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SPECIAL RECOGNITION ADDRESS

THE LIFE AND ACHIEVEMENTS OF PROFESSOR WILLIAM BRODBECK HERMS

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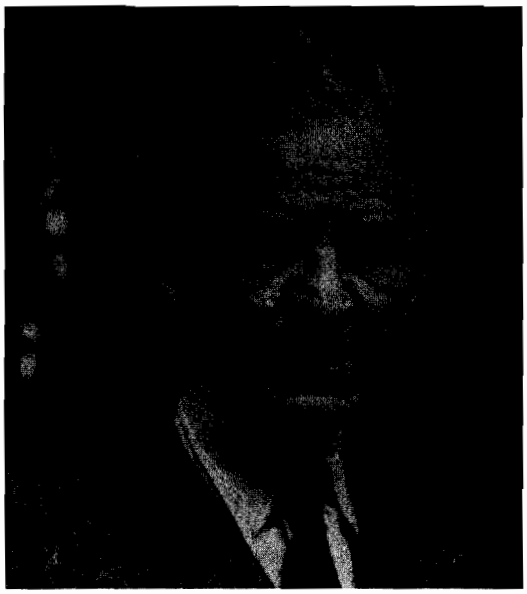

The invitation to present "The Life and Achievements of Professor William Brodbeck Herms" gave me an enjoyable opportunity to review his 136 publications (see Appendix to this article) and many volumes of newspaper clippings, pictures, and diaries that are in the Bancroft Library at Berkeley and the Archives of the California Mosquito and Vector Control Association at the Delta Vector Control District in Visalia.

I first met Professor Herms when I was a student in the 1930s and became one of his disciples. It is appropriate that this presentation is made at a joint meeting of the American and California Mosquito Control Associations as Herms presented his last scientific paper at the first joint meeting of these two organizations in Berkeley in 1949. His topic was "Looking Back Half a Century for Guidance in Planning and Conducting Mosquito Control Operations." To read this paper is an education in the principles that governed Herms' career. At that meeting an oil portrait of Professor Herms was presented to the Department of Entomology to be hung in Agriculture Hall (Gray 1949). The painting portrays him lecturing on malaria and its control.

I want to present Professor Herms to you as a scientist, educator and leading citizen of his community. He was born in Portsmouth on Ohio, September 22, 1876. After high school he worked for several years on farms and as an accountant. He also contracted malaria which persisted for six years and this he declared was the genesis of his interest in that disease. He entered nearby Baldwin-Wallace College and graduated with a BA degree in 1902. He won graduate fellowships at Western Reserve, Ohio State, and Harvard. He completed the MA degree at Ohio State in 1906 and was Acting Head of the Department of Zoology at Ohio Wesleyan College in 1906-1907. He attended Harvard from 1907-1908 as an Austin Fellow. While he did not complete a doctoral degree he received a solid education in biology and a pre-

medical curriculum. He was interested in becoming a medical missionary in China where he hoped to cure malaria cases with quinine. However, he returned to Ohio, married Lillie Magly and briefly taught accounting and biology at Baldwin-Wallace College. In 1908, he was offered an Assistant Professorship in Entomology at Berkeley at a salary of $1,500 per year. His former professors at Harvard advised him not to accept as the University of California was seen as a dead end academically and the population of California was uncultured. Fortunately, Herms rationalized that California was much closer to China than Ohio and Massachusetts and he might still become a missionary selling malaria and mosquito control in California. On arrival he found the Entomology Department was in an old-decrepit frame building. He and his wife found no reason to unpack their bathing suits as the sun wasn't shining through the Berkeley fog and the Pacific Ocean was very cold.

He was introduced to a program to control salt marsh mosquitoes initiated by Professors H.J. Quayle and C.W. Woodworth (Quayle 1906). However, he did not find this exciting as those mosquitoes were not carrying a disease. He was then assigned to join the first agricultural extension staff that was touring California from its southern to northern borders. The Southern Pacific Railroad provided a train and Herms was given half a railroad car to set up a demonstration and distribute information on medical entomology, parasitology, and health concerns on the farm. This experience quickly oriented him to the varied ecology, populations, and problems in California. It is interesting that Harold F. Gray, a student, was Herms' assistant on that train. This began a close relationship that continued for 40 years.

MALARIA CONTROL

Herms' studies on malaria began in 1909 and were the first demonstration in the United States that a community based project to abate malaria was feasible. This project was originated just 12 years after Ronald Ross announced that *Anopheles* mosquitoes were the vectors for malaria. It was the same year, 1909, that General William Gorgas announced his successful campaign against yellow fever and malaria in Panama.

In 1909, the California Board of Health documented 112 deaths and 6,000 cases of malaria in the state. There were many assessments of the problem in that time period (Gray and Fontaine 1959). An almost unbelievable sequence of events developed in the winter of 1909. A request came to the Department of Entomology from a citizen of Penryn, California, for assistance in combating malaria. Penryn, a small community in the foothills of the Sierra Nevada Mountains, had a real malaria problem. It took no time for Herms to arrive in Penryn. He had been looking for a problem to get his teeth into. On his first visit he organized a community educational program on malaria and mosquitoes, on his second visit he outlined a plan directed specifically at control of *Anopheles* mosquitoes in an eight square mile area surrounding the town. He wanted to extend the program to other towns in the region but encountered resistance. Many people, including some physicians and health officers, still believed malaria was caused by bad water and miasmas. There was opposition from real estate developers, chambers of commerce, and newspaper editors who didn't want publicity on a disease as it would be adverse to development of the region. As an example, a hand bill was distributed in Placer County in 1910 that said "Don't go to hear that Professor from Berkeley. Every time you stub your toe on a gold nugget it don't mean you will get malaria." Stanley B. Freeborn, a later member of the team, reported he was threatened to be ridden out of town on a rail or worse, tarred and feathered, if he didn't leave. In spite of such opposition, once control was started in Penryn, the pressure was off.

Actions that stand out in the Penryn campaign were that the Penryn Fruit Company provided a budget of $715 and space for a field laboratory, the Southern Pacific Railroad carried out permanent control measures along their right-of-way which went through town, and much volunteer labor was forthcoming. An intense educational program was directed at school children regarding elimination of mosquito sources. The initial program was directed at *Anopheles* mosquitoes and the permanent removal of breeding sites by drainage or filling, screening of houses and public buildings, and use of oil only when necessary. A community clean-up of junk that was a source of pest mosquitoes was added for comfort and to give both children and adults a project to do. Data were collected that documented a dramatic 45% decrease in school absenteeism from illness and a decrease in the malaria cases seen by physicians.

In the following year, a campaign began in Oroville. Again, the first step was educational. The first funds were raised by "Tag Day". A 10 cent contribution bought a tag. The project raised $600 to start the control program. This was the first "March of Dimes", preceding by 30 years the Franklin Roosevelt National Foundation for Infantile Paralysis program that raised millions of dollars for research and control of that disease.

The movement for malaria control quickly spread as Herms travelled the state with missionary zeal to initiate programs in Los Molinas and Bakersfield. Elated by this success Herms took a leave of absence in 1911 and

toured the southern United States, England, Germany, France, and Italy to visit the sites of original malaria research. He reported his studies at an international conference in Germany. On this tour he was not only known as Professor Herms but also as "Officer in Charge of Malaria Investigations for the California State Board of Health" - a title that impressed audiences.

In 1913, Herms published his first book "Malaria Cause and Control". This 163 page volume presented his first two projects and became the "Bible" for development of malaria control programs in California.

In 1906, New Jersey had enacted legislation that provided a mechanism for development of a state program to control salt marsh mosquitoes. Herms recognized the need for similar actions if mosquitoes and malaria were to be controlled in California. He drafted the Guill Bill in 1911 which was passed by both houses of the State Legislature but was vetoed by the Governor who was under pressure to do so by the real estate lobby. The second effort in 1915 was Assembly Bill 1565 which sailed through and was strongly supported by the real estate lobby which had become educated by Herms.

In 1915, the State Board of Health requested Herms to develop a state-wide survey of malaria using blood smears as a case finding method and to push for required reporting of malaria by physicians. Equally, and I believe most important, the Board requested that Herms organize a state-wide survey of the mosquitoes of California. Herms, assisted by Stanley B. Freeborn, organized the project so that it began in the summer of 1916. They surveyed 34 counties in northern and central California. This entailed a 95 day trip and 6,446 miles of travel. In those days, California was not covered by a network of freeways and pavements and most of the travel was on dirt roads or no roads. The total budget from the State Board of Health for the first summer was $2,145 which included no salaries. They purchased a Chevrolet Baby Grand touring car for $850 and it was a wreck by the end of the summer. The State Controller complained that a car should last at least two years but he had never been on a field trip with Herms and Freeborn. Bills were submitted for repairs including new springs, tires, brakes, batteries, radiator hoses, and a new jack. The budget provided 180 man days of per diem, $1 for a hotel and $2 for meals. Gasoline was 19 cents a gallon.

In 1917, the southern area of the state was surveyed. The two year project covered almost every county and from sea level to 8,000 foot elevation. The objective was to survey for *Anopheles* but in fact covered all species of mosquitoes, and the collection was the base for Freeborn's (1926) "The Mosquitoes of California".

THE CALIFORNIA MOSQUITO CONTROL ASSOCIATION

Herms was determined that the embryonic mosquito control program should be developed into a networking program that would cover most of California. He organized the first Mosquito Control Conference in Berkeley in 1920. His purpose was to develop a California Mosquito Control Association (CMCA). A small group of around 15 people met for two days in Berkeley, attended a laboratory on mosquito identification, discussed problems they had experienced, and attended a "round table smoker" that Freeborn organized. A smoker was when everyone went into a room in the evening, lit up their pipes, cigars and cigarettes, and got to know each other for better or worse, a sort of cancer promoting act that is unlikely to occur at meetings like these today. No follow-up meeting was held until 1930 when Herms and Gray organized a second conference attended by 28 persons. Formal action was taken to form the CMCA, officers were elected and annual meetings were planned. Today is the 64th such conference.

In Herms' introductory talk he clearly stated the objectives would be to provide technical training for mosquito control personnel by presenting laboratories on mosquito identification, to demonstrate new equipment or methods of control and to encourage interaction of workers in this field.

In November, 1933, Dr. F.C. Bishop of the United States Department of Agriculture wired Professor Herms that money in the Federal Civil Works Administration budget could be used to hire unemployed men for mosquito control projects. This was a critical time period for mosquito control programs in California as the country was experiencing a major depression. Herms took the initiative and responded with a detailed plan for action that would cost $415,000 over a three month period. The project would have a supervising staff from mosquito control agencies and the State Department of Health. The program was begun but was curtailed after eight weeks when Herms found support for a six week continuation from the California Civil Works Administration. Within this 14 week period 740 laborers were hired and provided shovels, boots, and transportation and 46 projects were completed largely by sweat, shovels, and brush hooks. Projects in 20 mosquito control districts had completed 57 miles of drainage ditches, 200,000 linear feet of stream banks were cleared of brush and thousands of *Gambusia* had been planted. The total cost was $87,299. Herms had provided a real "shot in the arm" for mosquito control in the middle of the depression.

I will not review further the development of the CMCA. The proceedings of its meetings are interesting reading and in the first 19 conferences Herms made 56 presentations.

After Professor Herms' death on February 9, 1949 he no longer was at CMCA meetings to act as a catalyst but two of his former students, Harold F. Gray and Richard F. Peters, emerged as his disciples. Their presence assured that the organization would continue and thrive. We can thank them. If Professor Herms was here today and faced this audience, he would feel rewarded and have tears in his eyes. His dream of a network of mosquito and vector control is realized. In 1930, 28 people representing 13 small districts met in Berkeley and formed an Association. Today the California Mosquito and Vector Control Association (CMVCA) is a network that represents 52 districts, a population of 24,000,000 people and covers 56,000 sq. miles of California.

PUBLICATIONS

The bibliography of 136 publications of Professor Herms, that I have organized, will be published in the proceedings of this meeting (see Appendix following this article). I learned a great deal in compiling this. As expected, 49 papers were on mosquitoes and mosquito-borne diseases but the majority were on other topics. As early as 1910 he became an "Agent and Consultant in Parasitology" to the California State Board of Health. This provided him an unusual outlet for publications in the Bulletin of the State Board of Health. His early interest in flies was expressed in 25 bulletins on their biology and control. One of his first papers in 1913 was on an unsuccessful attempt to transmit poliomyelitis virus to monkeys by the stable fly. His co-author was Dr. Wilbur A. Sawyer the State Health Officer who later became Director of the International Health Division of the Rockefeller Foundation. Also, a note was published in the Bulletin that stated "Contributions for a new pipe for Professor Herms will be gratefully received. Of all the pipes that ever reached the paradoxical stage of strong and vigorous old age and absolute decrepitude, his old incinerator is the worst. If it were not for adhesive tape it wouldn't hold together, and as for odor, even his mosquitoes couldn't stand it." This must have been a statement of true and unusual respect by a colleague.

Herms wrote six bulletins on the construction of septic tanks and control of typhoid fever on the farm. He published five papers on ticks and the newly discovered relapsing fever in the high mountains of California. His student Charles M. Wheeler (1935) described the tick vector *Ornithodoros hermsi*. Some 20 papers were on insect pests of agricultural crops. He wrote on insect pests of coconuts based on a trip by sailboat to Fanning and Washington Islands in the 1920s. It took longer to get to the islands and back than the time for the study. This was his first and last research in a tropical setting. I refer elsewhere to the books he published on malaria and medical entomology. His final major book "Mosquito Control - Practical Methods for Abatement of Disease Vectors and Pests", was co-authored with his close associate H.F. Gray in 1940 and a second edition in 1944. This book brought together the principles and practices of mosquito control in the pre-insecticide era. DDT and other synthetic insecticides are not even indexed in the first edition and received only two pages in the second edition. Every mosquito control district should have a copy of this book on the manager's desk as it stresses the methods of source reduction and biological control that are increasingly applicable today. It is out of print so I might sell my copies to the highest bidder.

