33	27	59	5	0		40	45	171	72	55	0
nymph		0	0	0	0	0	10		0	0	0	0	0	8
larvae		0	0	0	0	0	80		0	0	0	0	0	27

Figure 3. Seasonal distribution and abundance of *I. pacificus* in Lake Chabot Regional Park, 1994 and 1996.



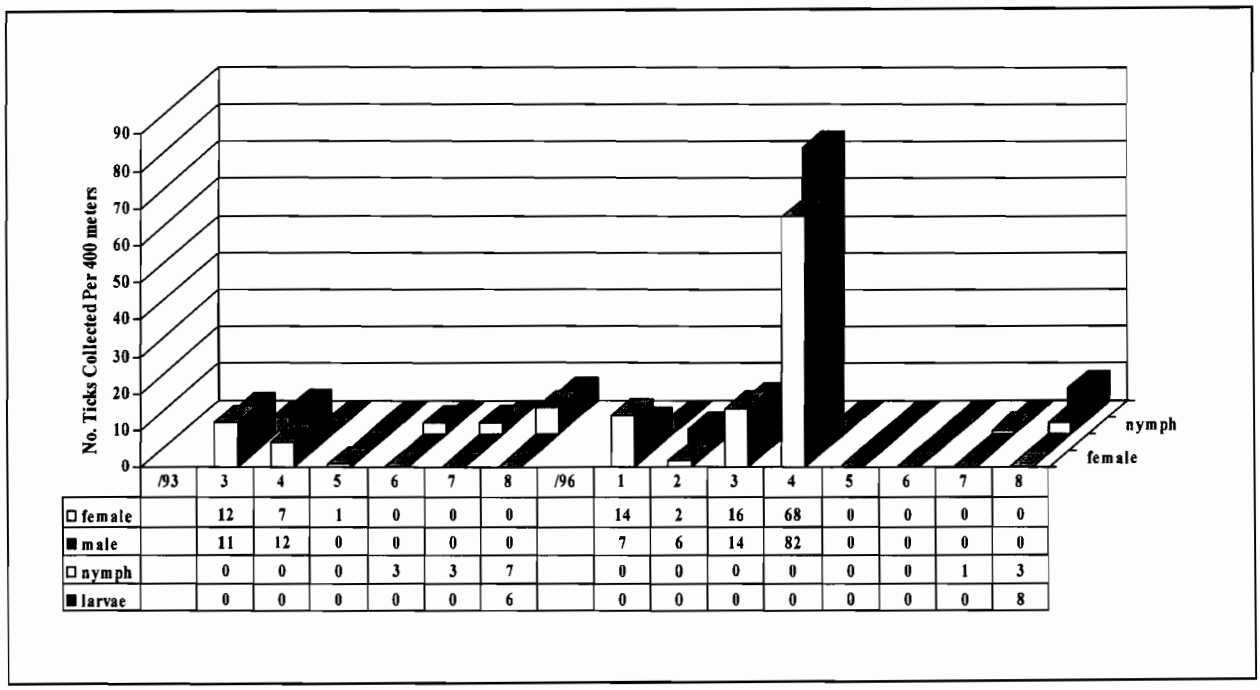
	/94	2	3	4	5	6	/96	1	2	3	4	5	6
female		0	10	37	19	4		1	0	12	34	330	24
male		2	12	16	24	4		0	0	10	43	324	19

Figure 4. Seasonal distribution and abundance of *D. occidentalis* in Lake Chabot Regional Park, 1994 and 1996.



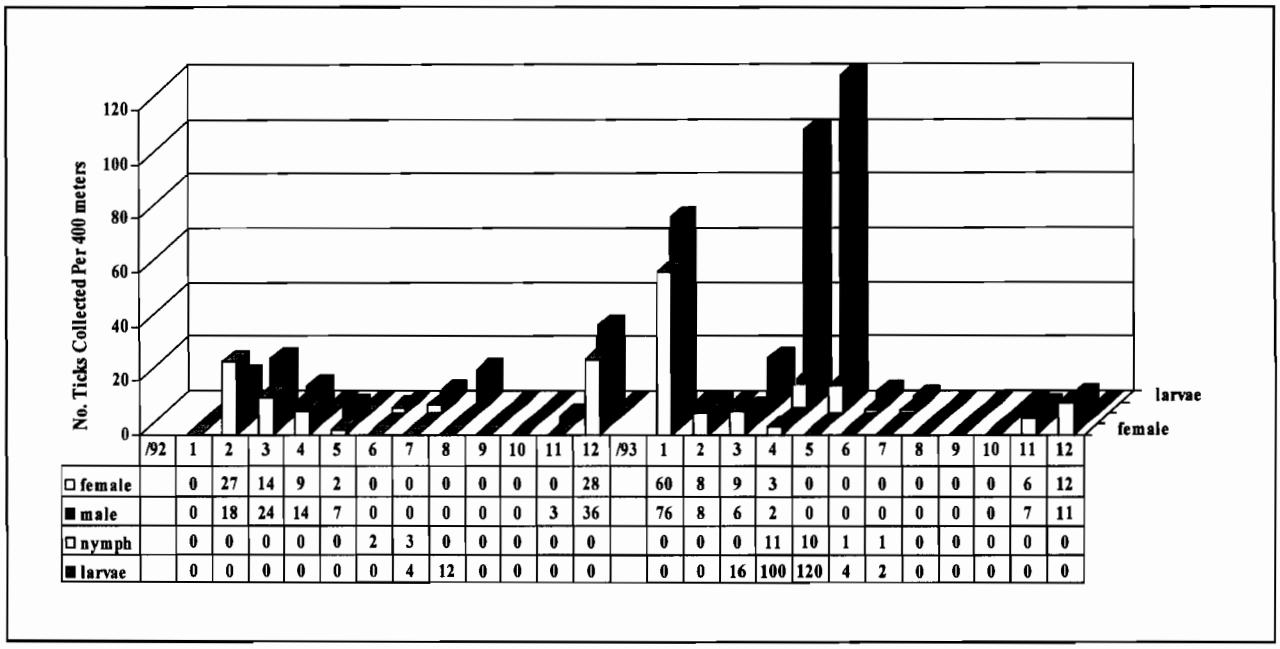
	/93	3	4	5	6	7	8	/96	1	2	3	4	5	6	7	8
□ female		3	5	0	0	0	0		32	36	25	37	7	0	0	0
■ male		6	4	0	0	0	0		21	18	54	33	9	0	0	0
□ nymph		0	39	105	58	65	5		0	0	0	0	19	11	3	0
■ larvae		0	77	415	12	169	0		0	0	0	0	55	41	0	0

Figure 5. Seasonal distribution and abundance of *I. pacificus* in Redwood Regional Park, 1993 and 1996.



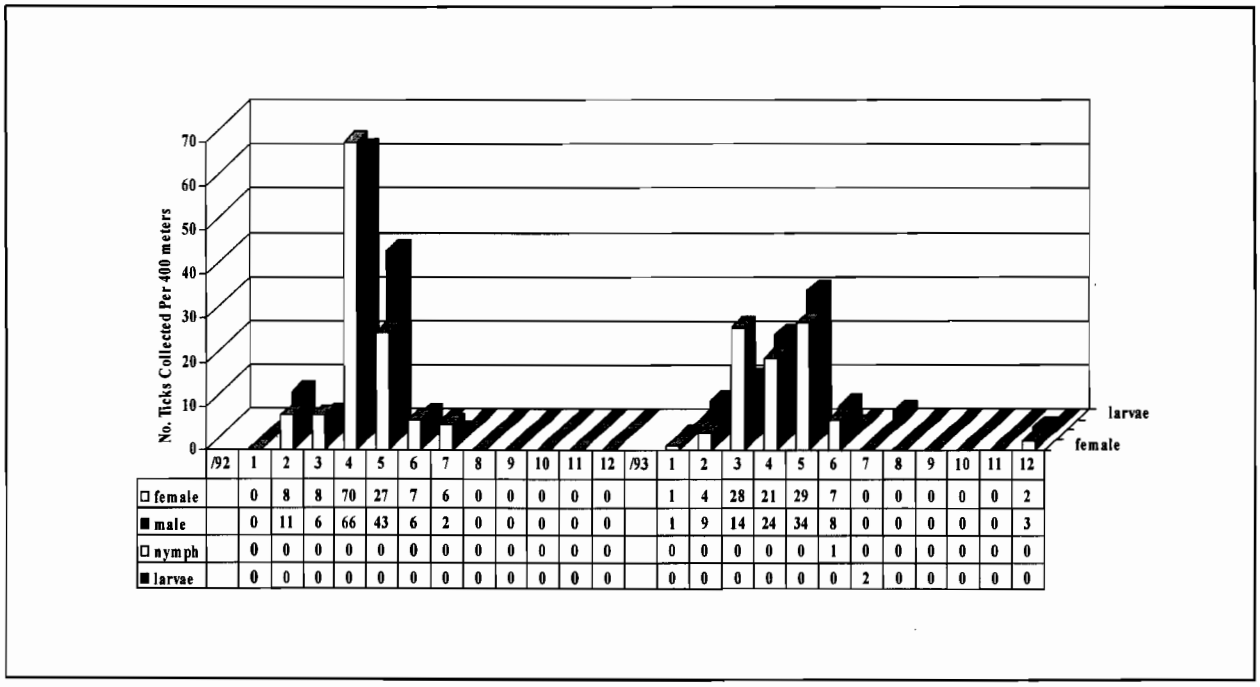
	/93	3	4	5	6	7	8	/96	1	2	3	4	5	6	7	8
□ female		12	7	1	0	0	0		14	2	16	68	0	0	0	0
■ male		11	12	0	0	0	0		7	6	14	82	0	0	0	0
□ nymph		0	0	0	3	3	7		0	0	0	0	0	0	1	3
■ larvae		0	0	0	0	0	6		0	0	0	0	0	0	0	8

Figure 6. Seasonal distribution and abundance of *D. occidentalis* and Redwood Regional park, 1993 and 1996.



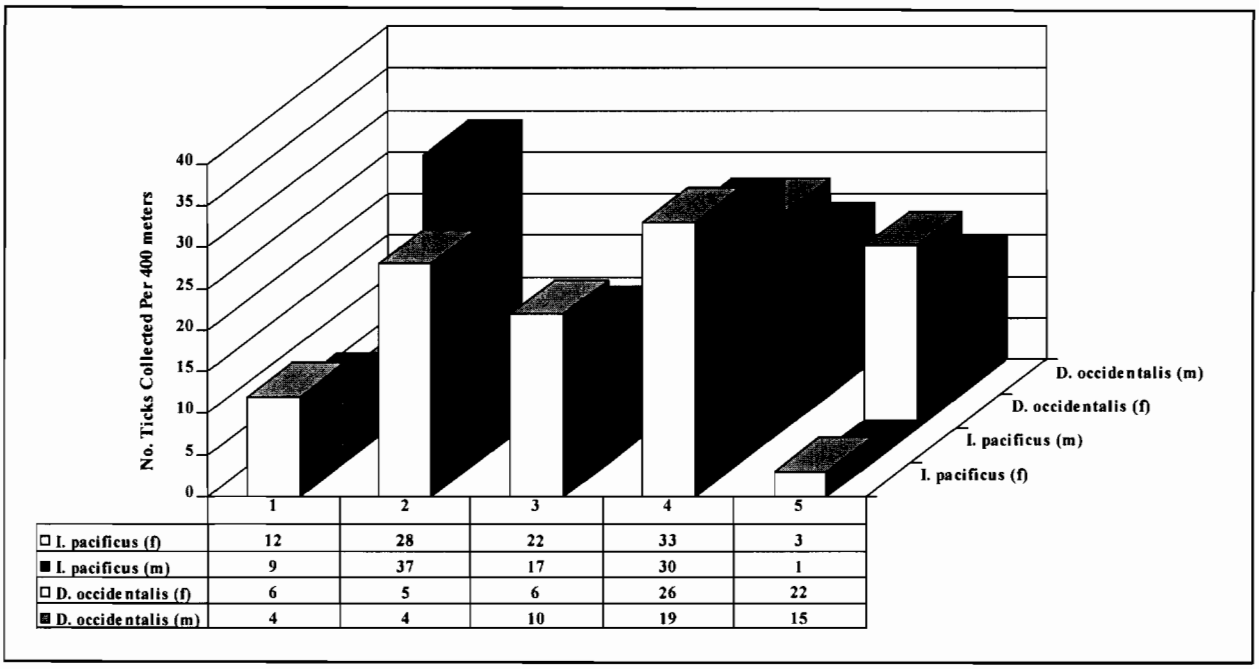
/92	1	2	3	4	5	6	7	8	9	10	11	12	/93	1	2	3	4	5	6	7	8	9	10	11	12
□ female	0	27	14	9	2	0	0	0	0	0	0	28		60	8	9	3	0	0	0	0	0	0	6	12
■ male	0	18	24	14	7	0	0	0	0	0	3	36		76	8	6	2	0	0	0	0	0	0	7	11
□ nymph	0	0	0	0	0	2	3	0	0	0	0	0		0	0	0	11	10	1	1	0	0	0	0	0
■ larvae	0	0	0	0	0	0	4	12	0	0	0	0		0	0	16	100	120	4	2	0	0	0	0	0

Figure 7. Seasonal distribution and abundance of *I. pacificus* in Roberts Regional Park, 1992 and 1993.



/92	1	2	3	4	5	6	7	8	9	10	11	12	/93	1	2	3	4	5	6	7	8	9	10	11	12
□ female	0	8	8	70	27	7	6	0	0	0	0	0		1	4	28	21	29	7	0	0	0	0	0	2
■ male	0	11	6	66	43	6	2	0	0	0	0	0		1	9	14	24	34	8	0	0	0	0	0	3
□ nymph	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	1	0	0	0	0	0	0
■ larvae	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	2	0	0	0	0	0

Figure 8. Seasonal distribution and abundance of *D. occidentalis* in Roberts Regional Park, 1992 and 1993.