TEACHING

I would like now to turn to Professor Herms the teacher. When he came to Berkeley in 1909 he immediately developed courses in veterinary and medical entomology. He also gave courses in veterinary parasitology at the Veterinary College at the San Francisco Campus of the University. In 1912, his title was changed to Assistant Professor of Parasitology and his annual salary was raised from $1,500 to $2,100. This new title made him the first person in the United States to hold an academic title in parasitology.

By 1911, Herms was offering nine undergraduate courses and several graduate courses. In 1915, he published the first edition of his text book "Medical and Veterinary Entomology." World War I interrupted his whirlwind of teaching as he took a commission in the U.S. Army Sanitary Corps for the period 1918-1920. He was stationed at Fort Sam Houston, Texas, and Newport News, Virginia, and carried out malaria control and environmental sanitation programs.

On return to Berkeley in 1920, at the end of the war, he was made head of a new combined Division of Entomology and Parasitology and his salary doubled to $4,000 per year. As you would expect he continued to carry a very heavy teaching and administrative load for the remainder of his career. In 1923, he published the 2nd edition of "Medical and Veterinary Entomology."

Herms had always gone by the respectful title Professor as he had not completed a doctoral degree at Harvard. In 1935, his alma mater Baldwin-Wallace

College, in collaboration with Western Reserve University conferred an honorary ScD degree upon him in recognition of his distinguished career in teaching and research. Regardless of the new degree, he remained Professor Herms to all his associates as that title fit him best. The one exception was Harold Gray, an associate of over 40 years, who on occasion would call him "Billy."

In 1942, Professor Herms interrupted his academic career for a second time when he accepted a commission as Lt. Colonel in the U.S. Army. It was unusual for a person 67 years old to do this. Some people accused him of doing it to escape his duties as Chairman of Draft Board No. 7 for the Berkeley region. I believe he did it as a patriot and that he wanted to join over 40 of his former graduate students who were in uniform at that time. He also felt challenged by the assignment to Carlisle Barracks in Pennsylvania, where he had the responsibility to train thousands of physicians, being inducted into the army, in the principles of environmental sanitation, medical entomology and parasitology, hardly subjects that had been treated as important in their prior medical education. It should be mentioned that Professor Herms was elected as President of the Entomological Society of America and the American Association of Economic Entomologists. This was a unique distinction and recognition of the breadth of his professional accomplishments.

My first recall of Herms as a teacher was as a student in his medical entomology class in 1938. The third edition of his book "Medical Entomology" was the text but the majority of his lectures did not follow the text - after all, you could read that. Instead, he spent time on anecdotes of personal experiences that illustrated practical approaches to problems in medical entomology or on recent reports in the literature. He also frequently inserted humor. In his first lecture he reported that an acquaintance had just sent him a reprint naming a louse parasitic on goats after him. The handwritten note that accompanied the reprint said naming the louse was not to be considered a compliment because "I always think of you as an old goat harboring this louse." Obviously the lesson was don't take yourself too seriously if someone names a bug after you. Shortly thereafter, a colleague named a scarab beetle I had collected *Phylophaga reevesi.* I had collected a pair that were mating in Death Valley. Herms appreciated it when I told him that a pair of dung beetles I had caught screwing around in Death Valley had been named for me. He said "I like your attitude."

To characterize Herms as a teacher - he was scholarly, friendly, approachable, and infinitely wise. His home was yours for evening seminars. His classes and the required curriculum were demanding. He attracted many excellent students. Dr. T.H.G. Aitken, who received the Belkin Award today, was Herms' last PhD candidate. Many big names in malaria and mosquito control came to Berkeley to see Herms - L.L. Williams, L.W. Hackett, J.M. Andrews, F.C. Bishop, E. F. Knipling, G.H. Bradley and H.H. Stage to name a few. As a student you met them and discussed your research.

When Herms retired Dr. Deane P. Furman, also a former student, replaced him and upheld Herms' high standards for the program in medical entomology and parasitology at Berkeley.

MALARIA TODAY

It should be of interest to this audience that several outbreaks of malaria have occurred in the mid 1980s in San Diego County just north of here (Maldonado et al. 1990). The cases were largely in hispanic migrant workers and residents of nearby affluent residential areas. The principle risk factors were sleeping outdoors, adjacent to fresh water lagoons where a new species of *Anopheles* mosquito was breeding. At almost this same time, Barr and Guptavanij (1988) were naming this new species *Anopheles* hermsi. Fritz and Washino (1993) have currently presented evidence this species also was the vector of malaria epidemics in New Mexico from 1926 to 1932. Professor Herms would have liked this series of events.

HERMS THE CITIZEN

I referred earlier to Professor Herms' dedication to the development and welfare of Berkeley and surrounding communities. In quick review, he was a primary mover for formation of the Alameda County Mosquito Abatement District in 1930. He circulated petitions for formation of the District and talked to every service club in the area. He then became the first Chairman and a member of the Board of Trustees of that District for almost 19 years. He was elected as Chairman of the Berkeley Board of Education for 8 years. He initiated and was Chairman of the Berkeley-Contra Costa Area Council of the Boy Scouts of America as well as counselor of Boy Scouts for the western states and Hawaii. He received the Silver Beaver award for his services to the Boy Scouts. He was consultant to the State Board of Health for 40 years, President of the California State Automobile Association, a member of seven community service clubs and Chairman of Berkeley Draft Board No. 7 in the early 1940s. Berkeley City acknowledged he was its outstanding citizen with the

Benjamin Ide Wheeler Service Award in 1937. In 1939, the Boy Scout Council renamed the Berkeley Boy Scout Camp as Camp Herms. This 22 acre facility, which he envisioned, has been used annually by 10,000 to 50,000 scout campers and other visitors. The original investment for the property was $8,000. The camp area is heavily wooded and nestled in an affluent residential area of Berkeley. For a short period, 1952 to 1959, the California Mosquito Control Association gave an annual "Herms Award" to send a needy scout to summer camp.

In conclusion, I hope you have enjoyed my introduction of Professor Herms. He was a unique man, a catalyst for action on any subject he adopted as worthy. We were fortunate he came to California and made history for us in the first half of this century.

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ARTICLE APPENDIX

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SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1993

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This brief report summarizes arboviral surveillance activities during 1993 and is the 24th report to the California Mosquito and Vector Control Association since 1969. The surveillance program involves cooperative efforts by many groups and individuals from local mosquito control agencies; the Arbovirus Research Program at the University of California at Berkeley; the California Mosquito and Vector Control Association (CMVCA) ; the CMVCA Research Foundation; county and local public health departments; the California Department of Food and Agriculture; physicians and veterinarians throughout California; and the Division of Communicable Disease Control, including the Vector Borne Disease Control Section, the Veterinary Public Health Section, the Disease Investigation Section, and the Viral and Rickettsial Disease Laboratory (VRDL) of the California Department of Health Services.

Announcements about the program and 30 weekly bulletins were distributed widely by FAX or mail during the season to provide detailed surveillance data. In addition to the weekly bulletins, positive findings were telephoned immediately to the agency which submitted the mosquito pools or sentinel chicken sera.

Following the recognition of early WEE viral activity in Sacramento County in mid to late June, a major effort was made to alert the medical community to watch for suspect cases of aseptic meningitis and encephalitis. Over 200 suspect cases were tested at the VRDL, and an unknown further number by other laboratories, revealing three SLE cases: (1) a 64-year old male resident of San Bernardino City, San Bernardino County, who became ill 8/12/93, was hospitalized from 8/15-8/23, and recovered completely. His only known recent travel had been within 20-30 miles of his home; (2) a 19-year old male resident of San Diego County, who became ill 9/11/93 with fever, headache, nausea, stiff neck and transient diplopia, but recovered completely. On 8/31, he had travelled to Imperial County and fished in the West Main Canal, the most likely source of mosquito exposure; (3) a third case, reported late in the season since the serologic test results were atypical and special extra testing was required, was a 30-year old male resident of Orange County at the time of illness onset 9/24/94. He was hospitalized three days but recovered completely. Probable place of exposure was San Bernardino County along the Colorado River south of Needles.

Extensive enzootic WEE virus activity was detected in sentinel chicken flocks and mosquito pools, especially in the Sacramento Valley northward. One fatal case of WEE occurred in a four month old quarter horse filly from Red Bluff, Tehama County, 8/21/93; and WEE infection was documented in a relatively new and exotic species in California, the emu, which is being raised commercially and is quite susceptible to the WEE virus.

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Despite special efforts to detect human cases of WEE, including tests on 87 patients with symptoms or signs suggestive of encephalitis or meningitis in the Sacramento Valley during 1993, no cases or even significant levels of WEE antibody were found.

The routine surveillance program utilizing sentinel chickens and mosquito collections for virus isolation has again proven to be a useful and successful means of providing early alerts of virus activity in various regions of the State and to help focus mosquito control efforts in the most critical areas. The 157 flocks of sentinel chickens were bled every two weeks, using a new filter paper blood collection technique, yielding over 17,000 blood samples for testing. Of these 91 were positive for SLE antibodies (Table 1) and 281 for WEE antibodies (Table 2). The dried blood samples were mailed at ambient temperature, saving significantly on dry ice and shipping costs. Of 3,800 mosquito pools tested at the VRDL, 168 were positive for WEE virus, and 5 were found positive for SLE virus (Tables 3 and 4). In addition, 595 pools were tested by the Arbovirus Research Laboratory (ARL), University of California, Berkeley, from the Coachella Valley and Imperial Valley study areas, yielding 12 WEE and 15 SLE isolates (Table 5). The filter paper collection method for serum testing and streamlined laboratory methods for virus isolation and identification have helped to reduce the costs and extend the surveillance network for this program.

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Table 1. SLE seropositive chickens during 1993 by location and semimonthly interval.

			End Date of Interval												
			June		July		Aug		Sept		Oct		Nov	
County	Location	City	15	30	15	30	15	30	15	30	15	30	15	Total
CHURCHILL	NORTH BROADWAY	FALLON											1	1
IMPERIAL	1477 ROSS ROAD	BARD					2			10				12
IMPERIAL	ARNIAZ	SEELEY							1		2	1		4
IMPERIAL	CHRISTOPHER	EL CENTRO		1				1			5	1		8
IMPERIAL	DOG POUND	CALEXICO									3			3
IMPERIAL	RIO BEND RV PARK	SEELEY						4	2		2			8
IMPERIAL	ROSARIO	WESTMORELAND						1			3			4
IMPERIAL	SALTON SEA NWR	SALTON SEA NWR									1	1		2
IMPERIAL	WISTER WR	WISTER WR							1	1	3	1		6
LOS ANGELES	BERNARD BIO STATION	CLAREMONT								1				1
LOS ANGELES	CAL POLY UNIVERSITY	POMONA						2						2
LOS ANGELES	CAL. COUNTRY CLUB	INDUSTRY								1		1		2
LOS ANGELES	HARBOR-TERM. ISLAND	LONG BEACH								1	1			2
LOS ANGELES	MONTEREY PARK CITY	MONTEREY PARK						3						3
LOS ANGELES	SANTA FE REC. PARK	IRWINDALE								4				4
LOS ANGELES	SEPULVEDA BASIN	ENCINO		2	2			1	2		2			9
RIVERSIDE	RANCHO JURUPA PARK	RIVERSIDE								1				1
RIVERSIDE	SALTON SEA STATE PK.	NORTH SHORE						1		1	1			3
SAN BERNARDINO	HORSE RANCH	COLTON								2	2			4
SAN BERNARDINO	PUMP STATION	CHINO HILLS				1	1							2
SAN BERNARDINO	SEWAGE TREAT. PLANT	NEEDLES						7		3				10
		Totals		3	2	1	3	20	6	25	25	5	1	91

Table 2. WEE seropositive chickens during 1993 by location and semimonthly interval.

County	Location	City	End Date of Interval												Total
			June		July		Aug		Sept		Oct		Nov		
			15	30	15	30	15	30	15	30	15	30	15		
BUTTE	ELMER STREET	CHICO				3									3
BUTTE	GREY LODGE	GRIDLEY				3	3	5							11
BUTTE	HONCUT ROAD	HONCUT				5	5	3							13
BUTTE	HIGHWAY # 32	CHICO	1				1		4						6
BUTTE	LORENE COURT	OROVILLE				2									2
BUTTE	RIVER ROAD	CHICO	3			1	1								5
BUTTE	THEBACH RANCH	BIGGS				1	4		2						7
CHURCHILL	NORTH BROADWAY	FALLON								1					1
COLUSA	GRUSSENMEYER RNCH.	COLUSA					5			1					6
CONTRA COSTA	EMERSON DAIRY	OAKLEY						1		2		3			6
FRESNO	FIREBAUGH	FIREBAUGH				1			1						2
GLENN	N.E. WILLOWS	WILLOWS			1	1	2	4	3						11
IMPERIAL	ARNIAZ	SEELEY			7										7
IMPERIAL	CHRISTOPHER	EL CENTRO			3							1			4
IMPERIAL	RIO BEND RV PARK	SEELEY		1	2	1	2			1					7
IMPERIAL	ROSARIO	WESTMORELAND		2	3		2								7
IMPERIAL	SALTON SEA RV NWR	SALTON SEA NWR			1										1
LAKE	MAD FISH POND	UPPER LAKE						3	1	2					6
LOS ANGELES	CHARMLEE PARK	MALIBU											1		1
MADERA	FARIA RANCH	FIREBAUGH							1						1
MADERA	HARMON RANCH	CHOWCHILLA								1					1
MERCED	NEWMAN DUCK CLUB	GUSTINE					1	1		1					3
RIVERSIDE	DEX-O-TEX DUCK CLUB	MECCA							1						1
SACRAMENTO	G. WHITNEY	HOOD				2	1	2	1						6
SACRAMENTO	N. BEACH LAKE	FREEPORT					1		1						2
SACRAMENTO	N. STONE LAKE #1	HOOD				1	2	3	1						7
SACRAMENTO	N. STONE LAKE #2	HOOD					2		2	1					5
SACRAMENTO	N. STONE LAKE #3	HOOD						2		1					3
SACRAMENTO	N. STONELAKE #4	FREEPORT						2	2						4
SACRAMENTO	N. STONE LAKE #5	FREEPORT					4	3		1					8
SACRAMENTO	NATOMAS	SACRAMENTO		2											2
SACRAMENTO	POPPY RIDGE	ELK GROVE						1			1				2
SACRAMENTO	S. RIGG	HOOD					2	4	3						9
SACRAMENTO	S. BEACH LAKE	FREEPORT				1		3	1	1					6
SACRAMENTO	T.JOHNSON	FRANKLIN				3		3	2						8
SAN BERNARDINO	SEWAGE TREAT. PLANT	NEEDLES						3		2					5
SAN JOAQUIN	BACON ISL. - MANTELL	HOLT						5	1						6
SAN JOAQUIN	MIDSECTION ROAD	THORNTON						3	3						6
SHASTA	MAD OFFICE	ANDERSON						1							1
SOLANO	CORDELLA	CORDELLA					2	5	1						8
SOLANO	GRIZZLY ISLAND	SUISUN						3	2	4					9
SONOMA	E. SONOMA	SONOMA						2	1						3
STANISLAUS	SEWAGE PLANT	MODESTO						1							1
STANISLAUS	VALLEY HOME	OAKDALE					1	3	1						5
STANISLAUS	VICTORIAS	CROW'S LANDING					1	1							2
SUTTER	BARKER	RIO OSO					1	4	1						6
SUTTER	DEAN RANCH	SUTTER					4	2	2						8
SUTTER	ROBBINS	ROBBINS		3			2	3							8
SUTTER	SHEPPARD	LIVE OAK				1	3	2							6
TEHAMA	MAD OFFICE	RED BLUFF					1	6							7
TULARE	MAD OFFICE	TULARE									1				1
WASHOE	HEREFORD RANCH	SPARKS							2	1					3
YOLO	MERRITT	WOODLAND					3	4							7
YUBA	ALBERTO'S	MARYSVILLE					1	5	1						7
YUBA	SR. CITIZEN CENTER	OLIVEHURST				2	3	3							8
		Totals	4	8	17	28	60	96	41	20	2	4	1		281

Table 3. Mosquitoes tested in the Viral and Rickettsial Disease Laboratory during 1993.

	Ae. melanimon		*Cx. pipiens*		*Cx. stigmatosoma*		*Cx. tarsalis*		Totals	
Agency	Pools	Mosq.	Pools	Mosq.	Pools	Mosq.	Pools	Mosq.	Pools	Mosq.
ALCO			1	13			6	174	7	187
BUCO			6	257	2	100	16	800	24	1157
CHLV							2	100	2	100
CLSA	2	100					65	2932	67	3032
CNTR	3	144					263	12713	266	12857
DLTA							33	1371	33	1371
FRNO							14	652	14	652
GLEN							16	716	16	716
KERN							186	8630	186	8630
KNGS							2	61	2	61
LAKE	9	409			10	483	100	4763	119	5655
LCHD							3	69	3	69
LONG			303	15270			220	11369	523	26639
LOSA			140	6586	10	295	256	12512	406	19393
MADR			38	1883			38	1900	76	3783
MARN							15	754	15	754
MERC	2	100	2	100			10	374	14	574
NAPA							12	106	12	106
NWST			51	2529	16	782	161	8038	228	11349
ORCO			103	3319			9	305	112	3624
SACR	49	2168			1	40	588	27761	638	29969
SANB							65	2710	65	2710
SAND					1	10	126	5861	127	5871
SANJ	1	12					32	1521	33	1533
SBOV			16	557	8	101	17	434	41	1092
SGVA			43	1528	2	21	15	255	60	1804
SHAS			1	50			44	1784	45	1834
SOUE			67	1693	6	156	151	5965	224	7814
STCL			1	44			23	1150	24	1194
SUYA	52	2182					255	10312	307	12494
TECO							20	618	20	618
TRLK	5	191	11	450	1	11	21	930	38	1582
VENT							35	1750	35	1750
WVAL			13	612	3	93	2	37	18	742
Totals	123	5306	796	34891	60	2092	2821	129427	3800	171716

Table 4. Mosquito pools positive for SLE and WEE viruses during 1993 by agency.

Virus	Agency Code	*Aedes melanimon*	*Culex tarsalis*	Totals
SLE	LONG	0	1	1
SLE	SANB	0	4	4
WEE	ACR	0	1	1
WEE	CLSA	0	14	14
WEE	CNTR	0	11	11
WEE	GLNN	0	3	3
WEE	IMP	0	1	1
WEE	LAKE	0	8	8
WEE	MADR	0	1	1
WEE	SACR	0	80	80
WEE	SANB	0	3	3
WEE	SANJ	0	1	1
WEE	SHAS	0	7	7
WEE	SSCU	1	0	1
WEE	SUYA	1	33	34
WEE	TECO	0	1	1
WEE	TRLK	0	2	2
	Totals	2	171	173

Table 5. Mosquito pools tested by the ARL in 1993 from Coachella Valley and Imperial County study areas.

Species	Mosq.	Pools	Isolates
COACHELLA VALLEY			
Aedes dorsalis	79	4	None
Culex tarsalis	5605	115	None
Culiseta inornata	3802	81	None
Totals	9486	200	
IMPERIAL COUNTY			
Aedes dorsalis	13	2	None
Culex tarsalis	17301	369	12 WEE 15 SLE
Culex quinquefasciatus	1016	21	None
Culiseta inornata	57	3	None
Totals	18387	395	

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ARTHROPOD-BORNE ENCEPHALITIS SURVEILLANCE IN THE SAN GABRIEL VALLEY DURING 1993

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During 1993, the San Gabriel Valley Mosquito Abatement District (SGVMAD) conducted its own first year of mosquito control and surveillance instead of contracting services to a neighboring district. Traditional as well as less conventional methods for the control and surveillance of mosquitoes were used in the District. Although the effects of several components of this control program cannot be quantified, it is felt that they were effective in reducing mosquito populations in the District. Standard mosquito population and encephalitis surveillance methods used by the District included the operation of carbon dioxide (CO_2)-baited traps and Reiter-Cummings gravid female traps for collection of mosquitoes, and collection of sera samples from sentinel chickens and feral birds for virus detection.

Perhaps the most effective tool of the program was communication. Discussion between surveillance and control staff and between the District and other agencies was very productive in reducing the number of breeding sources within the District. Any significant increases in mosquito populations discovered by surveillance staff were reported to district technicians, who would then find the source, eliminate it if possible, or treat it with a larvicide. Post-treatment adult trapping was done to verify treatment effectiveness. Large differences between pre- and post-larviciding adult counts indicated treatment success; conversely, small differences indicated the source(s) were not treated properly, or that another source was present.

The District also worked very closely with the code enforcement and/or health department of each member city or county area. Code enforcement staff would report to the District any breeding discovered in abandoned swimming pools, ornamental ponds, etc. The District would treat the source and both agencies would work to have the "nuisance" abated. No legal action was necessary and the number of "nuisance" swimming pools dropped from 700 to less than 40.

The District also worked for the second complete year with water control authorities to reduce the breeding of mosquitoes along rivers, channels, and flood control structures in the District. Vegetation at the margins of water sources was removed whenever possible and the District was notified whenever the Department of Public Works released water to spreading grounds to recharge the aquifers. In addition, the water was released at agreed upon cycles that allowed for ample drying time between releases. This minimized the time standing water existed at any given site.

These efforts prevented duplication of circumstances similar to 1991, when an impoundment of water went undetected until a large population of *Culex tarsalis* Coquillett developed (Fujioka et al. 1992). Fourteen of fifteen chickens in the sentinel flock near this site were positive for antibodies to St. Louis encephalitis (SLE) virus, and one case who was exposed to mosquitoes from the area was reported.

MOSQUITO COLLECTIONS

Adult mosquitoes were collected with CO_2-baited traps as part of the disease surveillance program. These mosquitoes were identified to species, counted, and submitted in pools of 10-50 to the State Viral and Rickettsial Disease Laboratory for encephalitis virus testing.

A total of 6,176 mosquitoes were trapped during the season. These consisted of 3,597 (58%) *Culex quinquefasciatus* Say, 1,855 (30%) *Culex tarsalis*, 301 (4.9%) *Culex stigmatosoma* Dyar, 333 (5.3%) *Culiseta incidens* Thomson, and 90 (1.4%) others. From these mosquitoes, 60 pools were submitted for testing, including 43 pools of *Cx. quinquefasciatus*, 15 pools of *Cx. tarsalis*, and 2 pools of *Cx. stigmatosoma*. No mosquito-borne encephalitis virus was found in the pools of mosquitoes that were tested.

Table 1. Sentinel chickens with antibodies to SLE virus from the San Gabriel Valley during 1991-1993.

Flock Site	1991	1992	1993
Irwindale	14/15	6/9	4/6
Monterey Park	no flock	3/5	3/6
Pomona	0/15	1/10	2/6
West Covina	no flock	5/5	0/6
Claremont	no flock	no flock	1/6
Industry	no flock	no flock	2/6

Table 2. Percent *Cx. quinquefasciatus* collected before and after seroconversions at 5 sites in the San Gabriel Valley during 1993.

	% *Culex quinquefasciatus*	
Site	Before	After
Claremont	16	66
Industry	89	79
Irwindale	94	97
Monterey Park	100	88
Pomona	55	83

CHICKEN SEROLOGY

Sentinel flocks of six chickens each were used at 10 locations in the District. Antibodies to SLE virus were detected in five of the flocks. For surveillance purposes, the percentage of a flock that is infected is not as important as whether they are infected. Using smaller flocks allowed for the placement of chickens at more sites than in 1992. Because of this, viral activity was detected at two additional locations. A comparison of sentinel flocks placed in the San Gabriel Valley indicates that smaller flocks are as successful at detecting encephalitis viral activity as larger flocks (Table 1).

The adult mosquito population near each sentinel flock was assessed by CO_2-baited trapping at least one week prior to bleeding and within one week of notification of any seroconversion. In all cases except one, *Cx. quinquefasciatus* was the dominant species trapped (Table 2). Many investigators have had success trapping *Cx. tarsalis* with CO_2-baited traps, and we feel that the comparatively low numbers of *Cx. tarsalis* trapped was due to smaller populations, rather than trap ability.

FERAL BIRDS

Sera from feral birds were collected biweekly from three sites and tested by the Orange County Vector Control District for antibodies to SLE and western equine encephalomyelitis (WEE) viruses by hemagglutination inhibition (HAI). A total of 644 birds were captured, banded, sampled, and released. There were 525 (81%) *Passer domesticus*, 115 (18%) *Carpodacus mexicanus*, and four (<1%) *Zonotrichia leucophrys*. Twenty-one sera (3.3%) from *P. domesticus*, the English house sparrow, and four sera (3.5%) from *C. mexicanus*, the house finch, contained antibodies to SLE virus. Only one bird was captured for a subsequent bleeding and it did retain its titer. It was not, however, captured a third time and therefore the length of time this bird retained an antibody titer was not determined. In 1992, one bird retained a titer for two consecutive bleedings. Six others were negative, positive, and negative again for antibodies to SLE virus during three successive bleedings (Brisco et al. 1993). The predictive value of seropositive feral birds is not certain, since there has not been an epidemic of SLE since 1984.

In spite of these questions, this system shows promise. Antibodies to mosquito-borne encephalitis virus may be detected in feral birds throughout the year, and were detected earlier in feral birds than in sentinel chickens both in 1993 (Fig. 1) and 1992 (Brisco et al. 1993). In 1992, both the Orange County Vector Control District (Bennett et al. 1993) and Los Angeles County West Vector Control District (Kovaltchouk et al. 1993) experienced year-round findings of antibodies to mosquito-borne encephalitis virus in feral birds.

HUMAN DISEASE

Although no cases of either SLE or WEE were reported to the Los Angeles County Department of Health Services, the incidence of encephalitis and aseptic meningitis exceeded the five-year-mean. Since tests to identify the etiologic agent frequently are not done, it is not known if any of these cases were caused by WEE or SLE virus. In addition, many cases of aseptic meningitis and encephalitis are not reported, making it impossible to determine the etiologic agent, and artificially reducing the incidence of disease.

A lack of cases does not preclude the need for vigilance; evidence that SLE virus has been present in the San Gabriel Valley has been found each year since 1991, and the only reported cases of SLE in Los Angeles County during this time period (one each in 1991 and 1992) (Fujioka et al. 1992 and Brisco et al. 1993, respectively) were residents of the San Gabriel Valley.

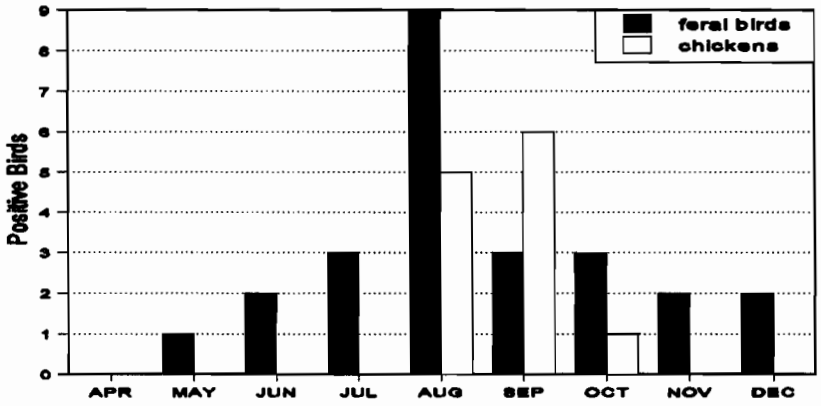

Figure 1. SLE antibody-positive chickens and feral birds in the San Gabriel Valley during 1993.