	1	2	3	4	5
I. pacificus (f)	12	28	22	33	3
I. pacificus (m)	9	37	17	30	1
D. occidentalis (f)	6	5	6	26	22
D. occidentalis (m)	4	4	10	19	15

Figure 9. Seasonal distribution and abundance of *I. pacificus* and *D. occidentalis* in Sunol Regional Park, 1994.

This observation is very different from the study at Tilden Regional Park (Lane 1996).

The Lyme disease infection rate of *B. burgdorferi* in *I. pacificus* ticks collected in this study ranged from 0.19% to 1.9%. This rate is comparable with the infection rate of 2% found in northern California (Burgdorfer et al. 1985). *I. pacificus* has been identified as a vector of *Ehrlichia equi/phagocytophila* in California (Richter et al. 1996; Barlough et al. 1997). The adult *I. pacificus* ticks collected from Lake Chabot Regional Park were tested PCR positive for *Ehrlichia equi/phagocytophila*. This preliminary test result sheds new light on the presence of emerging tick-borne diseases in the urban parks. Further study of HGE and HME from the area will be conducted in the near future.

Most visitors to the urban parks may be unaware of the existence of ticks and tick-borne diseases. When the public see the paved trails and groomed landscaped areas in most regional parks, Lyme disease or other tick-borne diseases may not be considered as a potential health risk. This study suggested that many trails with the combination of oak, bay woodland and mixed with grassland and shrubs as understory are a potential adult tick habitat. Adult ticks are most active in cooler months, especially from January to April at regional parks. Oak trees with a thick, accumulation of leaf litter under them are a potential habitat for immature ticks. Since the infection rate of Lyme disease in nymphs is much higher than adults (Clover and Lane 1995; Keith and Hui, in prep.), people appear to be mostly at risk in spring and early summer, when nymphal ticks are most abundant at regional parks.

ACKNOWLEDGEMENTS

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PUBLIC PERCEPTION PROBLEMS: CONSEQUENCES AND SOLUTIONS

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What is a Problem District?

A problem district or agency is one that the public or legislature perceives as broken and they want to fix it. The problems of one special district will have consequences for all special districts. If the public perception of the district is one of distrust, whether deserved or not, then all special districts will be looked upon as untrustworthy. Most of the time this is actually public misperception and totally undeserved. The public simply does not know enough about us so they assume the worst. The general public tends to think of special districts as unaccountable, because they do not understand how local governments operate; secretive, because they don't pay any attention to what we are doing until we do something they don't like; wasteful, because they think that all government agencies spend $500 for a toilet seat or $150 for a wrench; top heavy with bureaucrats, because they think that all government agencies are run like the federal government and unnecessary, after all, "we don't have any mosquitoes, why do we need you?". In some areas they also think of special district trustees as arrogant because they feel that their protests will go unheeded even though they may have never even attended one of your meetings. Unfortunately, they believe what they read in the media and most of the time that is negative. The California Governance Consensus Project has been doing public opinion polling and found a lot of ugly reactions to local governments.

Bad Examples

Why is this misperception happening? Because, in addition to the obvious generalizations made about government of any type, some of us are setting some mighty bad examples out there. In the past few years the public has been reading about us in the paper. They read about the water district over on the coast where the trustees were traveling to expensive resorts for retreats and spending the district's money in blatantly self-serving ways. They read about the mosquito abatement district manager who was indicted for embezzling district funds and misuse of district property. They read about the mosquito abatement district that allowed their reserves to build up to extremely high levels without any long range planning or purpose. These are some of the more obvious cases; there are smaller local scandals that also reflect upon us.

Consequences

What are the consequences of being lumped together as "bad" local government agencies and being totally misunderstood? Well, for one thing we get very little sympathy or help when our funding is threatened; as it has been by the State since 1993. The public doesn't think of us when they hear about local government cuts; they think of cities and counties. By the time they realize that they may lose our services, it's too late. Besides they don't even know what services we provide.

And what if your district is the "bad example"? Well, the public gets upset, or much more likely and far worse, a local politician out to make a name for himself gets upset and decides to "fix it". The media gets involved. The local and state politicians get involved. They call for forced consolidations of the districts to make them more "efficient". Studies have shown that most of the time a larger consolidated agency is not more efficient and is many times less efficient. Or they decide they don't need you anymore and they try to get the district dissolved. Or they notice that you have reserve funds and your district becomes the cash cow to fix some of the city or county's fiscal woes. Or they get really serious and try to reorganize all local government into regional governments. Once again, they replace several small efficient agencies with one large top-heavy bureaucracy that costs more than all of the

smaller ones combined and is big and unresponsive to public concerns. In other words they create the very thing they wanted to eliminate. To sum it up, if your district gets into trouble, the consequences can be as severe as the elimination of your district, and all of the other districts near you will have to deal with the bad press.

Are you next?

We all need to ask ourselves if our houses are in order. Do you know how the public feels about your district? Do you ever get bad press? Do your reserves look like a cash cow? Do you operate in an open and accessible manner? You need to know the answers to these questions.

One of the districts in the Bay area went to considerable trouble and expense to conduct a public opinion poll. The results were alarming. There was a large percentage of the population in their district that did not know they existed, much less whom to call if they had mosquito problems. So, they started a newsletter, expanded their public education and outreach efforts. They call the media more often and get some very positive press as a result. These are the types of efforts that are essential for all of us. Even though most of us know this, how many of our districts actually follow through with continuous public education programs?

Take a look at your district operation. Do you get bad press or unhappy citizens at your board meetings? If you do, then you need to be aware that you could be the next "bad example" we will be reading about in the paper. Identify any potential problems that your district may face and make sure that the district board allows the manager to manage the district. The board is responsible for fiscal oversight and setting policy that the manager carries out. If the manager is not allowed to manage the district, then trouble usually follows. This is because a board cannot be effective in the day to day management of the district operations and, if the manager is not allowed to do it, then no one is doing it.

We have a problem. Now what?

The first thing you have to do is identify your problems. Then you must deal with them in a positive and open way before anyone can make an issue of them. Start by investigating the possible solutions, enlist citizen participation and cooperation and enlist political participation and cooperation. Let me give you some examples.

A politician decided that there were too many water districts in his area. He wrote legislation to consolidate them into one agency. He expected the districts to come to him and discuss the issue. Instead the districts got adversarial right away and vowed to fight him. This only made the politician more determined to fix the problem. Instead of going toe to toe with the politician, it would have been a better approach to go and sit down with him and discuss the matter. The districts should have asked why he was concerned and then addressed those concerns. If the politician said that he thought they were inefficient because of the number of districts, then the districts could let him know that they don't think this is the case but they sure want the most efficient delivery of service for our constituents so they will pool their resources and commission a study to prove that they are more efficient as they are. The politician wins because he is making sure that the public is well served and he can tell them all he is doing for them. The districts avoid a messy political confrontation played out in the media where they would no doubt come off looking obstinate and bureaucratic. And most importantly, the politician drops his plans to force consolidations legislatively because the districts are working WITH him and a compromise can be reached over time.

A mosquito abatement district had too much money in their reserves and they did not have it earmarked for any specific purpose. They did not have long term goals for which the money was intended. They began getting bad press because someone noticed this and decided to make an issue of it. What could they have done to head off the consequences of being taken over for their money? Well, step one would have been to acknowledge the problem. Then they could have put together a citizen's group to help them explore the possible solutions including suspension of their tax revenues until the reserves were down to a reasonable level. And finally, they should have implemented a plan to lower their reserves. By enlisting the help of the citizens they would have a lot more support if the issue is pushed and they do not appear arrogant or unapproachable. Let the public know that you are doing the best you can and sometimes you might just

need a little help if things get out of control. If you make the public a party to solving the problem, then they will be on your side. The same thing goes with politicians. They aren't really very fond of listening to complaints but if you ask for their help and they can give it to you and get some good press out of it, then they will be on your side as well.

Understand that the power of constructive cooperation is awesome. Valleywide Park and Recreation District lost 70% of their funding in the ERAF tax shifts. This cut was big enough to put them out of business and they couldn't do an assessment or hold a special election because the laws either didn't allow it or it was too expensive. So, they said what can we do? They decided to hold public forums to discuss solutions. At first the public was angry until they realized that it wasn't the district's fault. Then the public offered to help solve the problem. They got organized and held community picnics and other fund raising activities. They did all sorts of things to help the district make up for the funding losses. The community embraced the problem as their own and set about the business of solving it.

We don't have a problem now. How can we prevent future problems?

If you look at your operations and you don't see eminent problems that does not mean that you can be complacent. Police yourselves and watch for the warning signs. Be aware of public perception. Never let bad press go unanswered but make sure that your response is positive and constructive. Don't be defensive, be constructive. Work on your public education or outreach programs. Make sure that the public knows who you are, what you do and WHY THEY NEED YOU. Work on your political contacts. If you have established a relationship with your local or state politician then he will be much more likely to work with you than to come after you.

If you aren't sure how to develop your political relationships, then reread the talks I've given in the past on political networking. Good luck! We're all going to need it.

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EVALUATION OF MOSQUITO AND ARBOVIRUS ACTIVITY IN ORANGE COUNTY DURING 1997

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ABSTRACT

The Orange County Vector Control District continued its surveillance of mosquito and arbovirus activity throughout 1997 by collecting blood samples from wild birds and sentinel chickens, as well as collecting adult mosquitoes from CDC/CO2, gravid and stable traps. There were no positive mosquito pools, sentinel chicken seroconversions, or human cases in Orange County during 1997. However, SLE-positive wild birds were found during the months of June, July, August, October and November, with a peak seroprevalence of 2.0% occurring in both July and October. Overall, 0.54% of the 1,297 sampled house finches and 0.57% of the 1,574 sampled house sparrows tested positive for SLE antibodies during the year. *Culex quinquefasciatus* was the most commonly trapped mosquito throughout Orange County, except for a freshwater wetland area of Irvine, where *Cx. tarsalis* was predominant.

The Orange County Vector Control District (OCVCD) encompasses about 780 square miles (all of Orange County) and has almost 3 million residents living within its borders. Most of the District is composed of suburban development with many typical, residential mosquto-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. All three important encephalitis vectors are collected in the county: *Culex quinquefaciatus* Say, *Culex tarsalis* Coquillett and *Culex stigmatosoma* Dyar. In 1997 the District continued its mosquito and encephalitis virus surveillance program throughout the year by collecting blood samples from wild birds and sentinel chickens, as well as collecting adult mosquitoes from a variety of trapping sites.

Mosquito Surveillance

Mosquitoes were collected every week at ten permanent sites in the county, using eighteen CDC/CO2 traps (Sudia and Chamberlain 1962), five gravid female ovipositional traps (Cummings 1992) and a single Australian crow trap (McClure 1984) modified into a stable trap to capture wild birds and the engorged mosquitoes which fed on them. Overall, mosquito numbers for all species were much lower in 1997 than the two previous years at both urban and rural habitats (Figures 1 and 2).

Culex quinquefasciatus was collected most frequently in the District, but varied in abundance according to the season and habitat. Populations of this species were sampled best with gravid traps in suburban areas of the county. Counts peaked in the summer and persisted in low numbers through the winter at all trap locations. Collections were highest in August at two sites, Central Park in Huntington Beach and OCVCD headquarters in Garden Grove, rising to 125 and 85 gravid females, respectively (Figures 3 and 4).

Culex tarsalis was collected in substantial numbers at only two, relatively small, undeveloped areas of the District. It has always been locally abundant in certain places of the county, such as the San Joaquin freshwater marsh in Irvine. Host-seeking *Cx. tarsalis* numbers at this site were highest in June (14 - 27 per trap night) and decreased gradually through the summer months, disappearing by the end of October for the remainder of the year

Table 1. Number of mosquitoes and mosquito pools submitted for SLE and WEE virus testing by species and trap type from Orange County CA., during 1997.

Species	Number of mosquitoes	Oviposition Traps (pools)	Modified Crow Trap (pools)	CDC Traps (pools)	Total Number of pools
Culex quinquefasciatus	2,482	84	18	2	104
Culex tarsalis	354	0	18	0	18
Totals	**2,836**	**84**	**36**	**2**	**122**

Table 2. Small bird seroconversions for SLE and WEE antibodies in Orange County, CA., during 1997.

Species	SLE	WEE	No. Blood Samples	% SLE	% WEE
House Finch	7	0	1,297	0.54	0.00
House Sparrow	9	0	1,574	0.57	0.00
Totals	**16**	**0**	**2,871**	**0.56**	**0.00**

(Figure 5B). In contrast, during 1996, *Cx. tarsalis* collections in the marsh were highest in July and August, averaging 140 - 250 per trap night. The most common mosquito in this habitat, *Culex erythrothorax*, peaked between 130 - 360 per trap night in June 1997, (Figure 5A) and were much less numerous than 1996 (1000 - 1300 per trap night during the highest months of March and April).

During the course of the year, 2,836 post-blood fed (gravid or engorged) encephalitis vector mosquitoes were selected for arbovirus testing from routine collections (nulliparous adults were not included). From this number, 122 pools were sent to the California State Department of Health Services' Viral and Rickettsial Diseases Laboratory at Berkeley (Table 1). The submissions included 104 pools of *Cx. quinquefasciatus* and 18 pools of *Cx. tarsalis*. None of these pools tested positive for Saint Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus.

Sentinel Chickens

The District maintained one sentinel chicken flock of 15 chickens during 1997. The flock was located at the San Joaquin Wildlife Sanctuary near a *Cx. tarsalis* - producing freshwater marsh. The chickens were tested biweekly for SLE and WEE antibodies by the State laboratory from April - October and the District laboratory for the entire year. None of the 15 chickens tested positive for either SLE or WEE antibodies during 1997.