SUMMARY

To summarize, mosquito-borne encephalitis was present in the San Gabriel Valley for the third consecutive year. Five of the ten sentinel chicken flocks in the San Gabriel Valley were infected with St. Louis encephalitis virus. Feral bird serology continues to produce interesting results, and may prove to be a useful surveillance tool as more data are accumulated. No mosquitoes that were tested were infected with encephalitis virus, and although no cases of either SLE or WEE were reported, the incidence of aseptic meningitis and encephalitis in Los Angeles County exceeded the five-year-mean. Despite our status as the new kids on the block with regard to mosquito control in Los Angeles County, we would like to think that our initial efforts as a functioning mosquito abatement district were successful in protecting public health and maintaining the comfort of residents of the district.

ACKNOWLEDGEMENTS

The authors thank Carrie Fogarty of Orange County Vector Control District for performing the HAI test on the feral bird sera. We also thank the Los Angeles County Department of Public Works and code enforcement and/or health departments of our member cities for their efforts and cooperation in reducing the number of mosquito breeding sources in the District.

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EVALUATION OF MOSQUITO AND ARBOVIRUS ACTIVITY IN ORANGE COUNTY DURING 1993

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ABSTRACT

The Orange County Vector Control District continued its surveillance of mosquito and arbovirus activity throughout 1993 by collecting blood samples from wild birds and sentinel chickens as well as collecting adult mosquitoes from CDC CO_2-baited and ovipositional traps. There were no positive mosquito pools or human cases in Orange County during 1993. In addition, there were no serconversions among the 20 sentinel chickens held in a single flock in Irvine. Wild birds were found SLE-positive every month of the year except June with 1.22% of the 981 sampled house sparrows and 1.85% of the 1,783 sampled house finches seroconverting to SLE virus. *Culex quinquefasciatus* was the most common mosquito collected except for rural areas of Irvine, where *Cx. tarsalis* was predominant. Gravid *Cx. quinquefasciatus* were collected from both suburban and rural sites using ovipositional traps with the most productive site (reaching 450 per trap-night in May) being Fullerton College.

In 1993, the Orange County Vector Control District (OCVCD) continued its mosquito and encephalitis virus surveillance throughout the year. Mosquitoes were collected at nine permanent sites throughout the county utilizing 16 CDC CO_2-baited traps as well as five Cummings gravid female ovipositional traps. Additional mosquitoes were collected inside a single Australian Crow trap, modified to retain any mosquitoes entering through slots on the sides (similar to a stable trap). No chicken-baited stable traps were used this year.

A total of 3,613 mosquitoes was collected from which 112 pools were submitted to the California State Department of Health Services' Viral and Rickettsial Diseases Laboratory at Berkeley for virus testing (Table 1). The collections included 104 pools of *Culex quinquefasciatus* Say and 8 pools of *Culex tarsalis* Coquillett. None of these pools tested positive for St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) viruses.

A single sentinel chicken flock (20 chickens), maintained at the San Joaquin Wildlife Sanctuary (formerly the 20 Ranch Duck Club) in Irvine, showed no seroconversions for SLE or WEE in 1993. In contrast, seroconversions for SLE virus in 1992 included 2 of 15 chickens bled on September 9 from the San Joaquin Wildlife Sanctuary; 1 of 5 chickens bled on September 23 from the Fullerton College stable trap, and 1 of 5 chickens bled on October 6 from the San Joaquin Wildlife Sanctuary stable trap (Bennett et al. 1993). Despite this detected SLE virus activity, no human cases of SLE or WEE were reported from Orange County in 1993.

Wild bird sera were tested for SLE and WEE antibodies by the OCVCD at the laboratories of the Orange County Health Department. Eight modified Australian Crow traps (McClure 1984) were used in 1993 to trap a total of 9,606 birds from which 2,780 blood samples were taken and tested (Table 2).

Of the 981 house sparrows sampled in 1993, 1.22% tested positive for SLE antibodies while 1.85% of the 1,783 house finches sampled were SLE-positive (3.43 and 1.34%, respectively in 1992). None of the 16 white-crowned sparrows sampled, were found SLE- or WEE-positive.

SLE-positive birds (sparrows and finches combined) were collected during every month of the year except June (no samples were taken in January) with the highest percentage of SLE-positives occurring in October

Table 1. Mosquito pools submitted from Orange County for virus testing during 1993.

Species	Modified crow traps	Ovipositional traps	CDC traps	Total pools
Culex quinquefasciatus	15	89	0	104
Culex tarsalis	8	0	0	8
Totals	23	89	0	112

with 3.4% of all birds found SLE-positive (Fig. 1). An abrupt increase from 0 to 2.0% in small bird SLE seroconversions occurred in June and early July, at approximately the same time as the first chicken SLE seroconversions in the neighboring Los Angeles basin, and remained between 2.0 and 4.0% through December.

Overall, house sparrows accounted for the highest percentage of seroconversions during February through July (Fig. 2), but house finches were highest from August to December (Fig. 3).

Mason Regional Park in Irvine had the overall highest percentage of SLE-positive house sparrows (10%) among the eight sampling sites in 1993 (Fig. 4). However, it should be noted that only 20 birds were collected from this site during 1993 with 11 sampled from February to March and 9 sampled from June to July.

A residential site in Huntington Beach had the overall highest percentage of SLE-positive house finches (3.6%) among the eight sampling sites in 1993 with 26 SLE-positive of 719 house finches sampled. This site also had the second highest percentage of SLE-positive house sparrows (1.8%) with 5 SLE-positive of 285 house sparrows sampled throughout 1993 (1.5% and 4.4%, respectively in 1992). The highest percentage of SLE-positive house sparrows at this site (50%) occurred in April (14% in April 1992), however, only two house sparrows were collected at that time (Fig. 5). At this same site, House finches were consistently SLE-positive from July through December (ranging from 3 to 9%) but little viral activity was noted prior to this time (Fig. 6). In contrast, in 1992 most SLE-positive house finches were collected from this site between January and April (ranging from 3 to 7%).

Culex tarsalis Coquillett was the most commonly collected mosquito at this site, reaching a high of 50-55 females per trap-night in April and disappearing by October (averaging 26-28 per trap-night in April 1992). *Culex quinquefasciatus* Say at this site reached a high of only 10-12 per trap-night during the year; much lower than in 1992 (28-34 per trap-night).

The total percentage of SLE-positive house sparrows (0.33%) and house finches (0.38%) from Central Park in Huntington Beach was considerably lower than in 1992 (5.5 and 1.2%, respectively) with house finches showing viral activity only in July and house sparrows only in September (Fig. 7). In 1992, viral activity was detected at this site in April, May, June, July, October, and December. Once again, *Cx. tarsalis* was collected in highest numbers at this site during May at 80 females per trap-night (32 per trap-night in 1992) and *Cx. quinquefasciatus* reached 14 females per trap-night during May (10 per trap-night in June and July of 1992), but remained below 5 per trap-night for the rest of the year.

The highest rates of SLE-positive birds at Fullerton College occurred in July and August at 12 and 6%, respectively; much lower than the observed highest rates in 1992 (20 and 22% in September 1992). Just prior to the bird seroconversions at Fullerton College, host-seeking *Cx. tarsalis* at this site reached a high of 8 females per trap-night and *Cx. quinquefasciatus* increased to 120 females per trap-night.

Culex tarsalis was more abundant in rural or suburban areas such as the San Joaquin Freshwater Marsh and the San Joaquin Wildlife Sanctuary (20 Ranch Duck Club), both in Irvine (Figs. 9 and 10). Populations of host-seeking *Cx. tarsalis* in the marsh were highest in May (65 females per trap-night), June (115 females per

Table 2. Small bird seroconversions for SLE and WEE antibodies in Orange County during 1993.

Bird Species	No. positive		#	% Positive	
	SLE	WEE	Sampled	SLE	WEE
House sparrow	12	0	981	1.22	-
House finch	33	0	1783	1.85	-
White-crowned sparrow	0	0	16	0	-
Totals	45	0	2780	1.62	

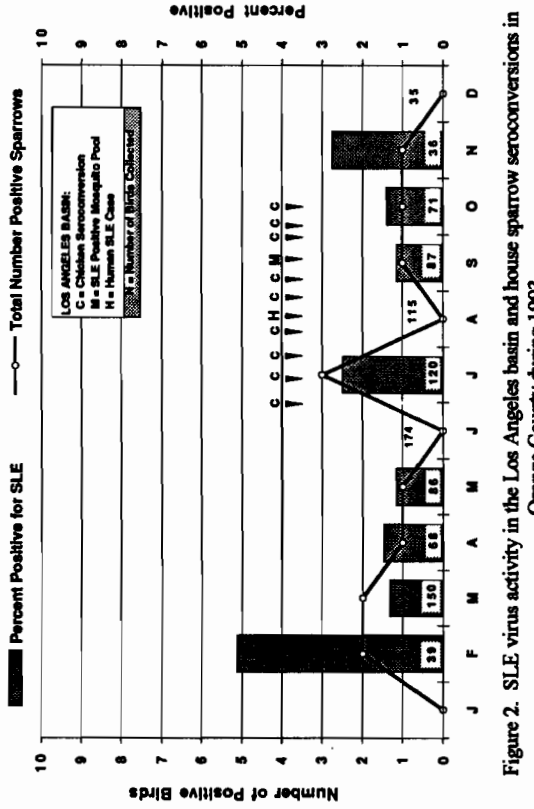

Figure 2. SLE virus activity in the Los Angeles basin and house sparrow seroconversions in Orange County during 1993.

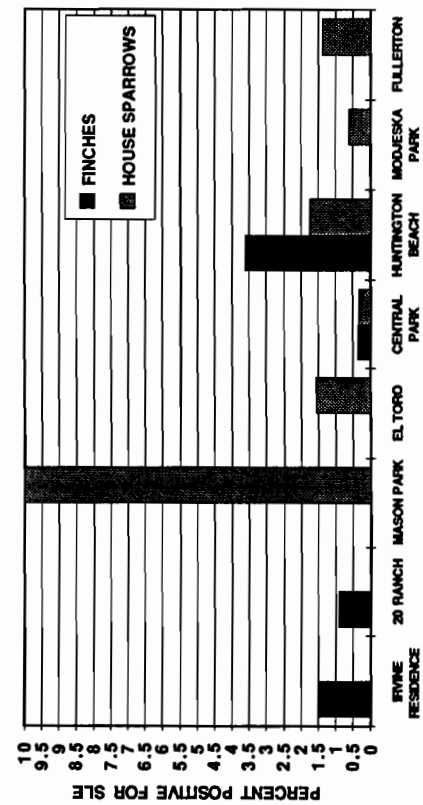

Figure 4. SLE virus activity in house sparrows and house finches at each sampling site in Orange County during 1993.

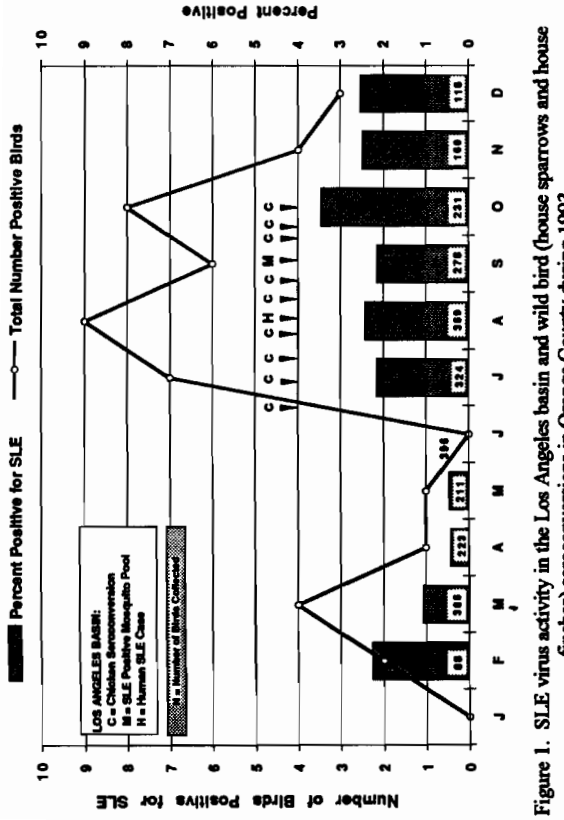

Figure 1. SLE virus activity in the Los Angeles basin and wild bird (house sparrows and house finches) seroconversions in Orange County during 1993.

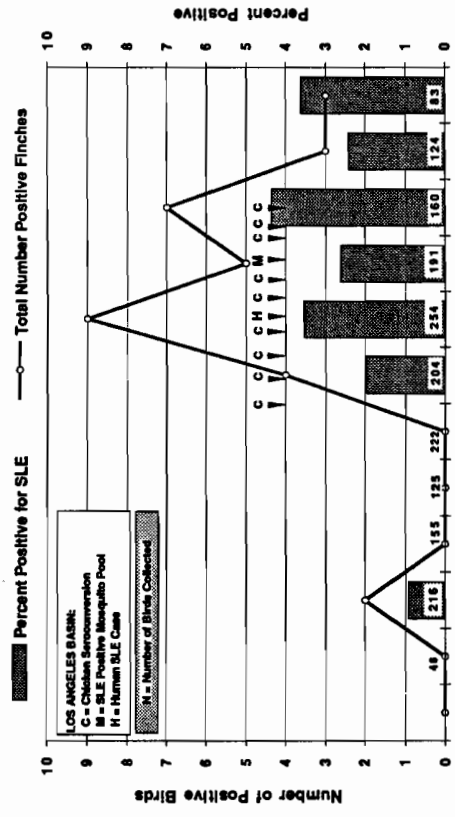

Figure 3. SLE virus activity in the Los Angeles basin and house finch seroconversions in Orange County during 1993.

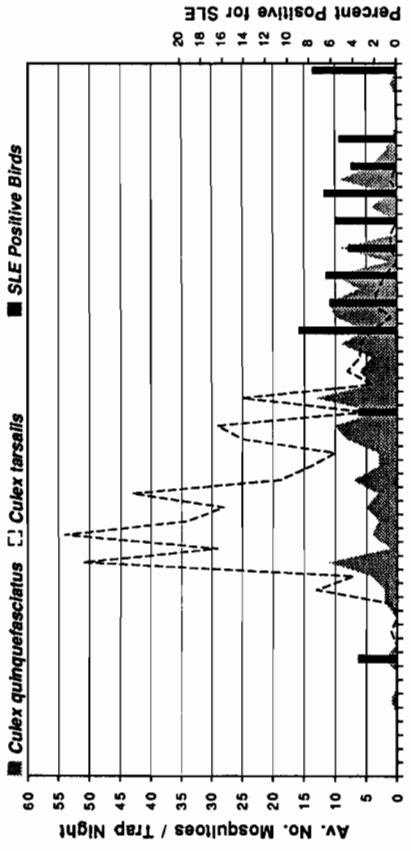

Figure 6. Host-seeking mosquito (CDC traps) and SLE activity in house finches from a residence in Huntington Beach during 1993.

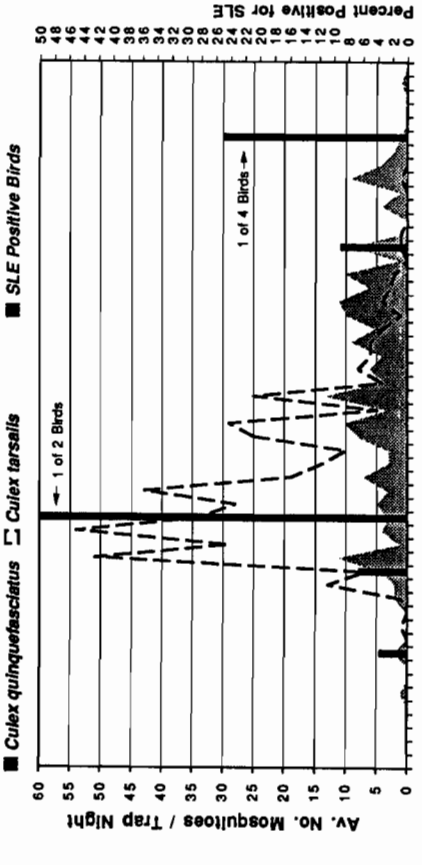

Figure 5. Host-seeking mosquito (CDC traps) and SLE activity in house sparrows from residence in Huntington Beach during 1993.

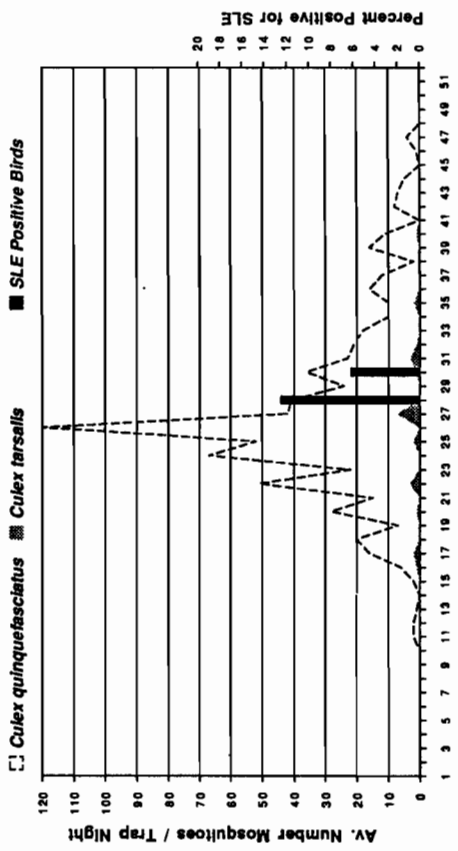

Figure 8. Host-seeking mosquito (CDC traps) and SLE activity in house sparrows from Fullerton College during 1993.

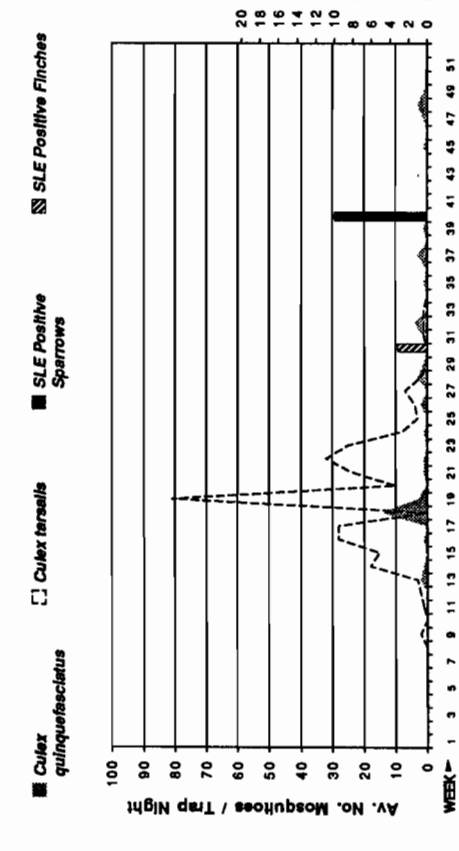

Figure 7. Host-seeking mosquito (CDC traps) and SLE activity in wild birds (house sparrows and house finches) from Central Park in Huntington Beach during 1993.

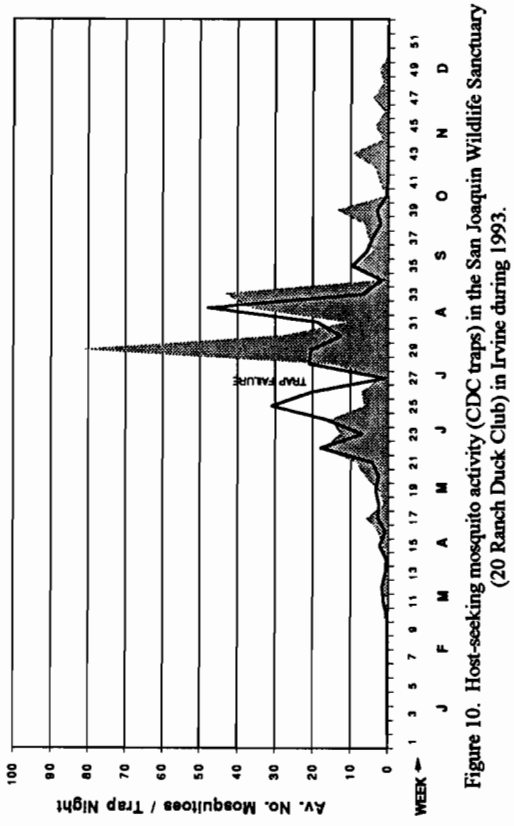

Figure 9. Host-seeking mosquito activity (CDC traps) in the San Joaquin Freshwater Marsh in Irvine during 1993.

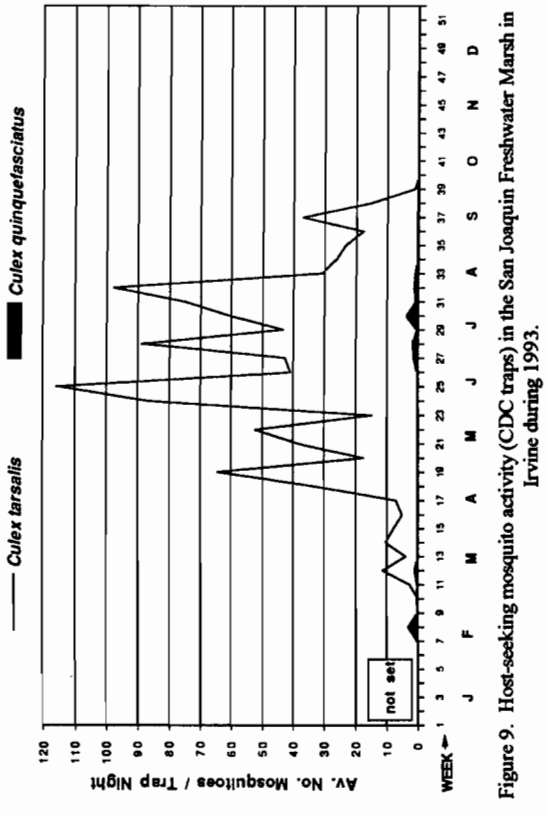

Figure 11. Gravid mosquito activity (Cummings ovipositional traps) in Irvine during 1993.

Figure 10. Host-seeking mosquito activity (CDC traps) in the San Joaquin Wildlife Sanctuary (20 Ranch Duck Club) in Irvine during 1993.

Figure 12. Mosquito activity (CDC and Cummings ovipositional traps) at a suburban residence in Irvine during 1993.

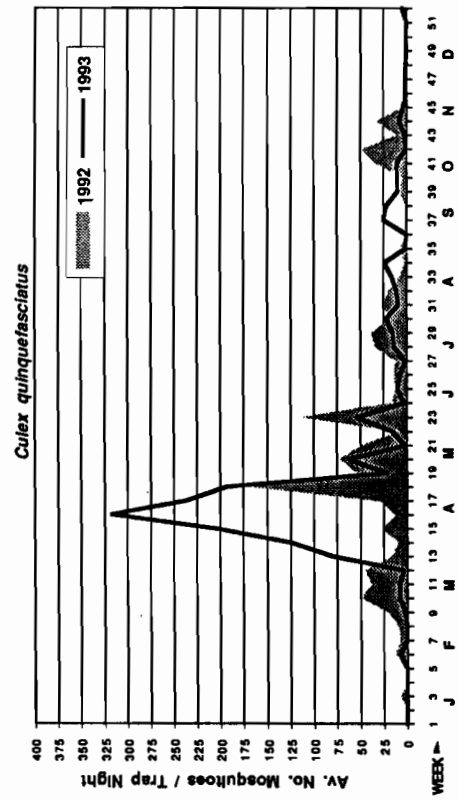

Figure 13. Gravid mosquito activity (Cummings ovipositional traps) in Central Park, Huntington Beach during 1992 and 1993.

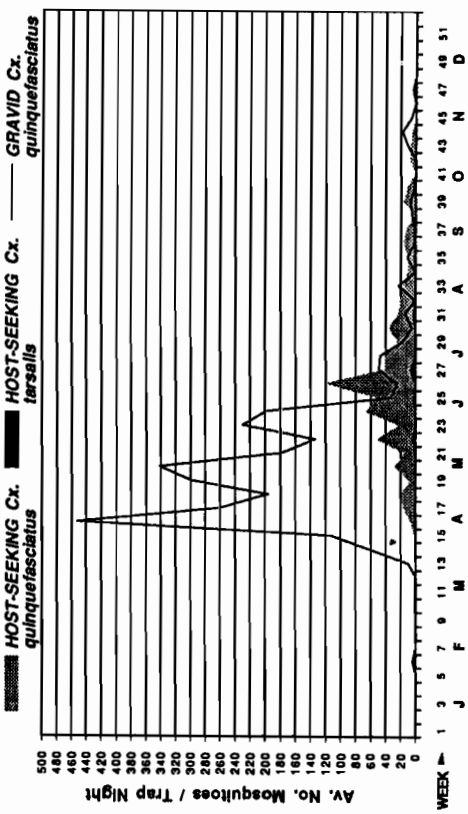

Figure 14. Mosquito activity (CDC and Cummings ovipositional traps) at Fullerton College in Fullerton during 1993.

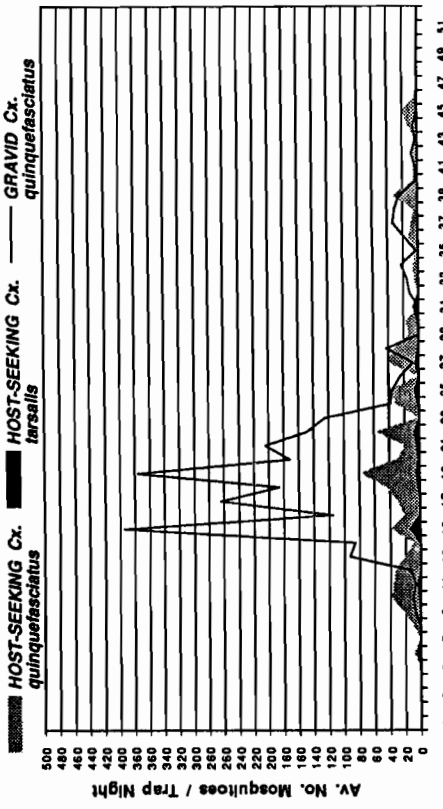

Figure 15. Mosquito activity (CDC and Cummings ovipositional traps) at Modjeska Park in Anaheim during 1993.

trap-night), July (90 females per trap-night), and August (100 females per trap-night). In 1992, this mosquito was considerably more numerous (240 females per trap-night in April, 525 per trap-night in May, and 175 females per trap-night in mid-June). Although *Cx. quinquefasciatus* is traditionally uncommon in the marsh, it was almost non-existent in 1993, averaging less than 5 females per trap-night between February and October (averaging 20 females per trap-night in 1992).

In the San Joaquin Wildlife Sanctuary, *Cx. tarsalis* was also less abundant in 1993, reaching a high of 48 females per trap-night in September while rarely averaging below 30 females per trap-night all year in 1992 with the highest counts that year occurring from May to June (100-200 females per trap-night). *Culex quinquefasciatus* also was less abundant in the Wildlife Sanctuary during 1993, never exceeding 20 females per trap-night except for week 29 in August when they peaked at 80 per trap-night and week 33 in September when they reached 43 per trap-night. In contrast, in 1992 *Cx. quinquefasciatus* collected from this site peaked in May at 70-110 per trap-night and rarely dropped below 30 females per trap-night between April and October.