Encephalitis Antibody Seroprevalence in Wild Birds

The District's wild bird encephalitis antibody seroprevalence program focused primarily on two abundant peridomestic passerines, House Sparrows (*Passer domesticus*) and House Finches (*Carpodacus mexicanus*). Birds were trapped in nine modified

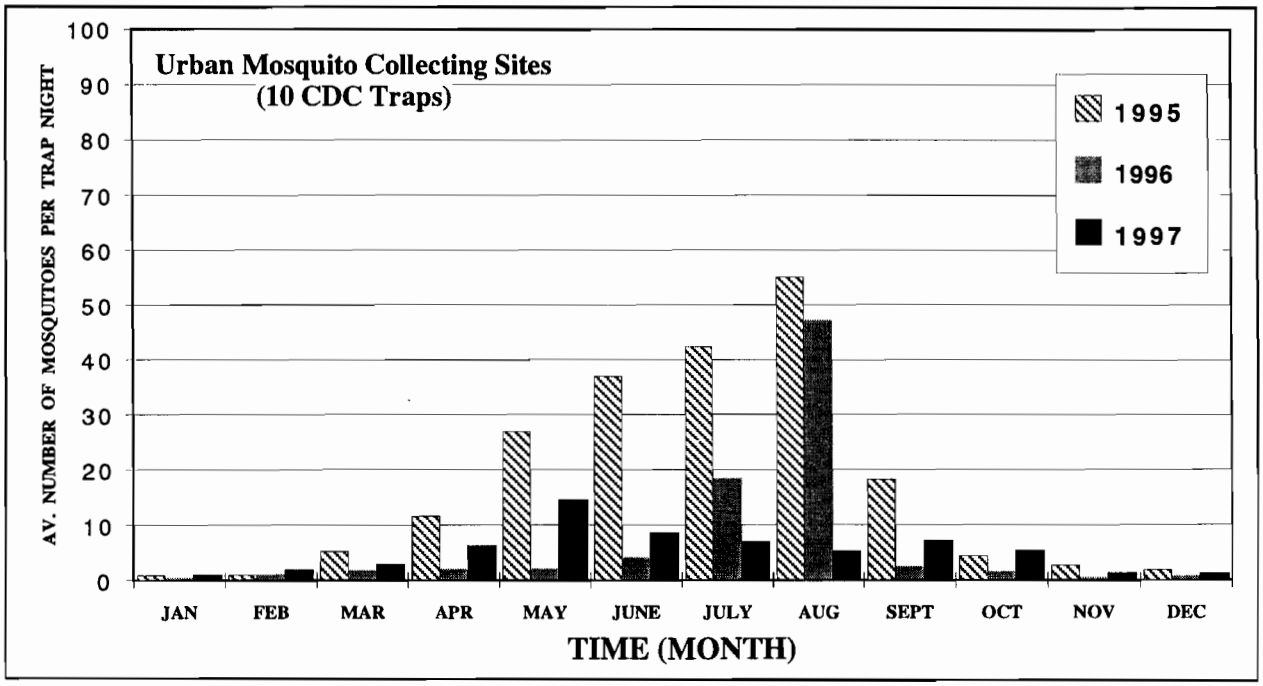

Figure 1. Host-seeking mosquito activity (CDC traps) in urban sites of Orange County, CA., 1995-1997.

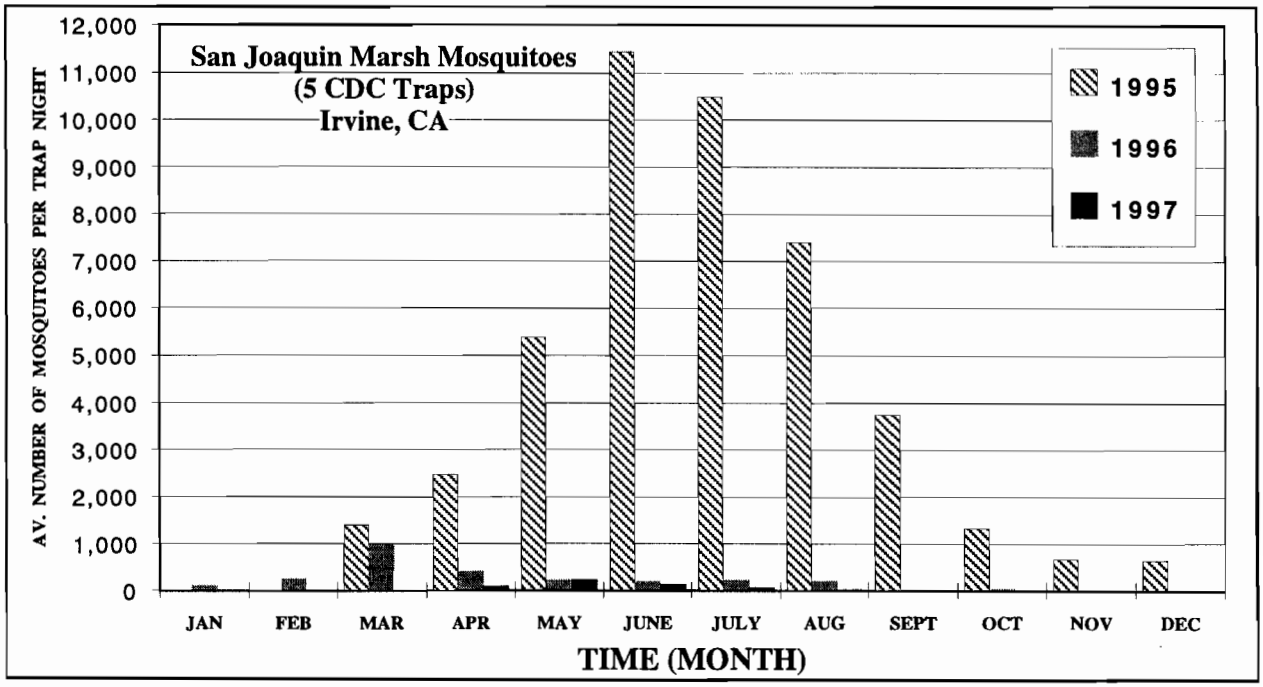

Figure 2. Host-seeking mosquito activity (CDC traps) in the San Joaquin Freshwater Marsh (rural site) Irvine, CA., for 1995-1997.

Australian Crow traps at sites also used to sample the adult mosquito population. Five trap sites were located in riparian corridors surrounded by suburban development. House Finches were the predominate avifauna caught and sampled at these localities. House Sparrows, on the other hand, were collected almost exclusively at the other four trap sites, which were located in highly developed residential communities with few open areas. At all nine trap locations, one species tended to be overwhelmingly abundant, and near-equal mixes of sparrows and finches were rare and temporary.

Birds were sampled at each trap site on alternate weeks, with five sites visited one week and the remaining four, the following week. Newly captured birds were banded and recorded, bled and then released. Blood samples (approximately 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 later in the District's laboratory using hemagglutination inhibition (HAI) techniques (Gruwell et al. 1988).

Of the 1,574 House Sparrows sampled in 1997, 0.57% (9 birds) tested positive for SLE antibodies, while 0.54% (7 birds) of the 1,297 House Finches sampled were SLE-positive (Table 2). Antibody positive birds were detected from June through November (except September), peaking in July and October at 2.0% each. Small bird seroprevalence was first noted in Orange County before and paralleled sentinel chicken seroconversions in neighboring Los Angeles County for 1997 (Figure 6).

Mosquito Abundance and SLE Seroprevalence

A residential site in Huntington Beach produced several positive House Sparrows (4 birds) in the District within a short time span (4 weeks) and the highest counts of *Cx. tarsalis* outside of a wetland area (Figure 7). Counts of host-seeking females rose in late April to 174 per trap night, and several House Sparrows tested positive for SLE antibodies several weeks later. Collections of *Cx. quinquefasciatus* were persistently low at this site, averaging less than 10 females per trap night throughout the year.

Elsewhere in the District, seroprevalence rates were temporally and spatially scattered, not distinctively correlated with vector abundance.

Long Term Trends

In the past five years, 1993 - 1997, the District's wild bird arbovirus surveillance program has experienced a period of relatively low antibody seroprevalence, in contrast to three very active years from 1990 to 1992 (Figure 8). 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), along with two human cases (Bennett et. al., 1992, 1993).

In general, mosquito counts from all sites in Orange County for 1997 were lower than in previous years. Nevertheless, data from the District's wild bird serosurveillance program suggests that enzootic transmission is continuous, even with the reduced numbers.

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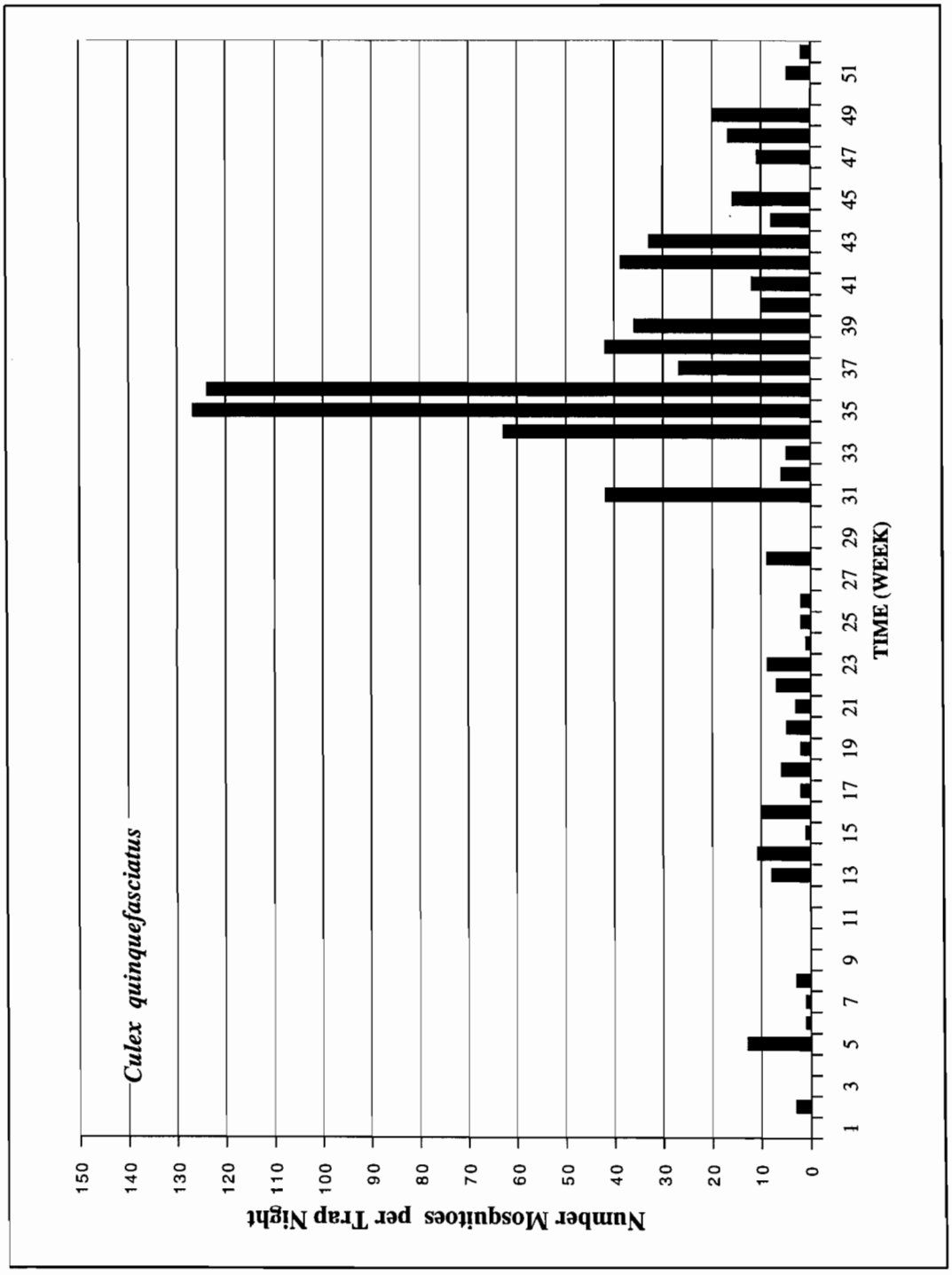

Figure 3. Gravid female mosquito activity at Central Park in Huntington Beach, CA., during 1997.

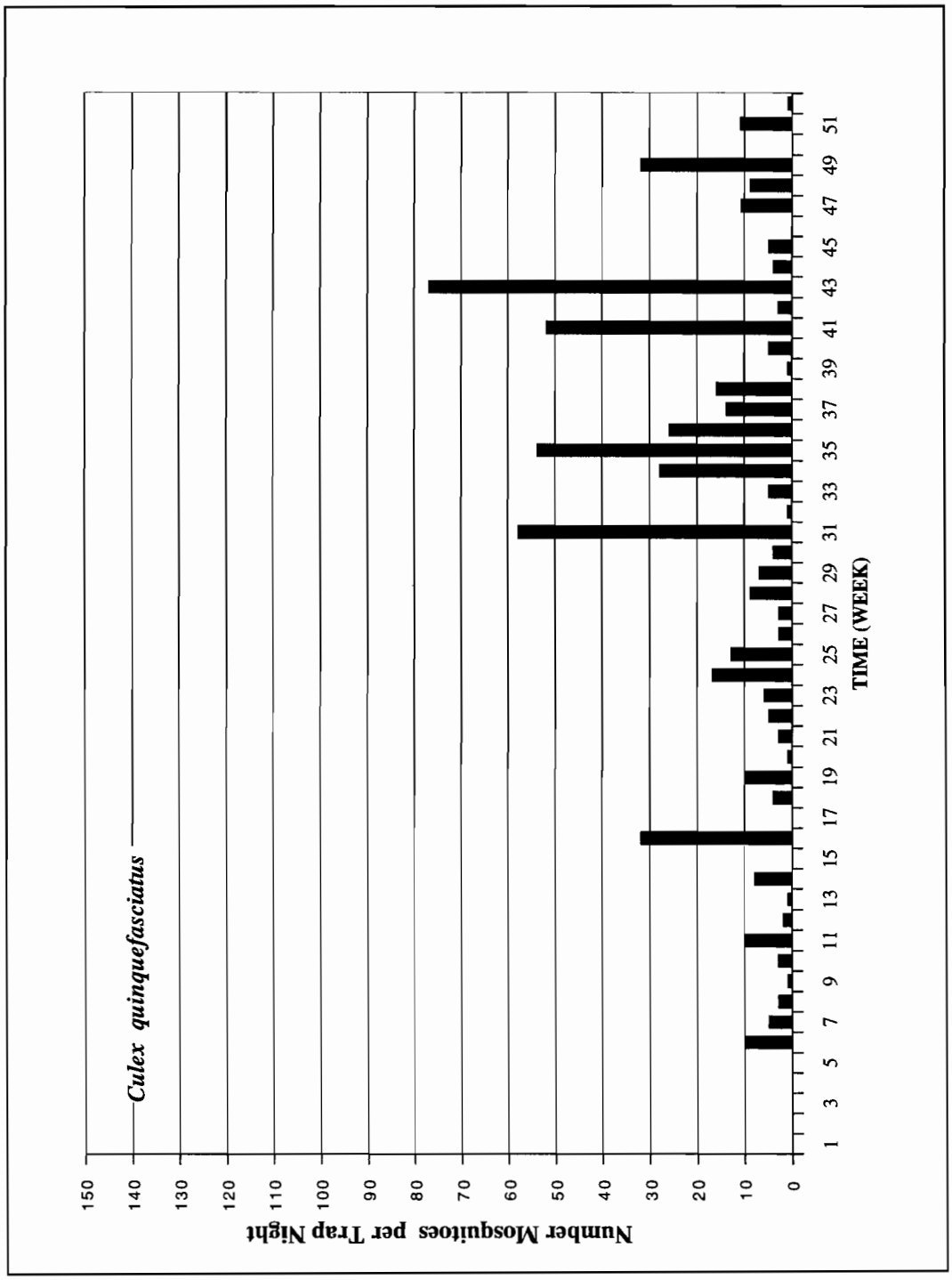

Figure 4. Gravid female mosquito activity at OCVCD in Garden Grove, CA., during 1997.