Throughout the year, gravid female *Cx. quinquefasciatus* were obtained using Cummings ovipositional traps at both suburban and rural sites. A comparison of ovipositional trap effectiveness at the San Joaquin Wildlife Sanctuary (a rural collection site) and a neighboring residential site in Irvine (a suburban collection site) six kilometers away (Fig. 11), indicates that seasonal collection patterns were similar at both sites, but the residential (suburban) site was more productive than the rural site during April and May; reaching as high as 225 gravid females per trap-night in May, whereas the rural site reached only 100 gravid females per trap-night during the same period. In 1992, the peak activity at both sites occurred in May.

The seasonal collection pattern of host-seeking *Cx. quinquefasciatus* at the Irvine residential site (Fig. 12) was virtually the same as in 1992, reaching 50 females per trap-night in May and 35 females per trap-night in mid-June (100 females per trap-night in April and 80 per trap-night in June 1992).

Numeric collections of *Cx. quinquefasciatus* at Central Park in Huntington Beach during 1993 varied from 1992, especially in April and May (100-320 per trap night for 1993 and 45-175 per trap-night for 1992), but for the most part seasonal fluctuations were very similar (Fig. 13). In contrast, less than 4 host-seeking females were collected each trap-night during 51 of 52 weeks in 1993 and 0-10 females per trap-night were collected throughout all of 1992.

The most productive site for gravid *Cx. quinquefasciatus* in 1993 was Fullerton College, peaking at 450 gravid females per trap-night in early May (Fig. 14). In 1992 this site produced a high of only 100 females per trap-night in June. Host-seeking *Cx. quinquefasciatus* at the Fullerton College site gradually increased from 20 per trap-night in May to 120 in July and back down to 10 per trap-night in October. *Culex tarsalis* at this site was present in lower numbers (2-7 per trap night) from May to October.

Modjeska Park in Anaheim was also quite productive for gravid *Cx. quinquefasciatus* in 1993 with 375-400 females collected per trap-night in April and May (Fig. 15); figures comparable to the highs of 400-460 females collected per trap-night in late May 1992.

ACKNOWLEDGEMENTS

Gratitude is sincerely extended to Drs. D.F. Moore and J.R. Greenwood of the Orange County Health Care Agency for laboratory space and supplies.

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MOSQUITO ABUNDANCE AND ARBOVIRAL ACTIVITY IN SAN BERNARDINO COUNTY DURING 1993

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ABSTRACT

Of the 23,185 mosquitoes collected in New Jersey light traps and CO_2-baited CDC traps in San Bernardino County during 1993, 85.5% were from the desert region (Needles) and 14.5% from the valley region (including <0.1% from the mountains). In the desert region, the dominant species were *Aedes vexans* (63.9%), *Culex tarsalis* (20.4%), and *Culiseta inornata* (15.2%). In the valley region, *Cs. inornata* (29.1%), *Cx. tarsalis* (28.2%), *Culex quinquefasciatus* (18.3%), and *Culex stigmatosoma* (17.6%) were the dominant species. Four of 65 *Cx. tarsalis* pools from the desert region tested positive for Saint Louis encephalitis (SLE) virus and 3 pools were positive for western equine encephalomyelitis (WEE) virus. All 46 mosquito pools from the valley and mountain areas tested negative for both viruses.

A sentinel chicken flock at Needles showed 7 seroconversions to SLE and 3 to WEE in August followed by 100% and 60% flock seroconversion to SLE and WEE, respectively, by October. Moreover, there were two human SLE cases; one in August 1993 from San Bernardino and the other is an out-of-county resident who possibly contracted the disease in the desert (Needles) area during September.

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 and other mosquito control activities in both the valley and desert 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 1.5 million county population 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 SLE in southern California during 1984, the only human case of encephalitis (SLE) in California during 1987 was reported from San Bernardino (Emmons et al. 1988). Of the two cases reported statewide in 1988, one was from the same San Bernardino site (Emmons et al. 1989). During the same period, both SLE and WEE virus activities were reported in the desert region, especially Needles, and adjourning 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 this county. Data generated in routine EVS activities are appraised here in relation to mosquito abundance and arboviral activity in San Bernardino County during 1993.

MATERIALS AND METHODS

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

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Adult Mosquito Population Dynamics.

The abundance of various mosquito species was monitored on a weekly basis through a number of New Jersey light traps. In the valley and mountain regions, the traps were stationed at seven different locations; Yucaipa, Highland, San Bernardino, Colton, Fontana, Ontario, Upland, and Jenkin Lake in the mountains. Within the valley region there were two trap 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 in all three regions, using dry ice (CO_2)-baited CDC traps to collect host-seeking adult female mosquitoes. Eight or more such traps were operated twice a month in the valley area, once a month in the Colorado River (desert) region and three times during the season (May through October) in the mountain area. Female mosquitoes collected overnight were anesthetized using triethylamine (TEA), counted, identified to species and sex; they were pooled by species with 10-50 adults per labelled vial. All pools (vials) were stored in dry ice in the field or in a deep freezer at -60°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 the encephalitis virus(es). Therefore, one sentinel flock consisting of ten white leghorn chickens was maintained in both the valley area in Colton and the desert area in Needles. The valley flock was stationed near a horse ranch at the northeastern corner of Meridian Avenue and Olive Street in the city of Colton. This site is located in the general area where the SLE human cases occurred in 1987 and 1988. The desert flock was maintained at the sewage treatment facility in the city of Needles. New Jersey light traps were regularly operated at both flock sites. Using the comb prick method, blood samples were taken from all sentinel chickens onto prelabelled filter paper strips on a bi-weekly basis during April through October 1993. These samples were then mailed to the State's VRDL for detection of arboviral activity.

RESULTS AND DISCUSSION

Of the 23,185 mosquitoes collected at all sites during the season, 85.5% were trapped in the desert area with only 14.5% being from the valley sites including <0.1% from the mountain area (Table 1). The most abundant mosquito in the desert region was *Aedes vexans* Meigen (63.9%) followed by *Culex tarsalis* Coquillett (20.4%), *Culiseta inornata* Williston (15.2%), and *Culex quinquefasciatus* Say (0.2%). Other species each accounting for ≤0.1% *Anopheles franciscanus* McCracken and *Culex erythrothorax* Dyar. *Culex*

Table 1. Percent species composition of female mosquitoes collected in New Jersey light traps and dry ice (CO_2)-baited CDC traps (and number of pools submitted) in San Bernardino County during 1993.

	DESERT REGION			VALLEY REGION		
Species	**N.J. traps**	**CO_2 traps**	**Totals**	**N.J. traps**	**CO_2 traps**	**Totals**
Aedes washinoi	0.0	0.0	0.0	0.2	1.8	0.9
Aedes vexans	4.3	86.0	63.9	0.0	0.2	0.1
Anopheles franciscanus	<0.1	0.1	0.1	1.2	0.0	0.6
Culex erythrothorax	0.1	0.1	0.1	0.0	0.8	0.4
Culex quinquefasciatus	0.9	<0.1	0.2	10.9	26.7 (9)	18.3
Culex stigmatosoma	0.1	0.0	<0.1	26.6	7.2 (18)	17.6
Culex tarsalis	45.9	11.0 (65)	20.4	36.4	18.8 (19)	28.2
Culiseta incidens	0.0	0.0	0.0	16.8	43.2	29.1
Culiseta inornata	48.5	2.8	15.2	7.9	1.3	4.8
Psorophora spp.	<0.1	0.0	<0.1	0.0	0.0	0.0
Totals	5,363	14,466 (65)	19,829	1,796	1,560 (46)	3,356
Positive pools		4 SLE			0 SLE	
		3 WEE			0 WEE	

tarsalis was present during the summer and fall months, whereas *Ae. vexans* populations peaked during September and October due to flood irrigation practices on the Arizona side of the Colorado River.

The most abundant mosquito species in the valley region was *Culiseta incidens* (Thompson) (29.1%) followed by *Cx. tarsalis* (28.2%), *Cx. quinquefasciatus* (18.3%), *Culex stigmatosoma* Dyar (17.6%), *Cs. inornata* (4.8%), *Aedes washinoi* Lanzaro and Eldridge (0.9%), *An. franciscanus* (0.6%), *Cx. erythrothorax* (0.4%), and *Ae. vexans* (0.1%). Earlier studies in this area, indicated *Cx. tarsalis* as the most abundant species, comprising as much as 72%, 62%, 86%, 55%, 70%, and 42% of the mosquitoes collected in 1986 and 1987 (Reisen et al. 1988), 1989, 1990, 1991, and 1992 (Mian and Prochaska 1990, 1991, 1992, and 1993), respectively.

A total of 111 pools of *Cx. tarsalis*, *Cx. quinquefasciatus* and *Cx. stigmatosoma* were sent to the VRDL for virus study. Of the 64 *Cx. tarsalis* pools submitted from the Needles (desert) region, four tested positive for SLE and three for WEE during the last week of August. All 46 mosquito pools submitted from the valley and mountain areas, however, tested negative for both viruses.

At the August 25, serosampling, the desert region sentinel chicken flock in Needles showed virus activity with seven seroconversions to SLE and three to WEE (two birds showed seroconversions to both viruses), thus collaborating virus activity detected in mosquito pools during the later part of August. Virus activity in this flock continued through the end of the season (October) with 100% flock seroconversion to SLE and 60% to WEE. Virus activity at the valley region site in Colton was apparent on the September 24 sampling with two seroconversions to SLE, followed by two more seropositives to SLE in October. Upon receipt of confirmation on seroconversions, both areas were posted with "Encephalitis Warning" signs followed by press releases advising residents to take necessary precautions during outdoor activities, especially at dusk and dawn in the affected areas.

In the wake of this high virus activity, several adulticidal applications of resmethrin (Scourge® 4-12, ULV) were carried out in several parts of the city of Needles. Moreover, at the request of the Mohave County (Arizona) Department of Health and Social Services, adulticidal applications were also carried out by the SBCVCP staff for the first time across the Colorado River at several locations in Mohave County. These applications were successful in significantly controlling adult populations of *Cx. tarsalis*.

During the 1993 mosquito season, there were two human cases of SLE reported in San Bernardino County. The first case was a 64-year old male resident of the city of San Bernardino who became ill on August 12, 1993, was hospitalized from August 15 to 23, and recovered completely. His travel 3 to 4 weeks prior to the onset of illness had been local, within 20-30 miles of his home. On certain occasions his job required him to close the shop during the evening hours. Also he and his wife, along with their dog, did go on evening walks regularly within a couple of miles of his home. Mosquito activity around his home as well as work site was low, yielding only two pools (10 *Cx. tarsalis* and 48 *Cx. quinquefasciatus*) from 16 CO_2-baited traps operated during two separate nights about two weeks after the onset of the illness. Both mosquito pools tested negative for both SLE and WEE viruses.

The second SLE human case in San Bernardino County was a 30-year old male resident of Orange County who along with 11 others had been to the Colorado River 10 miles south of Needles during the second week of September. Inspite of using mosquito repellent, he had a number of mosquito bites on his body. He became ill on September 24, was hospitalized for 3 days, eventually recovering completely. The probable area (Colorado River) where he may have contracted the disease had shown high activity of both SLE and WEE viruses during his time of exposure.

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TEMPORAL AND SPATIAL PATTERNS OF ARBOVIRUS ACTIVITY IN THE IMPERIAL VALLEY[1]

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ABSTRACT

Selected landscape features in the Imperial Valley were studied in an attempt to delineate foci important in the initiation and amplification of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses. The spatial pattern of WEE and SLE virus activity was generally similar from 1991 to 1993, beginning without association with a specific focus or habitat type and reaching most sites and habitats during the course of the transmission season. This pattern could be explained by the annual introduction of virus into different portions of the valley and/or by geographic variation in the amplification of widespread endemic virus within the relatively homogenous irrigated agricultural habitat that covers most of the Imperial Valley.

Landscape features and associated vegetative patterns markedly influence the spatial distribution of arbovirus enzootic transmission by bringing vectors and vertebrate hosts together in time and space. In the Coachella Valley, for example, the activity of both western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses always commences at marshes along the Salton Sea and then spreads to the northwest within the flood plain of the Whitewater channel (Lothrop et al. 1992, 1993; Reisen et al. 1992a). Previous and on-going research in the Imperial Valley has delineated the summer-early fall seasonality of WEE and SLE virus activity (e.g., Work et al. 1977a,b; Reisen et al. 1992b), but has failed to detect enzootic foci or specific habitats at which encephalitis virus activity is initiated each season. Delineating foci of virus initiation and amplification may allow future research as well as control efforts to concentrate on specific areas within the Imperial Valley.

From 1991 to 1993 three hypotheses were tested to determine the importance of specific landscape features in the initiation and amplification of arboviruses in the Imperial Valley. Research in 1991 determined if virus activity began at a large ciconiiform rookery and if virus activity was confined to riparian habitat along the New River. In 1992, the 1991 study area was expanded to determine if virus activity in riparian and agricultural habitats extended into adjacent residential areas, thereby increasing the risk of virus transmission to humans. In 1993, sampling determined if virus activity was initiated at marshes along the southern shore of the Salton Sea or was introduced into the New River riparian corridor from Mexico.

MATERIALS AND METHODS

Description of Study Area.

The Imperial Valley lies to the south of the Salton Sea in California's inland desert. The relatively homogenous landscape is dominated by irrigated row and fodder crops intersected by corridors of channeled riparian habitat associated with the New and Alamo Rivers which begin in Mexico and flow north, emptying

[1] A manuscript summarizing this study will be submitted for publication in the Journal of Medical Entomology.

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into the Salton Sea. Farm compounds and associated landscaping provide isolated islands of deciduous vegetation.

Sampling Methods.