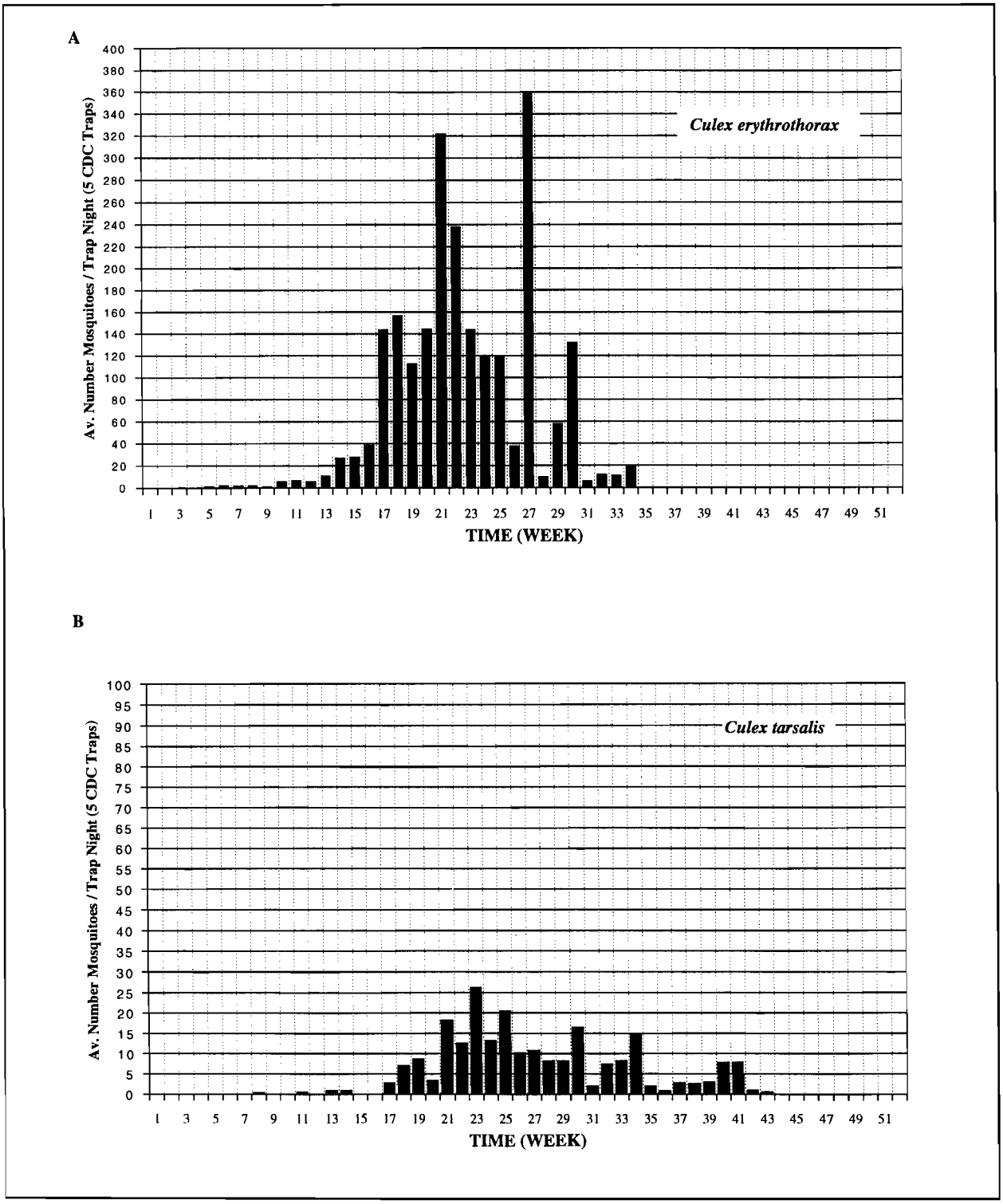

Figure 5. Host-seeking mosquito (CDC traps) activity in the San Joaquin Freshwater Marsh in Irvine, Ca., during 1997: A, *Cx. erythrothorax*; B, *Cx. tarsalis*.

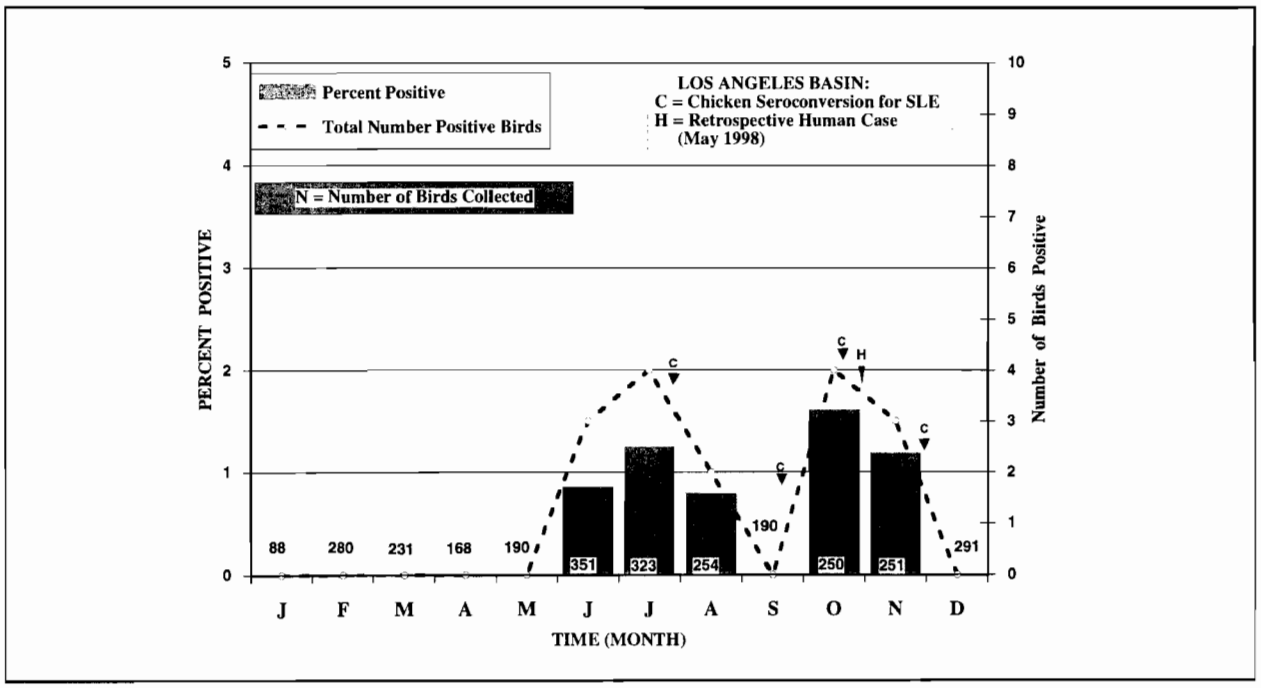

Figure 6. SLE virus activity in the Los Angeles Basin and wild bird seroconversions in Orange County, CA., during 1997.

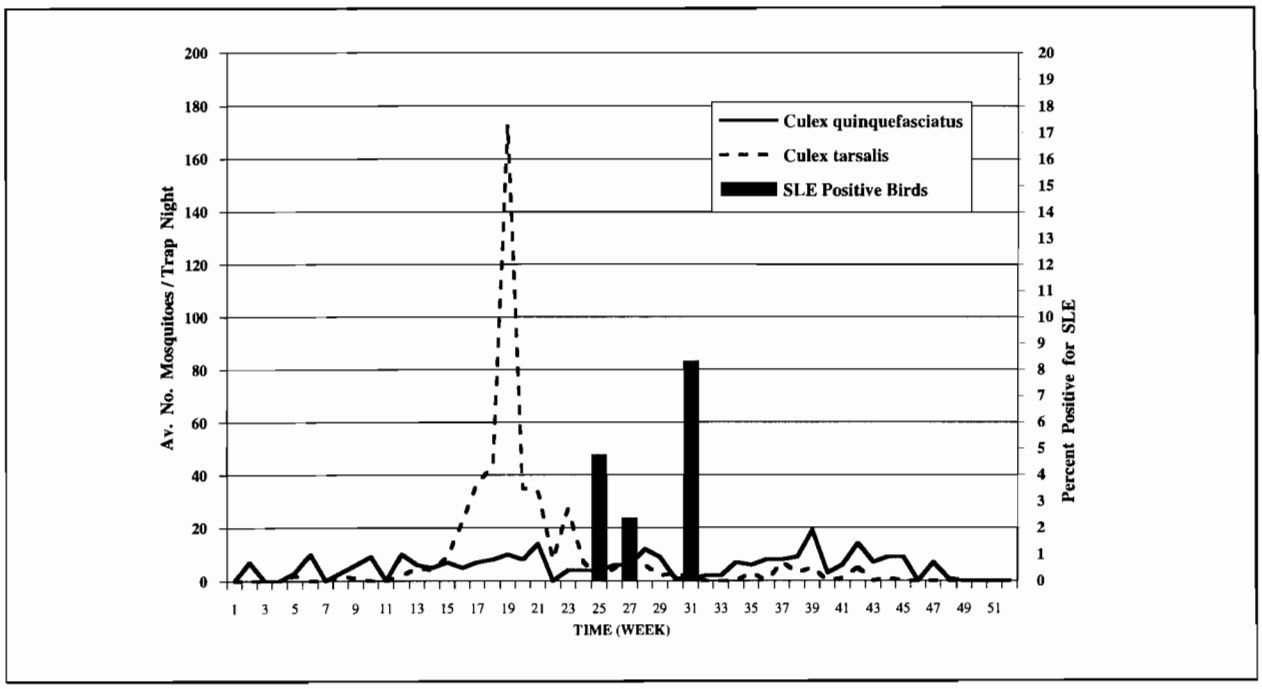

Figure 7. Host-seeking mosquito and SLE virus activity in house sparrows from a residence in Huntington Beach, Orange County, CA., during 1997.

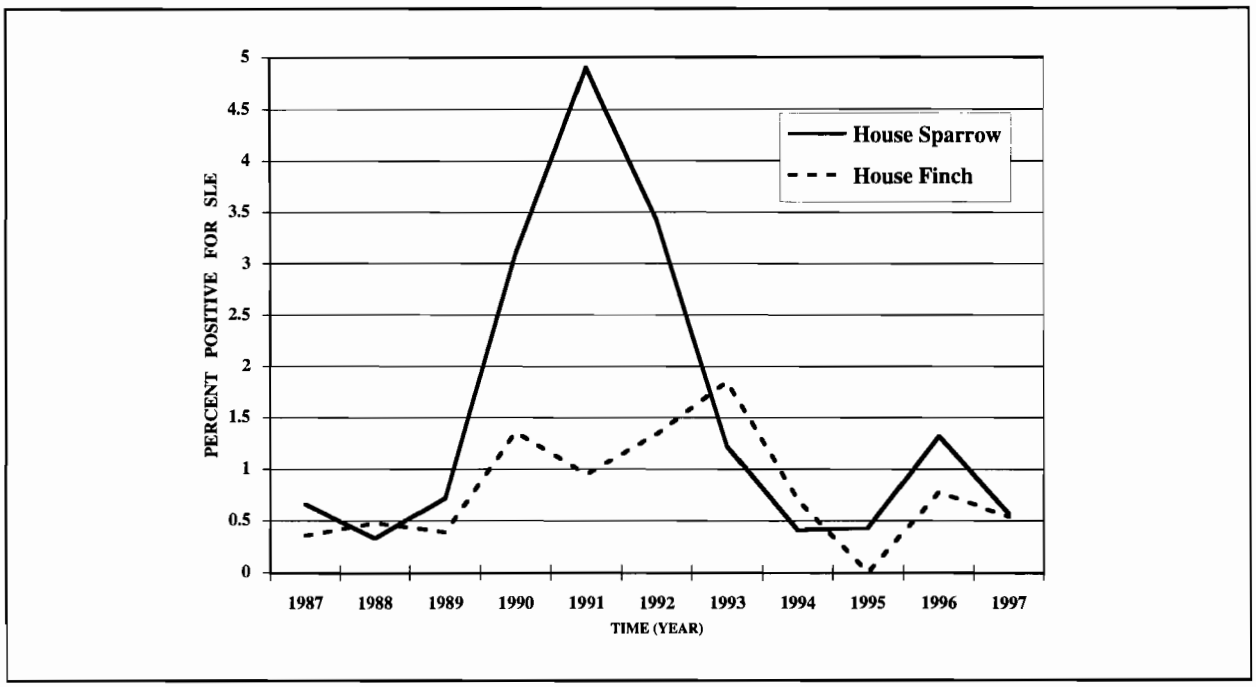

Figure 8. Small bird seroconversions for SLE antibodies in Orange County, CA., 1987 to 1997.

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SURVEY OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS PREVALENCE IN WILD BIRDS ON THE STONE LAKES NATIONAL WILDLIFE REFUGE, SACRAMENTO, CALIFORNIA

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ABSTRACT

Wild birds were captured using mist nets on the Stone Lakes National Wildlife Refuge from 1996 to 1997. Blood samples were collected from 450 wild birds and tested for the presence of WEEV antibody using hemagglutination inhibition. Nine birds (2% of those sampled) representing both resident and migrant species were antibody positive. It is speculated that resident species may be acting as reservoirs for WEEV on the Stone Lakes NWR at locations where natural wild bird habitat and the *Culex tarsalis* mosquito habitat are the same.

The western equine encephalomyelitis virus (WEEV) is maintained in western North America via enzootic transmission by the mosquito *Culex tarsalis* Coquillett and a number of passerine bird species that function as reservoir hosts (Reeves 1990). The WEE virus has been detected in the Sacramento Valley for the last four years using viral antibody tests on blood collected from sentinel chickens and by testing pools of adult *Culex tarsalis* for virus presence (Kramer et al. 1997, Kramer et al. 1996, Reilly et al.1995, and Emmons et al. 1994). Sentinel chickens positioned on the Stone Lakes National Wildlife Refuge (SLNWR) in Sacramento County also seroconverted during these same years (Dritz et al. 1994 and Dritz et al. 1996). Stone Lakes National Wildlife Refuge functions as a nesting and resting location for many species of birds. The Stone Lakes NWR Final EIS reports 111 species of birds associated with the refuge along with a large cormorant and heron rookery (SLNWR-EIS 1992) A recent census found 142 species of birds on the refuge (Sacramento Audubon Annual Christmas Count 1996) Wild birds can be effective indicators of arbovirus prevalence and if sufficient recaptures can be obtained, they can be excellent indicators of virus transmission (Reeves and Hammon 1962, Reeves 1990 and Crans et al. 1994).

The primary purpose of this study was to establish a WEEV antibody prevalence among wild birds on the Stone Lakes NWR and to investigate the probability of using base-line-negative recaptured wild birds as early warning sentinels of WEEV amplification in a surveillance program. The investigation also proposed to identify candidate avian reservoirs of WEEV based on antibody prevalence on the Stone Lakes NWR. Finally, we hoped to observe changes over time in avian species and abundance and to speculate on the likelihood of such habitats acting as a focus for WEEV maintainence.

METHODS AND MATERIALS

The Stone Lakes NWR, established in 1994 by the U.S. Fish and Wildlife Service (USFWS) is located in Sacramento County south of the town of Freeport and north of the town of Hood. The habitat includes valley oak and willow/cottonwood riparian woodlands along the Stone Lake slough and on the shore of Stone Lake, tule and cattail marshes adjacent

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to the slough and lake shore and vast open grasslands. The central attraction of the refuge is the approximately 70 hectare Stone Lake, an ancient oxbow lake of the Sacramento River with an estimated age at over 1,000 years. The lake's water is derived from Morrison and Laguna Creek watersheds after first flowing through the northern Beach Lakes complex.

Wild birds were collected using mist nest positioned within the thick oak and willow riparian woodlands located along the Stone Lake slough and in willow/cottonwood riparian woodlands associated with Stone Lake.

Five 2.6x12m (38mm) black nylon mist nets were opened for five hours three days each week from June 1996 to June 1997, exclusive of December through February due to normal winter flooding on the Stone Lakes NWR. Each day the nets were moved to a new location following a rotation that would return us to the start twice each month. The capture of wild birds included notes on the specific location and habitat, time of day, temperature and weather conditions, along with bird data such as age, sex, condition of the bird such as fat, molt and presence of observable diseases and a series of morphometric measurements that included culmen, exposed culmen, wing chord, tail, tarsus. overall length and bird weight. From each bird of appropriate size, those ten grams and over, a sample of blood (0.1cc) was extracted via jugular puncture using a fine needle (25 gauge to 28 gauge) and syringe. Each bird was banded according to protocol established by the U. S. Bird Banding Laboratory, U. S. Department of the Interior, Laurel MD, and released at the site of capture. Blood samples were diluted in the field with a phosphate-buffered bovine-albumin and saline solution (0.9cc) and then stored on blue ice. The blood samples were then centrifuged at 1.5 thousand rotations per minute for 30 minutes to separate the sera. Sera was then transferred via pipette to labeled 1mm cryogenic vials and stored in a Revco at -80 degrees Centigrade until tested. Frozen sera samples were sent to the National Veterinary Services Laboratory in Ames, Iowa for virus testing. The sera was tested using a hemagglutination inhibition (HI 1:10) test for WEEV antibody.

As a comparative indicator of WEEV activity in the area during the wild bird sample period, blood samples were collected from ten sentinel chickens positioned near the Stone Lakes NWR. In addition, adult female *Culex tarsalis* were collected on Stone Lakes NWR. Chicken sera and mosquito pools were sent to the California Department of Health Services-Viral and Rickettsial Disease Laboratory for WEE virus testing.

RESULTS

During the nine months (June through November 1996 and March through May 1997) of trapping wild birds on the Stone Lakes NWR, 450 individual birds from 45 species were sampled and tested for the presence of WEEV antibody (Table 1). Nine birds from eight species were found to be WEEV antibody positive, ie., equal to or greater than a titer of 1:20, three birds from three species had a WEEV antibody titer less than 1:20. All other birds tested negative for WEEV antibody. The overall antibody prevalence found on the Stone Lakes NWR during the sample period was 2.0%.

The nine positive birds included one house finch, one nuttall's woodpecker, one hermit thrush, one spotted towhee, one cooper's hawk, one golden-crowned sparrow, two cliff swallows and one song sparrow. The house finch was sampled on August 20,1996, the nuttall's woodpecker on September 9, 1996, the hermit thrush on October 1, 1996, the spotted towhee on March 3, 1997, the coopers hawk on March 13, 1997, the golden-crowned sparrow on March 27, 1997, the two cliff swallows on April 10, 1997 and the song sparrow was sampled on April 17, 1997. Ten percent of the birds banded on the Stone Lakes NWR were recovered during the sample period but none of the WEEV antibody positive birds were recovered during the same period. Nor were any of the WEEV negative birds that were subsequently recovered found to be positive. The WEEV antibody positive song sparrow was recovered twice after the end of the sample period and both times confirmed positive by EIA and neutralization tests.

All the positive birds were found to be after-hatching-year or adult birds by skull pneumatization. The spotted towhee, the house finch and the nuttall's woodpecker were all male birds, the gender of the other positive birds could not be determined. All the positive birds showed signs of good health, ie., overall healthy appearance, normal plumage, body size, body weight, and fat deposition. Upon release

Table 1. Number of wild bird species tested and found WEEV antibody (+) from Stone Lakes NWR.

No.	Bird Species	No. tested	WEEV (+)
1	Song sparrow - *Melospiza melodia*	75	**1**
2	Cliff swallow - *Hirundo pyrrhonota*	52	**2**
3	Yellow-rumped warbler - *Dendroica coronata*	36	0
4	Golden-crowned sparrow - *Zonotrichia atricapilla*	33	**1**
5	Spotted towhee - *Pipilo maculatus*	31	**1**
6	White-crowned sparrow - *Zonotrichia leucophrys*	23	0
7	Hermit thrush - *Catharus guttatus*	21	**1**
8	American goldfinch - *Carduelis tristis*	19	0
9	Lincoln's sparrow - *Melospiza lincolnii*	18	0
10	Brown-headed cowbird - *Molthrus ater*	17	0
11	Brewer's blackbird - *Euphagus cyanocephalus*	17	0
12	Ruby-crowned kinglet - *Regulus calendula*	11	0
13	Black phoebe - *Sayornis nigricans*	10	0
14	House finch - *Carpodacus mexicanus*	9	**1**
15	Black-headed grosbeak - *Pheucticus melanocephalus*	7	0
16	Fox sparrow - *Passerella iliaca*	7	0
17	Ash-throated flycatcher - *Myiarchus cinerascens*	6	0
18	Common yellowthroat - *Geothlypis trichas*	6	0
19	Nuttall's woodpecker - *Picoides nuttallii*	6	**1**
20	Pacific-slope flycatcher - *Empidonax difficilis*	5	0
21	Bewick's wren - *Thryomanes bewickii*	4	0
22	Swainson's thrush - *Catharus ustulatus*	4	0
23	Blue grosbeak - *Guiraca caerulea*	3	0
24	Red-winged blackbird - *Agelaius phoeniceus*	3	0
25	Western kingbird - *Tyrannus verticalis*	2	0
26	MacGillivray's warbler - *Oporornis tolmiei*	2	0
27	Marsh wren - *Cistothorus palustris*	2	0
28	Orange-crowned warbler - *Vermivora celata*	2	0
29	Tree swallow - *Tachycineta bicolor*	2	0
30	Western scrub jay - *Aphelocoma californica*	2	0
31	Western tanager - *Piranga ludoviciana*	1	0
32	Yellow warbler - *Dendroica petechia*	1	0
33	Dusky flycatcher - *Empidonax oberholseri*	1	0
34	Bushtit - *Psaltriparus minimus*	1	0
35	Savannah sparrow - *Passerculus sandwichensis*	1	0
36	Dark-eyed junco (oregon) - *Junco hyemalis*	1	0
37	Cooper's hawk - *Accipiter cooperi*	1	**1**
38	Western meadowlark - *Sturnella neglecta*	1	0
39	California quail - *Callipepia californica*	1	0
40	European starling - *Sturnus vulgaris*	1	0
41	Lazuli bunting - *Passerina amoena*	1	0
42	Bullock's oriole - *Icterus galbula*	1	0
43	Wrentit - *Chamaea fasciata*	1	0
44	Warbling vireo - *Vireo gilvus*	1	0
45	Wilson's warbler - *Wilsonia pusilla*	1	0
	TOTAL	**450**	**9**

each bird flew away demonstrating normal vigor.

From the mosquitoes collected on the Stone Lakes NWR, all *Culex tarsalis* pools collected between July and September 1996 tested negative for WEEV. Three pools of *Aedes melanimon* Dyar collected between June and August 1996 also tested negative for WEEV. Eight of ten sentinel chickens positioned in a coop near the Stone Lakes NWR seroconverted for WEEV antibody during the 1996 sample period.

DISCUSSION

The two percent WEEV antibody prevalence in wild birds found on the Stone Lakes NWR indicates that birds residing and resting on the refuge had been exposed to infected mosquitoes. Antibody presence alone however cannot indicate when and where these birds were infected as it is not known how long the various species of wild birds retain antibody titer.

Wild birds tend to move, sometimes, depending upon species, considerable distances. The two antibody positive cliff swallows, for example, may have been infected during their annual migration to the neotropics or they may have been infected while nesting on the Stone Lakes NWR. Cliff swallows return each year to the same nesting site where they rear their young in a social colony (Brown and Brown 1996). The fact that two of the cliff swallows were positive from the same breeding colony may indicate local infection as it seems less likely that randomly dispersed migrating individuals from the same colony would become infected than more stationary birds in a breeding colony.

The antibody positive golden-crowned sparrow and hermit thrush spend the warmer summer months in either coastal or mountain habitats only visiting the valley habitats like Stone Lakes NWR in the cooler spring, winter and fall months. The golden-crowned sparrows tend to return each year to the same wintering grounds. It seems somewhat more likely that these birds would contact WEEV infected *Culex tarsalis* mosquitoes in the valley habitat of Stone Lakes NWR than in their summer habitats of coastal or high mountains.

The other WEEV antibody positive birds, including the house finch, nuttall's woodpecker, cooper's hawk, spotted towhee and the song sparrow are all year-long resident species and very likely became infected in the local area. Some species like the house finches may move several miles to forage during the non-breeding season. The nuttall's woodpecker may move up-slope after breeding in the lowlands. Spotted towhees and song sparrows are very likely to remain in the local area throughout their lives.

Our valley song sparrows, *Melospiza melodia mailliardi* Wilson are especially local residents. Second-year birds establish breeding territories in tule or cattail marshes, nesting in the spring on the ground or above water in the tules, and remain there year-round. If conditions in their territory are good, an individual may hold a territory throughout its life. It is extremely likely that our antibody positive song sparrow was infected where it was trapped on the Stone Lakes NWR.

The valley song sparrow was, by far, the most abundant species encountered during our trapping on Stone Lakes NWR (Table 1). The very high pattern of recovery of this species throughout the sampling period indicates their permanent residence at this site. In the early spring we observed song sparrows on the Stone Lakes NWR with enlarged male cloacal protuberances and fully engorged female brood patches demonstrating full breeding conditions. We also captured increasing numbers of hatching-year song sparrows in the late spring and early summer. This indicates a healthy breeding population of song sparrows on the refuge. Once vast wetlands and marshes in the Central Valley of California constituted optimum habitat for these birds and their numbers must have been equally vast. Today few habitats suitable for the valley song sparrow exist and their populations are vulnerable. Breeding populations such as exists on the Stone Lakes NWR and perhaps a few other similar isolated habitats may represent relics of the once vast and continuous population in the Central Valley of California.

The presence of WEEV antibody in five Stone Lakes NWR resident species strongly indicates that transmission of WEEV among wild birds is occurring on the refuge. Historical and concurrent chicken seroconversions on and off the Stone Lakes NWR support the occurrence of virus transmission and the collection of a *Culex tarsalis* positive pool on the Stone Lakes NWR in 1994 (Dritz et al. 1996) along with the presence of passeriformes and piciformes blood from engorged *Culex tarsalis* collected from the refuge (Dritz, personal communication) implicate *Culex tarsalis* as the WEEV vector on the refuge and

resident birds as the likely reservoirs. Song sparrows, the most abundant resident species on the refuge and therefore the most available host for *Culex tarsalis* stands out as a clear candidate for a local WEEV reservoir.