We presumed that virus activity would appear first at discrete enzootic maintenance foci and that a combination of testing sentinel chicken flocks for seroconversions and *Culex tarsalis* Coquillett, the primary enzootic vector, for infection in a carefully planned sampling grid would delineate temporal patterns of virus initiation, amplification and dissemination. Flocks of ten white leghorn hens were bled at 4-week intervals during 1991 and 1992, and at 2-week intervals during 1993. Blood samples were tested for antibodies to WEE and SLE viruses using an enzyme immunoassay (EIA). Chickens that died or seroconverted were replaced.

Host-seeking mosquitoes were collected biweekly by two dry ice-baited CDC traps (CO_2 traps) operated on fixed standards near each chicken flock to determine abundance and virus infection rates. Up to ten pools of 50 *Cx. tarsalis* females per site per sample date were tested for virus using a modification of the in situ EIA described by Kramer et al. (1992).

RESULTS AND DISCUSSION

In 1991 five sites were established along the New River and two in the surrounding agricultural habitat near Seeley to test the hypothesis that virus activity was initiated or amplified at a large ciconiiform rookery at Rio Bend (RB, Fig. 1A,B). The onset of WEE virus activity in early July was sudden and widespread with virus detected in 16 of 27 mosquito pools from 6 of 7 sites; only site 3 was negative. By August positive mosquito pools and positive chickens were found at all sites and transmission to chickens continued into late September. Virus activity was detected last in late October by seroconversions at sites RB, 3 and 4.

SLE virus activity began gradually and was first detected by one seroconversion at site 4 in late June. By late July positive mosquito pools were detected at all sites, and seroconversions were detected at sites 2, 3, 5, and RB. Chickens seroconverted at all sites by late August, and activity continued to be widespread through mid-September. Although positive mosquito pools were not found after September, seroconversions continued until mid-November.

In 1991 the onset of both WEE and SLE virus activity was widespread at study sites in both riparian and agricultural habitats, indicating that virus initiation and amplification was not restricted to the rookery at Rio Bend or to riparian vegetation along the New River. Therefore, in 1992 sites 2 and 3 were eliminated along the New River and sites 7, 8 and 9 were established at residential habitat along a transect from the New River east into El Centro (Fig. 1C,D). This sampling design increased the interval between sites and sampled residential habitat to assess the risk of transmission to humans.

Activity levels for both viruses were much lower in 1992 than in 1991. Mosquito pools positive for WEE virus occurred sporadically and did not correlate well with chicken seroconversions. WEE virus was first detected in June by seroconversions at sites 1, 8, and RB. Virus activity peaked in late July when it spread to include sites 4 and 5. Final seroconversions were detected in late August at sites 1, 6, and RB. Positive mosquito pools were collected at RB through early October when detectable WEE virus activity ended. WEE virus was not detected at sites 6, 7 and 9 in 1992.

SLE virus activity was first detected in late July when a single chicken at site 1 seroconverted. This site remained active until late September, when virus spread to sites 4, 5, 6, and 8. SLE virus was last detected by seroconversions at sites 5 and 8 in mid-October. Positive mosquito pools were recovered in September from sites 1, 4, and RB. SLE virus activity was not detected at sites 7 and 9.

Similar to 1991 both viruses were distributed widely in 1992, and the pattern of virus initiation did not support the hypothesis of an enzootic virus focus within the study area. Neither virus was detected at urban sites 7 or 9, but both viruses were detected at site 8 on the outskirts of El Centro. During both 1991 and 1992 virus activity was detected at marshes along the north shore of the Salton Sea in Coachella Valley prior to the Seeley area in Imperial Valley. Therefore, in 1993 sampling was expanded to include sites 10, 11, and 12 along the southern shore of the Salton Sea, site 13 at Brawley intermediate between the Salton Sea and the Seeley area, and site 14 at Calexico to detect possible virus movement northward along the New River from Mexico (Fig. 1E,F). Temporal sensitivity was enhanced by bleeding sentinels biweekly.

Virus activity was detected first in mid May, 1993 when a single pool of *Cx. tarsalis*, positive for WEE virus, was collected at site 8 near El Centro. Transmission to chickens was detected in late June at widely separated sites RB and 12. By mid-July seroconversions to WEE virus occurred at sites 1, 8, 11, 12, and RB. WEE virus activity then decreased rapidly, but was detected sporadically in mosquito pools until early September and by two seroconversions from August

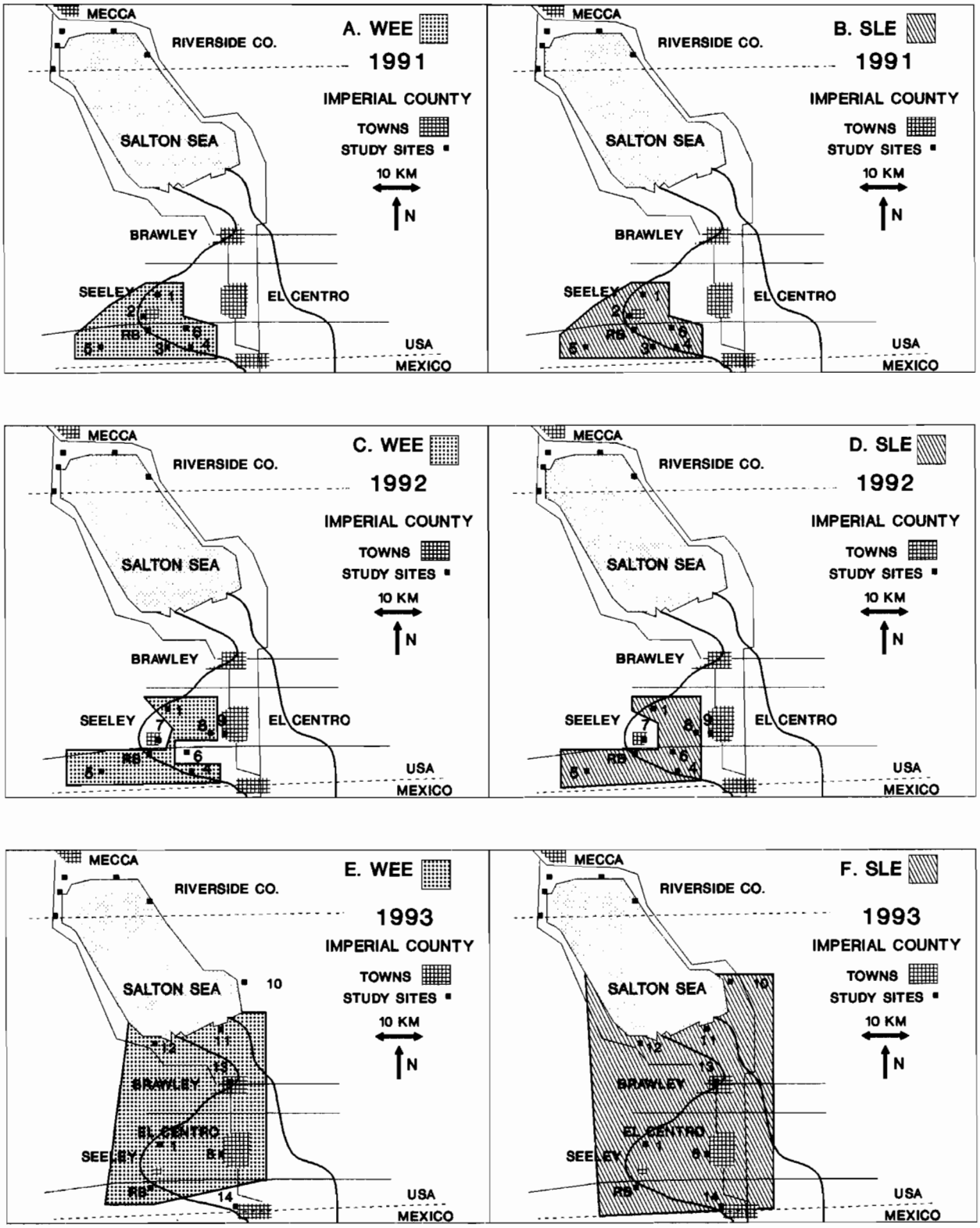

Figure 1. Distribution of study areas and seasonal WEE and SLE virus activity in Imperial Valley during 1991 to 1993. Sites were positive if >1 sentinel chicken seroconverted or >1 pool of *Culex tarsalis* tested positive for virus.

to October. WEE virus was not detected at sites 10, 13 and 14 (Fig. 1E).

SLE virus was first detected in late June by a single seroconversion at site 8 and by one positive mosquito pool at site RB. Virus activity subsequently remained sporadic in mosquito pools until late August, when chickens at sites 8, 12, and RB seroconverted. Virus activity peaked in late October when seroconversions were detected at all sites except 13. Virus activity was last detected in mid-November at sites 10 and 11. SLE virus was not detected at site 13 (Fig. 1F).

The onset of both WEE and SLE virus activity again was not associated with specific landscape features or habitats. Sampling biweekly enhanced temporal sensitivity, but did not clearly indicate the existence of maintenance foci of either virus. WEE virus activity initially was widespread at residential, riparian, marsh and agricultural habitats, whereas SLE virus activity commenced at low levels at residential and riparian habitats and then spread to marsh and agricultural habitats.

Temporal Patterns.

Despite changes in the distribution of our study sites from 1991 to 1993, the seasonality of the enzootic transmission of WEE and SLE viruses to sentinel chickens in the Imperial Valley was remarkably consistent (Fig. 2). Seroconversions to WEE virus peaked in July and generally preceded seroconversions to SLE virus which peaked in September. Similar temporal relationships were described previously for these viruses in the San Joaquin Valley (Reeves 1990). The geographical patterns of WEE and SLE virus activity was generally similar during all three years, beginning without association with a specific focus or habitat type and reaching most sites and habitats during the course of the transmission season. This heterogeneous pattern of virus initiation and amplification may be interpreted by two hypotheses:

1) Virus was amplified at a site or sites distant from Imperial Valley and then introduced by bird movements or as "rolling epizootics" into widely separated parts of Imperial Valley.
2) Both viruses overwinter throughout the Imperial Valley at levels undetectable by our sampling methods. Virus appearance may be related to focal increases in mosquito abundance and/or to the spatial distribution of herd immunity among reservoir bird species, rather than to the clustering of transmission activity at specific landscape features.

We hope to test these hypotheses further during 1994 by sampling along the Alamo River drainage in the eastern portion of the Imperial Valley and at additional

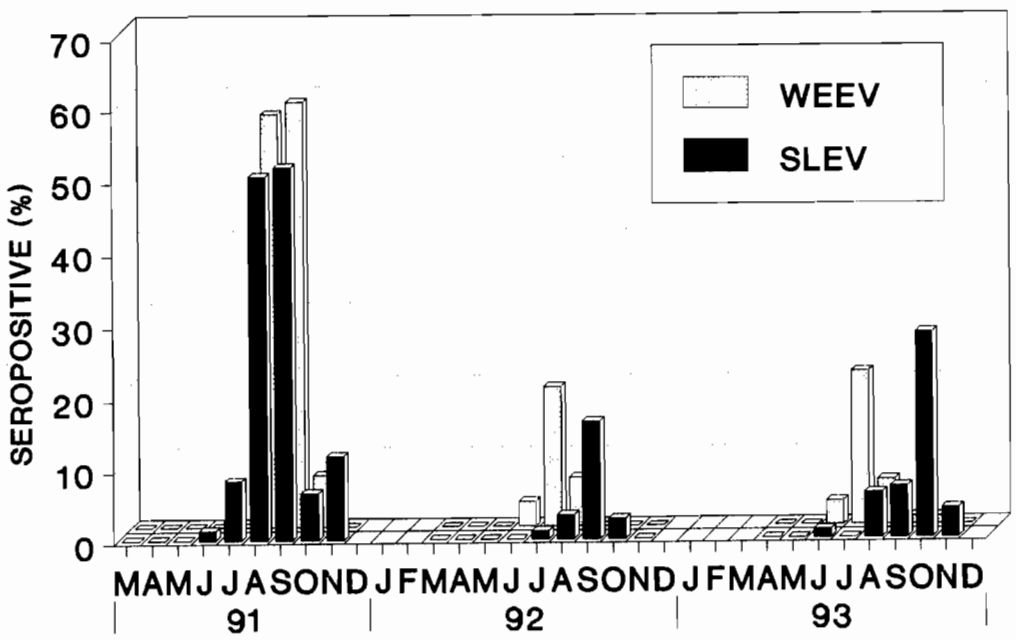

Figure 2. Percentage of sentinel chickens in Imperial Valley seroconverting to WEE or SLE viruses during each month in 1991 to 1993.

sites along the Mexican border.

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1994 Proc. Calif. Mosq. Vector Control Assoc. 62:37-38

U.S. NAVY DISEASE VECTOR AND ECOLOGY CONTROL CENTER EMERGENCY RESPONSE ROLE IN THE 1993 MIDWEST FLOODS

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As a result of constant, torrential rains throughout much of the Mississippi River Valley, the worst floods in recorded history occurred throughout the spring of 1993, continuing throughout the summer. Damage estimates range from $10-20 billion and large portions of the following states were declared federal disaster areas; Missouri, Minnesota, Nebraska, North and South Dakota, Wisconsin, Iowa, Illinois, and Kansas. The final impact is still to be felt, as whole communities are presently being bought out by the federal government, rather than being permitted to rebuild in flooded areas. In late July 1993, when the U.S. Navy Disease Vector Ecology and Control Center (DVECC) became involved, more than 500 square miles in Missouri and 640,000 acres in Illinois were flooded. In addition, 53 counties in Nebraska, 17 counties in North Dakota, all 99 counties in Iowa, and 12 counties in Kansas were affected by flooding and declared federal emergency areas.

One flood-related problem was, of course, mosquitoes involving two distinct public health problems, mosquito-transmitted viruses and pest mosquitoes. Not only was there severe flooding throughout extensive areas, but there had been higher than normal rains the entire spring and summer in the entire midwest region. Two arthropod-borne viruses capable of causing widespread epidemics are enzootic in the region; St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE). Although arbovirus activity had been low in the preceding years, no one was sure what impact the massive flooding would have. In addition, levee work and flood recovery activities were being severely hampered by nuisance mosquitoes.