CONCLUSIONS

The tule and cattail marsh adjacent to the oak and willow forest provides optimum habitat for the resting and breeding of migrant and resident species of wild birds. This same habitat also functions very well as harborage and breeding habitat for *Culex tarsalis* mosquitoes. The proximity of these two natural elements provides conditions for a focal enzootic transmission of WEEV. *Culex tarsalis* mosquitoes as vectors of the virus, and resident birds, such as the song sparrow, as reservoirs of the virus could act together to maintain and recycle the virus on the refuge. The periodic amplification and subsequent spread of WEEV from local bird reservoirs to various hosts could be mediated by annual variations in *Culex tarsalis* populations.

Several more years of trapping on Stone Lakes NWR will be necessary in order acquire a greater number of base-line-negative birds to allow the observance of seroconversions within the resident wild bird population. Further trapping will also allow the repeated detection of antibody in resident birds that can indicate which bird species are acting as reservoirs.

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MOSQUITO ABUNDANCE AND ARBOVIRAL ACTIVITY IN SAN BERNARDINO COUNTY DURING 1997

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ABSTRACT

Of the 8,534 mosquitoes collected in New Jersey light traps in San Bernardino County during 1997, 64.3% were from the desert region (Needles) and 35.7% from the San Bernardino valley area. In the desert region, the dominant species were *Culex tarsalis* (80.3%) and *Culiseta inornata* (17.1%). In the valley area, *Cx. tarsalis* (36.0%), *Culex stigmatosoma* (30.6%), *Culiseta incidens* (8.8%) and *Culex quinquefasciatus* (3.8%) were found in significant numbers. All 34 pools of culicine mosquitoes submitted for testing were negative for both Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses.

Both the San Bernardino and Redlands sentinel chicken flocks showed seroconversions in July and August, respectively. The posting of encephalitis warning signs, press releases and increased mosquito control activities were carried out in both areas.

As part of the state-wide encephalitis virus surveillance (EVS) program in California, the San Bernardino County Vector Control Program (SBCVCP) has carried out EVS in both the San Bernardino valley (from Yucaipa to Upland) and desert (Needles) areas of San Bernardino County for several years. Geographically, the county consists of three distinct regions; the desert, mountain and valley regions. Demographically, the valley region houses over 80% of the nearly 1.6 million human population in the County with the remaining scattered over various parts of the desert and mountain regions. Historically, cases of both Saint Louis encephalitis(SLE) and western equine encephalomyelitis(WEE) have been reported in the desert and valley regions from time to time.

After experiencing 26 human cases of Saint Louis encephalitis in southern California during 1984, the only human case of this disease in southern California during 1987 was reported from San Bernardino (Emmons et al. 1988). Of the two cases reported state-wide in 1988, one was from the same San Bernardino site (Emmons et al. 1989). Recently, two of the three cases reported state-wide in 1993 and one case in 1994 were found to have been contracted in San Bernardino County (Emmons et al. 1994, Reilly et al. 1995). During the same period, activities of both SLE and WEE viruses were reported in the desert region, especially Needles, and adjoining areas along the Colorado River. Due to the periodic incidence of encephalitis disease, mosquito control and EVS activities have been routinely carried out in the desert and valley regions of the county. Data generated in these activities in 1997 are appraised here in relation to mosquito abundance and arboviral activity in San Bernardino County.

MATERIALS AND METHODS

EVS procedures described by Mian and Prochaska (1990) were continued in these studies as follows:

Adult Mosquito Population Dynamics: The abundance of various mosquito species was monitored weekly by the use of New Jersey light traps. In the valley region, the traps were stationed at

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Table 1. Percent species composition of adult female mosquitoes caught in New Jersey light traps in San Bernardino County during 1997.

Mosquito Species	Mosquito Composition					
	Desert Area*		Valley Area**		Total	
	Number+	%	Number++	%	Number	%
Aedes increpitus	0	0	1	<0.1	1	<0.1
Aedes vexans	3	<0.1	0	0	3	<0.1
Anopheles franciscanus	15	0.3	28	0.9	43	0.5
Anopheles freeborni	1	<0.1	1	<0.1	2	<0.1
Culex erythrothorax	74	1.3	32	1.1	106	1.3
Culex quinquefasciatus	14	0.3	116	3.8	130	1.5
Culex stigmatosoma	26	0.5	932	30.6	958	11.2
Culex tarsalis	4,407	80.3	1,097	36.0	5,504	64.5
Culiseta incidens	2	<0.1	570	18.7	572	6.7
Culiseta inornata	938	17.1	268	8.8	1,206	14.1
Psorophora columbiae	9	0.2	0	0	9	0.1
Total Number Caught	5,489	100	3,045	100	8,534	100
Area Total	5,489	(64.3)	3,045	(35.7)	8,534	(100)

*Needles area along the Colorado River.
**Yucaipa to Upland.
+Total collected over 3x259 trap-nights.
++Total collected over 8x259 trap-nights.

eight different locations; Yucaipa, Redlands, Highland, San Bernardino, Colton, Fontana, Ontario and Upland. Within the valley region there were at least two traps sites each in urban, suburban and rural environments. In the desert region (Needles area), one trap each was operated in urban, suburban and rural areas along the Colorado River. Adult mosquitoes collected weekly in all traps were counted, sexed and identified to species with the Adult Mosquito Occurrence Reports submitted to the California Department of Health Services.

Arboviral Activity in Female Mosquitoes: Arboviral activity in local mosquito populations was monitored by using CO_2-baited CDC traps to collect host-seeking adult female mosquitoes. Eight or more of such traps were operated twice a month in the valley area. Female mosquitoes collected overnight were anesthetized using triethylamine (TEA), counted, identified to species and sex then pooled by species and sex with 10-50 adults per vial. All vials were stored on dry ice in the field or in ultra-low temperature deep freezer (-70°F) in the laboratory before being shipped in dry ice-packed containers by overnight express mail to the Viral and Rickettsial Disease Laboratory (VRDL) in Berkeley.

Arboviral Activity in Sentinel Chickens: Both wild and domestic birds are known to play a significant role in the epidemiology of mosquito-borne encephalitides by acting as reservoir hosts for these arboviruses. Therefore four sentinel flocks, each consisting of ten white leghorn chickens, were used to monitor arbovirus activity in the area. Three of the flocks were maintained in the valley area in Colton, Redlands, and San Bernardino and one in the desert area in Needles. The Colton flock was stationed at the southeastern corner of Rancho Avenue and La Cadena Drive in the city of Colton. This site is located in the general area of previous SLE human cases reported in 1987 and 1988. The Redlands flock was maintained along the Santa Ana River between Alabama and Nevada Streets. The San Bernardino flock was stationed by the County flood control basins at the northeastern corner of 40th Street and Waterman Avenue. The desert flock was

Table 2. Frequency of blood samples taken from sentinel chicken flocks during 1997.

Sampling Date (Week of)	Colton Flock			Needles Flock			Redlands Flock			San Bernardino Flock		
	Chickens Per Flock	Number Sampled	Number Sero + ive	Chicken Per Flock	Number Sampled	Number Sero + ive	Number Per Flock	Chicken Sampled	Number Sero + ive	Number Per Flock	Chicken Sampled	Number Sero + ive
April 28	10	10	0	10	10	0	10	10	0	10	10	0
May 12	10	10	0	10	10	0	10	10	0	10	10	0
May 28	10	10	0	10	10	0	10	10	0	10	10	0
June 9	10	10	0	10	10	0	10	10	0	10	10	0
June 23	10	10	0	10	10	0	10	10	0	10	10	0
July 7	10	10	0	10	10	0	10	10	0	10	10	1[a/]
July 21	10	10	0	10	10	0	10	10	0	10	10	1
August 4	10	10	0	10	10	0	10	10	0	10	10	1
August 18	10	10	0	10	10	0	10	10	1[b/]	10	10	1
September 1	8	8	0	10	10	0	8	8	1	5	5	1
September 15	8	8	0	10	10	0	8	8	1	5	5	1
September 29	8	8	0	10	10	0	8	8	1	5	5	1
October 13	8	8	0	10	10	0	7	7	1	5	5	1
October 27	8	8	0	10	10	0	7	7	1	5	5	1
November 10	8	8	0	10	10	0	7	7	1	5	5	1
November 24[c/]	8	8	0	10	10	0	7	7	1	5	5	1
December 12	8	8	0	10	10	0	7	7	1	5	5	1
December 30	8	8	0	10	10	0	7	7	1	4	4	1

[a/] Bird bled July 8, one sero-converted to SLE.
[b/] Birds bled August 21, one sero-converted to SLE.
[c/] Last week of regular bird feeding.

kept at the sewage treatment facility in the city of Needles. Using the comb prick method, blood samples were taken from all sentinel chickens and placed on prelabeled filter paper strips on a bi-weekly basis during the season. These samples were then mailed to the VRDL for detection of arboviral activity. New Jersey light traps were regularly operated at all flock sites.

RESULTS AND DISCUSSION

Of the total 8,534 mosquitoes collected at all sites during the season, 64.3% were trapped in the desert area and 35.7% from the valley sites (Table 1). The most abundant mosquito in the desert region was *Culex tarsalis* Coquillet (80.3%), followed by *Culiseta inornata* Williston (17.1%), *Culex erythrothorax* Dyar (1.3%), *Culex stigmatosoma* Dyar (0.3%), *Culex quinquefasciatus* Say (0.3%), *Anopheles franciscanus* McCracken (0.3%) and *Psorophora columbiae* Dyar and Korab (0.2%). Similarly, the most abundant species in the valley area was *Cx. tarsalis* (36.0%), followed by *Cx. stigmatosoma* (30.6%), *Cs. incidens* (18.7%), *Cs. inornata* (8.8%), *Cx. quinquefasciatus* (3.8%), *Cx. erythrothorax* (1.1%), *An. franciscanus* (0.9%), *Aedes increpitus* (<0.1%), and *Anopheles freeborni* (<0.1%). Earlier studies in this area indicated *Cx. tarsalis* as the most abundant species, comprising as much as 35% of the mosquitoes collected in 1989 (Mian and Prochaska, 1990).

A total of 34 pools of *Culex* mosquitoes (*Cx. tarsalis*, *Cx. quinquefasciatus* and *Cx. stigmatosoma*) collected in CO_2-baited CDC traps in the valley area was sent to VRDL for virus study. All pools tested negative for both SLE and WEE viruses.

The results on chicken serology showed one sero-conversion to SLE each in San Bernardino and Redlands flocks (Table 2). The sero-conversion in the San Bernardino flock was found early in July (bled July 8, 1997), whereas the sero-conversion in the Redlands flock was reported about a month and a half later (bled August 21, 1997). There were no sero-conversions detected in the Colton and Needles flock.

Upon receipt of confirmation on sero-conversions in both San Bernardino and Redlands flocks, the areas were posted with "Encephalitis Warning" signs followed by press releases to local newspapers advising residents to take necessary precautions during outdoor activities especially at dusk and dawn in the affected areas. In the wake of virus activity, mosquito source reduction and control activities were intensified in both areas. During the 1997 EVS season, there ware no human cases of mosquito-borne encephalitis in the SBCVCP territory.

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PLAGUE SURVEILLANCE IN SAN BERNARDINO COUNTY: A DECADE (1988-97) IN REVIEW

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ABSTRACT

During 1988-97, the San Bernardino County Vector Control Program carried out 146 plague surveys in the foothill and mountain areas of San Bernardino County. The average number of animals per survey per year ranged from 5.7 to 17.4 and the average number of fleas per animals per year ranged from 3.2 to 20.2. Of the total 1,716 animals trapped, 91.8% were *Spermophilus beecheyi*, 3.5% *Spermophilus lateralis*, 3.0% *Tamias merriami*, 0.5% *Neotoma fuscipes*, 0.2% *Dipodomys merriami*, and 1.0% other species. Of the total 7,437 fleas identified from these animals, 76.5% were *Dimanus montanus*, 18.0% *Hoplopsyllus anomalis*, 0.1% *Oropsyllus idahoensis*, <0.1% *Nosopsyllus fasciatus*, and 5.3% other species.

In rodent serology 1,602 sera were tested for plague antibody. Plague activity was detected every year except 1991 and 1992, during which small numbers of samples were taken. Seropositivity ranged from 1.2% to 48.4% per year with an overall mean 5.4%. Of the total fleas tested during the last 10 years, only 0.2% were positive for *Yersinia pestis* by animal inoculation.

Plague is an enzootic rodent disease communicable to humans. The disease, caused by a bacterium, *Yersinia pestis*, is transmitted to humans and other animals through the bite of infected fleas. Humans are exposed to the disease if they enter plague infected areas or if the disease is transmitted from feral rodents to commensal rats or cats that cohabit human environments. The occurrence of rural plague cases has been attributed to the extension of human habitations into previously wilderness areas.

Historically, plague is reported to have originated in Central Asia from where it spread to almost all continents of the world. This spread is evidenced from the first pandemic of AD 542, which involved Arabia, Europe, and North Africa. The second pandemic, the "Black Death" of the Middle Ages (1300's) covered parts of both Asia and Europe. The third and last pandemic originated in Asia (Southwest China) and spread to South Africa and South America by 1899, then to North America (San Francisco) and Australia (Brisbane and Sidney) by 1900 (Twiggy 1978, Kettle 1995).

Following the introduction of plague in North America, there have been four major urban epidemics in California: 1900-1903 and 1907-1909 in San Francisco; 1919 in Oakland; and 1924 in Los Angeles. Since that time, sporadic human cases in endemic areas have been traced to wild rodents and their ectoparasitic fleas. Plague infection in wild rodents is widely distributed in California including the coastal counties south of San Francisco Bay; inter-mountain valleys of northern California, the Sierra-Nevada from Lassen Peak to the Kern Plateau, and the Tehachapi, San Gabriel, San Bernardino, San Jacinto Mountains and mountains of San Diego County of southern California (Salmon and Gorenzel 1981, Anonymous 1983).

Known foci of plague epizootics are distributed throughout the mountains and foothill areas of San Bernardino County. The mountain ranges along with natural recreational lakes provide a wide variety of camping, hiking and water sport facilities to both local and out-of-county visitors. To safeguard public health and safety in these areas, the San Bernardino County Vector Control Program (SBCVCP) in collaboration

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Table 1. Data on animal plague surveys in San Bernardino County during 1988-97.

Year	Number of surveys	Animals trapped (M+F)[a/]	Animals per survey	Number of fleas collected	Fleas per animal
1988	14	244	17.4	3,219	13.2
1989	5	60	12.0	412	6.8
1990	17	138	8.1	2,783	20.2
1991	3	28	9.3	186	6.6
1992	9	51	5.7	393	7.7
1993	16	148	9.2	468	3.2
1994	23	314	13.6	1,170	3.7
1995	24	271	11.3	932	3.4
1996	18	165	9.2	550	3.3
1997	17	297	17.4	1,262	4.2
Total	146	1,716	113.2	11,375	72.3
Mean			11.32		7.23
Chi-Square (P = 0.05)			11.89[ns]		37.7*

[a/] (Male + Female)
[ns] not significant
* significant

with the California Department of Health Services-Vector-Borne Disease Section (CDHS-VBDS) and the United States Department of Agriculture-Forest Service (USDA-FS), carries out routine surveillance in plague enzootic areas during the season (April through November). The data generated in routine plague surveillance during the past 10 years (1988-97) are presented in this paper.

MATERIALS AND METHODS

In routine plague surveys, the general method used was similar to that of Lang and Wills (1991). During a typical daily survey, 30-35 Tomahawk #103 live traps (Tomahawk Live Trap Co., Tomahawk, WI) baited with peanut butter and rolled oats were set at appropriate locations in the survey area. In a campground situation, traps were also set up near picnic tables that attract wild rodents especially ground squirrels and chipmunks. Trap locations were flagged with orange nylon ribbon. Traps were set up in the morning and picked up in the early afternoon the same day.

Traps with live animals were brought to a central shady location for processing. The animal was transferred into a 45 x 90 cm. clear polyethylene bag (3 mil.). A ball of cotton drenched in ethyl ether was introduced into the bag which was kept tightly closed with a rubber band until the animal was anesthetized. The animal was then taken out of the bag and transferred to a white enamel pan (30 x 20 x 5 cm. deep), where the fleas were combed out using a stiff bristled brush. The fleas from each animal were collected in labeled 2 ml propropylene screw cap tubes containing a 2% saline solution. Next, through cardiac puncture, a 3 ml blood sample was drawn from each animal using a 23 gauge syringe. Pertinent data such as species, sex, and reproductive stage were recorded. The animal then was released back into its habitat. Survey site information was also recorded before leaving the area.

Blood samples were brought back to the laboratory where they were centrifuged for 20 minutes at 2000 rpm then the serum from each sample was transferred to labeled 2 ml polypropylene screw cap tubes. The sera and flea samples, along with completed paperwork, were sent on blue ice by overnight mail to the California Department of Health Services-Vector-Borne Disease Section (CDHS-VBDS) in Sacramento for laboratory analysis.

The laboratory at CDHS-VBDS, Sacramento, informed us via telephone of plague-positive samples. In the event of plague-positive sample confirmation, the standard plague epizootic protocol as outlined by Mian (1995) was followed. The protocol included posting the area with "Plague Warning" signs, follow-

Table 2. Rodent fauna collected in plague surveys in San Bernardino County during 1988-97.

Year	Number (% females) by Species						
	Spermophilus beecheyi	*Spermophilus lateralis*	*Tamias merriami*	*Neotoma fuscipes*	*Dipodomys merriami*	Other Species	Total
1988	225 (52)	0	17 (30)	0	0	2[a] (00)	244 (50)
1989	49 (51)	0	11 (36)	0	0	0	60 (48)
1990	132 (56)	0	2 (00)	4 (33)			138 (54)
1991	17 (53)	4 (50)	4 (25)	0	0	3[b] (67)	28 (50)
1992	50 (52)	1 (100)	0	0	0	0	51 (53)
1993	123 (53)	8 (50)	2 (50)	3 (67)	2 (100)	10[c] (60)	148 (54)
1994	305 (63)	4 (75)	1 (00)	1 (100)	1 (100)	2[d] (100)	314 (63)
1995	258 (60)	10 (60)	3 (100)	0	0	0	271 (61)
1996	142 (67)	15 (47)	7 (71)	0	0	1[e] (100)	165 (65)
1997	274 (58)	18 (39)	5 (60)	0	0	0	297 (57)
Total	1,575 (58)	60 (50)	52 (42)	8 (50)	3 (100)	18 (61)	1,716 (58)
%	91.8	3.5	3.0	0.5	0.2	1.0	100

[a] *Microtus* sp.
[b] *Neotoma* sp. (male), *Peromyscus* sp. (female), and *Silvilago audoboni* (Lagomorph (female).
[c] *Ammospermophilus leucurus* female, 5 *Neotoma lepida* (3 females), and 4 *Peromyscus californicus* (2 females).
[d] *Dipodomys agilis.*
[e] *S. audoboni.*

ed by public education and press releases (if warranted). It required evacuation (if a campground), followed by ectoparasite control and, if needed, rodent control. A post-treatment evaluation of flea index was made prior to re-opening the area for public use, especially at a campground or public park.

RESULTS AND DISCUSSION

During routine sylvatic plague surveillance, 146 surveys were carried out in the mountains and foothill areas of San Bernardino County over the 10-year period, 1988-97 (Table 1). A total of 1,716 animals were trapped. The average number of animals per survey year ranged from 5.7 to 17.4. The number of fleas collected from these animals varied significantly with the average number of fleas per animal per year ranging from 3.2 to 20.2.

Of the total animals caught in live traps, 91.8% were *Spermophilus beecheyi*, 3.5% *Spermophilus lateralis*, 3.0% *Tamias merriami*, 0.5% *Neotoma fuscipes*, 0.2% *Dipodomys merriami*, and 1.0% other species (Table 2). The other rodent species included *Ammospermophilus leucurus*, *Dipodomys agilis*, *Microtus* sp.,. *Neotoma lepida*, *Peromyscus californicus* and a lagomoph, *Silvilagus audoboni*. Of the 11,375 fleas collected from the trapped animals, 7,437 were identified to species. The species composition included 76.5% *Dimanus montanus*, 18.0% *Hoplopsyllus anomalis*, 0.1% *Oropsyllus idahoensis*, <0.1% *Nosopsyllus fasciatus*, and 5.3% other species (Table 3). The other flea species were *Anomiopsyllus mudatus*, *Atheca wagneri*, *Ctenocephalides felis*, *Echidnophaga gallinacea*, *Malaraeus telchinus*, *Monopsyllus* sp., and *Rhabdinopsyllus sectilis*. The variations in flea numbers were due in large part to a variation in the number of surveys and the number of rodents captured.

The data on rodent serology and fleas tested for plague are presented in Table 4. A total of 1,602 serum samples were tested for plague antibody. Plague activity was detected every year except 1991 and 1992 when sample sizes were relatively small. Of the total fleas tested for plague, only one pool (24 fleas) was

Table 3. Fleas collected from rodents in plague surveys in San Bernardino County during 1988-97.

	Number (%females)							
Year	*Dimanus montanus*	*Hoplopsyllus anomalis*	*Nosopsyllus fasciatus*	*Oropsyllus idahoensis*	Other Species	Total Identified	Unidentified Fleas	Total
1988	2,114 (59)	817 (56)	0	3 (33)	285[a] (63)	3,219 (59)	0	3,219
1989	325 (54)	14 (50)	0	0	7[b] (43)	346 (54)	66	412
1990	762 (52)	195 (56)	0	0	47[c] (06)	1,004 (51)	1,779	2,783
1991	-	-	-	-	-	-	186	186
1992	-	-	-	-	-	-	393	393
1993	265 (62)	0	0	2 (50)	0	267 (62)	201	468
1994	809 (70)	17 (59)	0	0	0	826 (69)	344	1,170
1995	839 (50)	89 (51)	0	0	0	928 (50)	4	932
1996	227 (64)	91 (64)	3 (100)	5 (60)	16[d] (50)	342 (64)	208	550
1997	347 (60)	119 (61	0	0	39 (51)	505 (59)	757	1,262
Total	5,688 (58)	1,342 (56)	3 (100)	10 (50)	394 (54)	7,437 (58)	3,938	11,375
%	76.5	18.0	<0.1	0.1	5.3	100		

[a] Includes 1 *Anomiopsyllus mudatus* (female), 2 *Atheca wagneri* (female), 1 *Ctenocephalides felis* (male), 233 *Echidnophaga gallinacea* (146 female), 8 *Malaraeus telchinus* (7 females), 39 *Monopsyllus* spp. (23 females), and 1 *Rhabdinopsyllus sectilis* (female).
[b] *Monopsyllus* spp.
[c] 1 *A. wagneri* (female) and 46 *E. gallinacea* (2 females).
[d] *E. gallinacea*.

Table 4. Data on rodent serology and fleas tested for plague in San Bernardino County during 1988-97.

Year	Number of sera tested	Number (%) of sera plague positive [a]	Number of fleas tested
1988	169	9 (5.3)	3,219
1989	26	4 (15.4)	412
1990	141	5 (3.5)	2,783
1991	25	0 (0)	186
1992	46	0 (0)	393
1993	148	12 (8.1)	468
1994	314	15 (4.8)	1,170
1995	271	32 (11.8)	932
1996	165	3 (1.8)	550
1997	297	6 (2.0)	1,262 [b]
Total	1,602	86	11,375
Mean	160.2	8.6 (5.4)	1,137.5

[a] An antibody titer of 1:16 or higher is diagnostic for exposures to the plague pathogen.
[b] Plague positive fleas are indicators of active plague. Only 1997 had 2.4 (0.2%) fleas positive for the plague antibodies.

found positive for the plague pathogen, *Yersinia pestis*, through animal inoculation and it was obtained during 1997.

Apart from rodent serology, carnivore blood samples taken on nobuto strips were tested during 1988 and 1990. In 1988, samples from one gray fox and eight coyotes tested negative for plague. However, in 1990, samples from three gray foxes from the Mill Creek area tested positive for plague antibody. In 1995, there was a canine plague case at the Cedar Lake camp in the Big Bear area. The dog survived after antibiotic therapy. During the period, there were no feline or human cases of plague reported in San Bernardino County.

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Table 4. Data on rodent serology and fleas tested for plague in San Bernardino County during 1998-97.

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VECTOR MONITORING OF SYNTHETIC GEOTEXTILE COVER AT A SANITARY LANDFILL IN SAN BERNARDINO COUNTY

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ABSTRACT

The San Bernardino County Vector Control Program (SBCVCP) carried out studies on arthropod and vertebrate vectors attracted to landfill covered with soil or with a synthetic geo-textile (tarp) cover at the California Street Landfill, City of Redlands, over a 12-month period during 1996-97. Data generated in these studies showed that the mean weekly fly grill counts at the tarp cover remained well below the threshold number, 6 flies/grill count, as described in the performance standards, section 17683 of the California Code of Regulations. Fly counts at the tarp were comparatively lower than those at the soil cover maintained as a control. As expected fly activity was affected by temperature, winds, and rain. In monthly fly species surveys, the faunal composition consisted of the pomace fly, *Drosophila* sp., common housefly, *Musca domestica*, false stable fly, *Muscina stabulans*, little house fly, *Fannia canicularis*, and green blowfly, *Phaenicia sericata*. The data on monthly rodent surveys showed no domestic rats trapped at the site. Other rodents trapped in these surveys included mainly deer mice with one house mouse and a ground squirrel. In conclusion, the tarp cover did not pose any significant health risk with respect to attracting and propagating disease vectors such as filth breeding flies and domestic rats.

Sanitary landfills or solid waste disposal facilities operate under permits by local enforcement agencies and comply with all applicable laws and regulations as defined by the California Code of Regulations (CCR) and the California Integrated Waste Management Board (CIWMB). Pursuant to Section 17683, Title 14 of CCR under the performance standards, the trash at sanitary landfills is compacted and covered daily with a 6 inch thick layer of soil on the top, sides and active face of the trash lift. Besides other uses, the daily soil layer is used to deter the attraction and propagation of flies, rodents and other vectors of diseases at these facilities. To offset the cost of soil and soil hauling to the site, many landfills in southern California have been faced with evaluating alternate materials such as green leaf waste, synthetic geo-textile cover, etc., as substitutes to the daily soil cover especially at the active face of the lift. Besides other requirements, the substitute cover must meet the performance standards pursuant to Section 17683 of CCR.

In evaluating the use of a synthetic geotextile cover (referred to as tarp cover) as an alternate daily cover at the California Street Sanitary Landfill in the city of Redlands, the San Bernardino County Vector Control Program (SBCVCP) in an agreement with the city of Redlands Municipal Utilities Department, carried out the monitoring of various vectors observed on the cover. In accordance with the performance standards criteria, this paper presents data on the vector monitoring of the tarp cover used at the above-mentioned landfill during a 12-month period, May 8, 1996 - May 7, 1997.

MATERIALS AND METHODS

A study was conducted comparing vector attractancy of landfill covered with soil or a synthetic

geotextile cover at the California Street Sanitary Landfill in Redlands, California. The study site and vector monitoring procedures used in the study were as follows:

Study Site: The study site was situated in the California Street Landfill located along the Santa Ana River between California and Nevada Streets, City of Redlands, CA. Of the total 155 acre city-owned property, 65 acres were used for refuse disposal with 43 acres being used as active refuse disposal and fill site. The entire site had an active gas collection system. To the south of the active disposal site, there was a low lying excavated area which under rainy conditions might collect run-off water leading to potential mosquito breeding. This area was regularly monitored for water ponding and mosquito breeding.

The landfill operation was based on the area-fill method where daily refuse was placed in 7-10 feet thick lifts with maximum perimeter slopes of 3:1 (base to height). Pursuant to Title 14, CCR, a minimum of 6 inch thick cover soil was placed daily on the top and sides of each advancing lift. Refuse and cover soil were compacted and graded to drain run-off water from rain. More detailed information on the California Street Landfill, City of Redlands, can be found in the report of disposal site information (Kleinfelder 1994).

Survey Methods: The monitoring methods of various vectors used in these studies were in accordance with the performance standards in Section 17683, Title 14, CCR. The vectors monitored in these studies included domestic muscoid flies, domestic rats, field rodents, mosquitoes and other vectors such as cockroaches, wasps, etc.

A. Flies:

Flies under surveillance in these studies included members of the families Anthomyiidae, Muscidae, Calliphoridae, Sarcophagidae and Drosophilidae.

Prior to fly grill observations, weather data such as temperature, relative humidity, wind velocity and sky conditions were recorded. Temperature and relative humidity were measured using a VWR digital Humidity/Temperature Meter (model #35519-043, Control Company, Friendswood, Texas) and wind velocity was read from an anemometer (Davis Turbo Meter, Wind Speed Indicator, David Instruments, Hayward, California).

Weekly fly grill surveys were carried out during the afternoon hours when the cover was available at the active site. The grill used in these surveys was constructed at SBCVCP with construction similar to the typical Scudder fly grill (Scudder 1949); it consisted of 24 slats each measuring 36 x 0.75 x 0.25 in., and placed 0.75 in. apart on a Z-shaped frame. Each observation consisted of placing the grill at one spot over the cover for 4 minutes and then during the next approximately 30 seconds all flies landing on the grill were counted. A fly landing repeatedly on the grill was counted only once. Using different spots on the cover, ten fly grill counts were made. Of the 10 counts, five with the highest counts were averaged to obtain the mean value for the survey. For each grill count at the tarp cover, fly data on grills placed on the soil cover 15 to 20 feet away from the tarp cover served as a control for comparison.

During weekly surveys for flies, visual observations on other vectors such as cockroaches, wasps, etc. were recorded along with information on other animals especially birds and mammals, if any, found at the active site. Moreover, mosquito breeding, if any, at the low-lying southern area of the landfill was monitored during these surveys.

For monthly fly composition studies, six 22.4 x 1.6 in. sticky tapes (Aeroxon FlyCatcher™ Roxide International, Inc., New Rochelle, New York) were hung near the active site. These tapes were picked up after one week. Due to windy conditions and dust interfering with the efficacy of sticky tapes, monthly four sweeps of on-wing flies at the cover were also carried out using standard insect sweep net. Flies collected on sticky tapes or in sweep nets and killed in an insect killing jar, were brought to the laboratory. All specimens were identified to family, genus and species using both descriptive and pictorial keys by Ecke (1963), Pratt et al. (1976) and Axtell (1986).

B. Rodents:

To monitor rodent activity at the landfill, two trap lines each consisting of twenty traps were operated overnight almost every month during 1996-97. Each trap line in turn had equal number of both National and Sherman type live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin). Traps baited with rolled oats were set up approximately 20 ft. apart. Of the

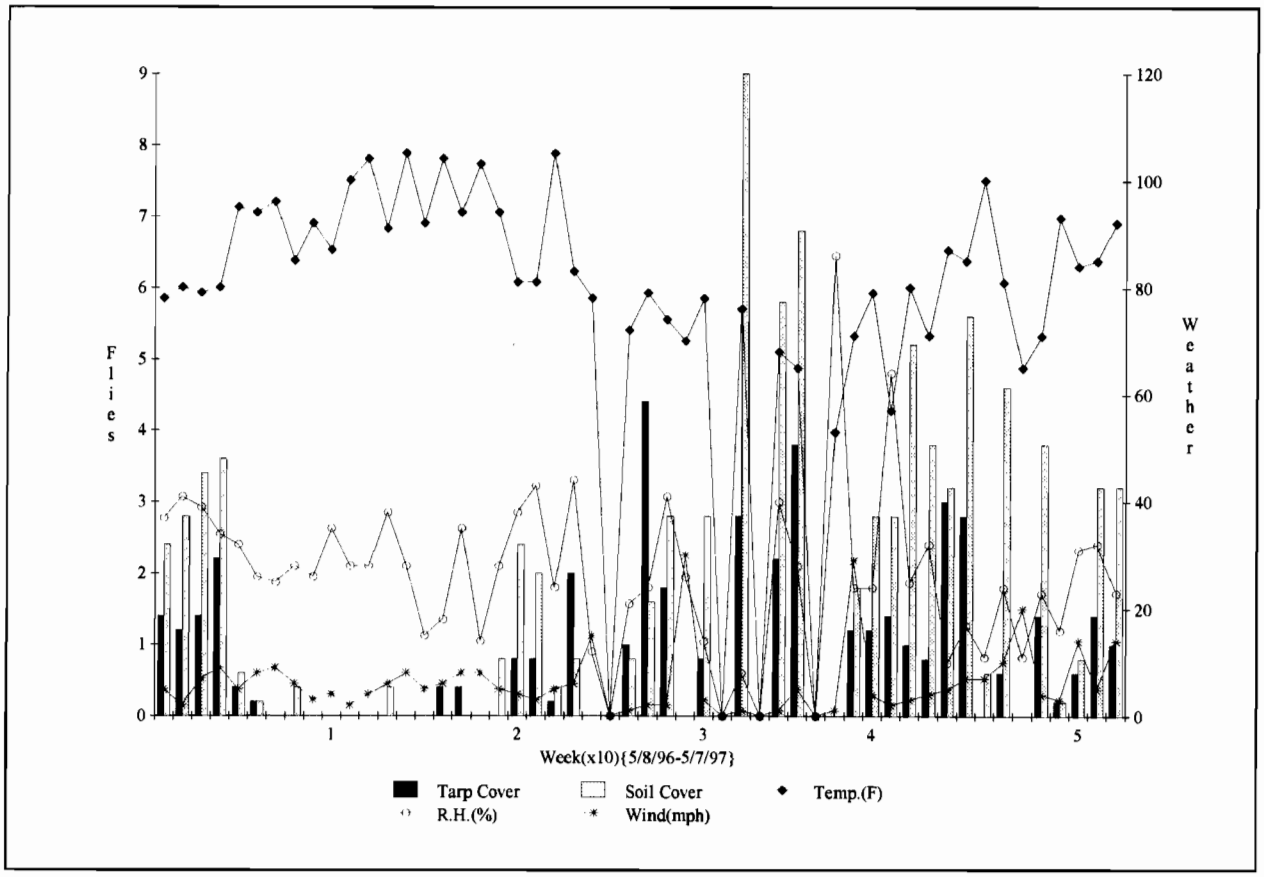

Figure 1. Weekly comparison of flies attracted to landfill covered with soil cover or tarp along with weather data at the California Street Landfill, Redlands, during 1996-97.

two trap lines, one was operated close to the active surface and the other was run on the periphery in a suitable location at the landfill. During the trapping activity, visual observations of field rodents or other animals were also recorded. Identification of collected rodents was accomplished using available identification keys (Burt and Grossenheider, 1976; Zeiner et al. 1990).

Last but not least, proper safety precautions were duly taken by the SBCVCP staff during all vector monitoring activities at the landfill. The staff always wore a hard hat and orange vest while at the landfill. Moreover, during monthly rodent surveys, the survey personnel followed all necessary precautions by wearing a Tyvek suit and proper respirator (battery powered air pressure type with HEPA 10 filter) to guard against Hantavirus or other air-borne pathogens carried by wild rodents.

RESULTS AND DISCUSSION

The data on mean fly counts varied significantly from week to week during the study period (Table 1). These variations were caused by changing weather parameters such as temperatures, humidity, and wind (Fig. 1).

The effect of temperature and wind at certain times was clearly evident. A temperature range of 85° to 100°F, combined with RH $\leq$25% and wind speeds of 8-10 miles per hour or higher during June through October, kept fly numbers at substantially low levels. During the cold months, November through March, at optimum temperatures (upper 70°s F), and RH (25-40%), wind had a significantly noticeable effect in reducing fly activity at both covers. This is evident from zero fly numbers at weeks #24 (10/23/96), #29 (11/26/96), and #47

Table 1. Mean [a/] weekly fly grill data taken at tarp (synthetic geotextile cover) and soil cover at the California Street Landfill, Redlands, CA during 1996-97.

Date	Week Number	Mean number of flies at Tarp	Soil Cover	Date	Week Number	Mean number of flies at Tarp	Soil Cover
05/08/1996	1	1.4 bcde	2.4 fghi	11/06/1996	27	1.0 abcd	1.2 abcd
05/15/1996	2	1.2 abcd	2.8 fgjij	11/13/1996	28	4.4 klm	1.6 cde
05/22/1996	3	1.4 bcde	3.4 ijk	11/20/1996	29	1.8 cdef	2.8 fghij
05/29/1996	4	2.2 efgh	3.6 ijk	11/26/1996	30	No tarp due to windy conditions	
06/05/1996	5	0.4 ab	0.6 ab	12/04/1996	31	0.8 abc	2.8 fghij
06/12/1996	6	0 a	0 a	12/11/1996	32	No data were taken	
06/19/1996	7	0 a	0 a	12/18/1996	33	2.8 fghij	9.0 p
06/26/1996	8	0 a	0 a	12/25/1996	34	No data were taken	
07/03/1996	9	No tarp due to holiday		12/30/1996	35	2.2 efgh	5.8 n
07/10/1996	10	0 a	0 a	01/09/1997	36	3.8 ijkl	6.8 n
07/17/1996	11	0 a	0 a	01/15/1997	37	No data were taken due to rain	
07/24/1996	12	0 a	0 a	01/22/1997	38	No data were taken due to rain	
07/31/1996	13	0 a	0 a	01/29/1997	39	1.2 abcd	1.8 cdef
08/07/1996	14	0 a	0.4 ab	02/05/1997	40	1.2 abcd	2.8 fghij
08/14/1996	15	0 a	0 a	02/12/1997	41	1.4 bcde	2.8 fghij
08/21/1996	16	0 a	0 a	02/19/1997	42	1.0 abcd	5.4 lmn
08/28/1996	17	0.4 ab	0 a	02/26/1997	43	0.8 abc	3.8 ijkl
09/04/1996	18	0.4 ab	0 a	03/05/1997	44	3.0 ghij	3.2 hijk
09/11/1996	19	0 a	0 a	03/12/1997	45	2.8 fghij	5.6 mn
09/18/1996	20	0 a	0.8 abc	03/19/1997	46	0 d	0.6 ab
09/25/1996	21	0.8 abc	2.4 fghi	03/26/1997	47	0.6 ab	4.6 klm
10/02/1996	22	0.8 abc	1.6 cde	04/02/1997	48	No tarp due to windy conditions	
10/09/1996	23	0.2 ab	0.4 ab	04/09/1997	49	1.4 bcde	4.4 klm
10/16/1996	24	2.0 defg	0.8 abc	04/16/1997	50	0.2 ab	0.8 abc
10/23/1996	25	No tarp due to windy conditions		04/23/1997	51	0.6 ab	0.8 abc
10/30/1996	26	No tarp due to rain		04/30/1997	52	1.0 abc	3.2 hijk
				05/07/1997	53	1.0 abc	3.2 hijk

a/ Mean of five fly grill counts.

b/ Means followed by the same letter(s) of are not significantly different from one another at P~0.05 (Multiple range test, Duncan 1955). Due to zero values in the data the analysis of variance was carried out on transformed data using the square root ($\sqrt{x+1}$) transformation method.

(4/2/97). Three high fly counts between 3 and 5 at the tarp cover occurred at weeks #27 (11/13/96), #35 (1/9/97) and #43 (3/5/97). Similarly, high fly counts at the soil cover were found at weeks #32 (12/18/96), #34 (12/30/96), #35 (1/9/97), #42 (2/26/97), and #44 (3/12/97). Rain also affected fly activity at weeks #25 (10/30/96), #36 (1/15/97), and #37 (1/27/97). In both cases of covers, temperature, wind, and rain clearly regulated fly activity at the site.

As a whole the data clearly show that throughout the study period the mean fly grill counts at the tarp cover remained well below the threshold number of 6 flies per grill count as described in the Performance Standards, Section 17683 of CCR. Moreover, fly counts at the tarp cover were comparatively lower than those taken at the soil cover maintained as a control, thus making the former evidently superior to the latter in terms of lower fly counts. Unlike the

former, the soil cover on two occasions even showed fly counts higher than the threshold number.

In monthly fly samples collected on sticky tapes or in sweep nets, the faunal composition included the pomace fly, *Drosophila* sp. (Drosophilidae), the common house fly, *Musca domestica* L. (Muscidae), false stable fly, *Muscina stabulans* (Fallen) (Muscidae), little house fly, *Fannia canicularis* L. (Muscidae), and green blowfly, *Phaenicia sericata* (Meigan) (Calliphoridae). In relative abundance these flies in descending order could be arranged as 246 *Drosophila* sp., 105 *M. domestica*, 16 *M. stabulans*, 8 *P. sericata*, and 7 *F. canicularis*. Moreover, the seasonal abundance of these flies was similar to fly distribution reported in earlier studies in the area (Mian 1994).

Monitoring of other insect vectors such as mosquitoes, cockroaches, bees, wasps, etc., was also carried out during the weekly samplings. No other insect vectors except for a few bees landing on the tarp were observed during the period.

The data on monthly rodent surveys showed no domestic rats belonging to the genus *Rattus*. During 10 trapping episodes involving 400 traps, only 18 rodents were collected. Of these 16 (88.9%) were deer mice, *Peromyscus maniculatus* (Cricetidae), a known vector of the causative agent of Hantavirus pulmonary syndrome (HPS). The other two rodents included a house mouse, *Mus musculus* and a ground squirrel, *Spermophilus beecheyi* (Sciuridae), a competent reservoir of sylvatic or rural plague.

In light of the foregoing discussion on the vector monitoring of the tarp cover as an alternate daily cover on the active surface, it is quite evident that the tarp cover does not pose any significant health risk with respect to the attraction and propagation of disease vectors such as filth breeding flies and domestic rats. It also needs to be emphasized that the daily time period (late afternoon through next morning 7:00 a.m.) during which the tarp cover will remain on the active surface minimizes the chances of fly activity to be noticeably significant since flies are active during the daytime (diurnal).

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