On July 27, 1993 the State of Missouri in cooperation with the Centers for Disease Control and Prevention (CDC), Ft. Collins, requested vector control assistance from our unit, DVECC-Jax. Their request was forwarded from the Federal Emergency Management Agency (FEMA) through the Disaster Coordinating Office for that region, to the Department of Military Support at the Pentagon, and then to FORSCOM, Ft. McPherson, Georgia. The official tasking from FORSCOM for our assistance was received as a flash message on August 2, 1993.

In the meantime, my boss, Commander Wooster, and I had been invited by the CDC to attend a regional vector surveillance/control meeting hosted by the CDC in Kansas City, Missouri on July 29-30, 1993. At that meeting, state epidemiologists and entomologists from the nine affected states met to conduct basic planning of how to approach potential disease and nuisance problems. CDC representatives also established the ground rules for determining if FEMA support would be given to a state for mosquito control (Table 1).

As a consequence of the Kansas City meeting and phone conversations with Captain Richard Gorham, of the United States Public Health Service (USPHS), Director of Emergency Support Function 8 (ESF-8, Medical) in St. Louis, the initial request for vector control assistance was changed to a request for vector surveillance. Several factors affected that decision, not the least of which was the total lack of baseline mosquito surveillance data. In the event mosquito control efforts were needed later, it became apparent that baseline data must be developed. In addition, vector control efforts, short of aerial treatment by the Air Force, would have been temporary, driven by politics vice science and logistically impossible due to the locations of affected cities and towns along the flooded rivers. Furthermore and most importantly, by surveying the mosquito population for SLE virus, it would be possible to develop an accurate picture of the arbovirus situation before human illness or death could occur.

In response to the immediate need for mosquito control assistance to aid flood relief workers, DVECC-Jax provided ESF-8 the following: insecticide purchasing and label information, assistance in the purchase (with FEMA funds) of 30 hand-held P-1 ULV machines (later donated to flood-affected county health departments), and

CRITERIA FOR FEMA AUTHORIZATION TO COMMIT FUNDS FOR MOSQUITO/PEST CONTROL (Developed by Centers for Disease Control and Prevention - C. Moore and R. Nasci)
1. Disease Threat (present or imminent) to Humans and/or Animals.
2. Mosquitoes Interfering with or Interrupting Recovery Activities.
3. Disruption of Normal Community Services (e.g., police, fire, etc.).
4. Increased Stress Levels on Already Stressed Population (i.e., large numbers of secondary infections in children due to bites).

wrote a 2-page manual to explain proper use of the P-1s.

On August 4, 1993 a six man DVECC-Jax team reported to Mr. Joe Lamb, Emergency Support Function-8 Director, in St. Louis. Meetings were held with Dr. Satalovich, Missouri State Epidemiologist, Dr. Roger Nasci, CDC, and Mr. Lamb to fine tune details of the mosquito surveillance program.

Basically, communities within four separate regions along the Missouri and Mississippi Rivers were surveyed; Kansas City, Hannibal, St. Louis, and Cape Girardeau. The St. Louis area was covered by St. Louis County mosquito control personnel. Navy personnel worked in Kansas City, Hannibal and Cape Girardeau.

Our three teams reported to their respective areas, were in operation the evening of August 6, 1993 and continued to collect six nights per week from that time on. Each team consisted of a medical entomologist and a preventive medicine technician. Within each area, three distinct areas (routes) were checked each week, two nights each week.

Bioquip light equipped CO_2-baited traps were set in six sites each day (in each of the three regions) in the late afternoon and picked up early the next morning. The average driving time to cover each route was 1.5-2 hours, and that was done twice daily. Upon return to the respective work site each morning, the tedious job of sorting and identification was carried out. Our primary concern was with *Culex* mosquitoes, *Culex tarsalis* Coquillett or *Culex pipiens* Linneaus. Specimens of these species were placed in vials and submitted twice weekly for testing.

The first two weeks' mosquito collections were delivered by courier to the Ft. Collins CDC laboratory and tested for the presence of SLE antigen using an antigen capture ELISA analysis. After August 20, 1993, all *Culex* from the four regions were sent to a laboratory at Southeastern Missouri University in Cape Girardeau.

Replacement personnel from DVECC-Alameda and NEPMU-2 (Norfolk, Virginia) began the turnover process August 25, 1993 and completed the process September 2, 1993. Other entomologists involved in this 50 dav operation included Lieutenant Commander Breaud and Lieutenant Commander Lluberas of DVECC-Jax, Lieutenant Commander Annis at NEPMU-2, Norfolk, and Lieutenant Schoeler and Lieutenant Bartholomew of DVECC-Alameda. Preventive medicine technicians included HMCS DeCristofaro, HM1 Wolfert and HM2 Roman of DVECC-Jax; HM1 Hogue NEPMU-2, Norfolk; and HM2 Hill and HM2 Zollicoffer of DVECC-Alameda.

During our activities in Missouri, more than 350,000 mosquitoes were captured, sorted, and identified. The program established in Missouri was much more extensive than that established in any of the other eight states, and our program accounted for more than 50% of the vector mosquitoes captured and tested for virus activity in the midwest area. From those mosquitoes more than 900 pools of potential vector specimens were prepared for laboratory analysis. In addition, more than 24,000 miles were logged while conducting the surveillance program, equal to the earth's circumference at the equator. Two and one-half tons of dry ice were used.

No pools of *Culex* mosquitoes were positive for SLE. In fact, no positive pools were found anywhere in the nine state region. By late August, the rapid risk assessment process had determined that the risk for arboviral disease was very low in the disaster area.

Though no positive pools were found, our efforts were integral to developing an accurate picture of the SLE situation in this most extraordinary flood situation. The overwhelming opinion heard again and again from farmer to health practitioner was "I'm glad for once to see the federal government doing something worthwhile". From a cost-benefit basis, the total cost for surveillance in the entire nine state region was about $100,000 (CDC estimate). If rapid risk assessment surveillance had not been developed, considerably more cost would have been incurred in conducting prophylactic mosquito control operations. Though not well understood, human encephalitis cases appear to increase the year following flood situations. For example, flooding in Illinois in 1963 was followed by 56 SLE cases in 1964 and major flooding along the upper Mississippi and Ohio rivers in 1973 was followed by epidemics of SLE and WEE in 1975. So, it is very important that good baseline data is now available and that each of the affected states conducts a thorough and extensive disease surveillance program in 1994-95.

1994 Proc. Calif. Mosq. Vector Control Assoc. 62:39-43

PLAGUE SURVEILLANCE AND DISEASE ACTIVITY IN CALIFORNIA DURING 1990-1993

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ABSTRACT

Bubonic plague became entrenched in wild rodent populations in California following the apparent introduction of the disease in the early 1900s and continues to persist within a series of geographical disease foci. Epizootics of plague occur among susceptible wild rodents in a cyclic nature within disease foci, at times leading to associated human cases. Delayed-treatment bubonic plague cases are always of concern since untreated cases lead to secondary plague pneumonia and potential pneumonic infection of patient contacts. One case of bubonic plague occurred in 1992 in California from exposure to infected wild rodents fleas in the Sierra Nevada Mountains. Plague was confirmed among wild carnivores, domestic pets, wild rodents and fleas in 28 California counties during 1990-1993. Plague continues to persist in endemic regions in California many of which are now under development or heavily used for recreational purposes. In disease endemic regions of California there is a definite need for health and landuse agencies to develop plague management strategies and preventive programs.

The earliest documented plague activity in California occurred in the early 1900s, consisting of urban rat epizootics and ensuing human epidemics with over 50% mortality (Link 1955). The disease became entrenched in wild rodent populations following this apparent introduction and continues to persist in California in a sylvatic cycle, sustained through the interaction of the disease bacteria, the rodent hosts, the vector fleas, and environmental conditions within the framework of a series of geographical disease foci. Cyclic plague epizootics among susceptible rodent populations occur within a given focus, alternating with inter-epizootic quiescent periods. This pattern has been observed to occur in the following geographical regions of California: Modoc Plateau, Cascade Mountains, Sierra Nevada Mountains, North Coast Ranges, Inter-mountain valleys (Northeastern California), Kern Plateau and Greenhorn Mountains, Piute and Tehachapi Mountains, Central Coast Ranges (San Francisco Bay to Ventura), San Gabriel Mountains and urban fringe of Los Angeles, San Bernardino Mountains, San Jacinto Mountains, Palomar and Cuyamaca Mountains

HISTORY AND HUMAN CASES

Since the last epidemic of plague in California in 1924, periodic human plague cases have been associated with epizootics in each of the above geographical regions, with the exception of the Palomar and Cuyamaca Mountains. Cases may be contracted from bites of infected fleas, by direct contamination with infected animal tissue or blood, or in some instances by pneumonic transmission from infected animals, such as domestic cats. Modern travel allows for infection in one locale, and illness, death and potential pneumonic secondary cases in a second locale, great distances away. As demonstrated by Mann et al. (1982), persons infected in plague endemic regions and then travelling to plague free areas have higher fatality rates than non-travellers. Delayed-treatment cases are always of concern since untreated cases of secondary plague pneumonia could lead to primary pneumonic cases among patient contacts through direct respiratory infection. The last person-to-person transmission of pneumonic plague in the United

States occurred in California in 1924 (Link 1955).

In the past 23 years in California, since 1970, there have been 32 human cases, seven resulting in fatality. Sixteen cases (50%) were exposed in recreational settings away from their home environment, 12 involving extensive travel. In one fatal case, illness, hospitalization and death occurred in San Diego, 600 miles from the point of exposure during an epizootic among ground squirrels in the Sierra Nevada Mountains. Fifteen cases involved exposure in the home or work environment. Four cases involved backyard exposure during epizootics among ground squirrels and chipmunks at residences or vacation homesites in mountainous regions of the state.

The encroachment of development into formerly rural areas and the ensuing development of peridomesticity in certain important species of wild rodents in California allows for the continuing potential of human cases under backyard peridomestic circumstances (Nelson et al. 1986). Four cases involved direct exposure to infected domestic cats. One case was a primary pneumonic transmission from an infected cat to the cat owner where both died (Werner et al. 1984).

DISEASE ACTIVITY

One human plague case occurred in California between 1990-1993 (Table 1). This case occurred in August, 1992 in a 6-year old boy, resident of Fresno, who had visited the Ansel Adams Wilderness Area near Yosemite with his family on a wilderness horseback trip. The boy became ill three days after arriving home. He was hospitalized in Fresno with fever, malaise, vomiting, headache, and a painful, tender, swollen right groin. Aspirate from the inguinal swelling was confirmed as *Yersinia pestis* on culture by the California Department of Health Services, Microbial Diseases Laboratory. The boy was treated with a prescribed regimen of antibiotics and subsequently recovered (Anonymous 1992).

Dead ground squirrels had been observed by the family at one of the high altitude campsites in the wilderness area. The boy had evidence of fleabites. A thorough investigation of the actual campsite was not possible due to the remoteness of the area. The U.S. Forest Service, Sierra National Forest, was alerted and trailheads were posted with plague warnings. All wilderness users and rangers were given plague precautionary information.

PLAGUE SURVEILLANCE

As required by the International Health Regulations of the World Health Organization, and the California Health and Safety Code, all human plague cases in the state are investigated epidemiologically. Plague surveillance and control is maintained to protect the public, as consistent with the letter and intent of the above regulations.

An inter-agency cooperative plague surveillance and suppression program, coordinated by the California Department of Health Services, Vector-Borne Disease Section, consists of the following integrated activities:

- Epidemiological and epizoological investigation of human cases.
- Education of health and landuse agency personnel

Table 1. Positive plague evidence among humans, carnivores, rodents and fleas in California during 1990-1993. Carnivore and rodent sera tested positive by passive or indirect hemagglutination test, flea pools tested positive by mouse inoculation and culture test, and rodent carcasses tested positive by fluorescent antibody and culture tests.

Sampling Method	Number Positive/Number Sampled* (Percent Positive)				
	1990	1991	1992	1993	Totals
Positive Human Cases	0	0	1	0	1
Carnivore Sera	96/463 (20.7)	24/170 (14.1)	14/163 (8.6)	14/232 (6.0)	148/1028 (14.4)
Rodent Sera	38/1980 (1.9)	31/979 (3.2)	46/720 (6.4)	107/1326 (8.1)	222/5005 (4.4)
Flea Pools	10/195 (5.1)	3/130 (2.3)	7/77 (9.1)	3/80 (3.7)	23/481 (4.8)
Rodent Carcasses	17/79 (21.5)	2/56 (3.6)	9/65 (13.8)	9/104 (8.7)	37/304 (12.2)

* Table does not include material tested by Los Angeles County Department of Health Services, Comparative Medical and Veterinary Services Laboratory for 1992 and 1993.

and the public.

- Maintenance of an intelligence network of trained cooperators who report suspicious disease activity and submit suspect material for laboratory testing.
- Serological surveillance of disease activity among wild rodents using wild carnivores and domestic pets in endemic regions as sentinels.
- Direct surveillance and sampling of disease hosts and vectors in endemic regions.
- Laboratory testing.
- Investigation of epizootics.
- Vector suppression.
- Disease management and prevention.

SURVEILLANCE RESULTS

In the integrated surveillance program in California, plague positives have been recorded in 40 of the state's 58 counties since 1962 (California Department of Health Services records). In the period 1990-1993, evidence of plague was found in 20 counties in 1990, 13 counties in 1991, 18 counties in 1992, and 22 counties in 1993 (Table 2). Plague epizootics, as documented by bacterial culture from rodent hosts or vector fleas, or clusters of high antibody titers among rodents, occurred in a variety of habitats including the Montane Forest of the Sierra Nevada Mountains, the coastal interior oak woodland and chaparral of central California, the Yellow Pine forest of the Transverse Ranges and San Jacinto Mountains in southern California, and the juniper-sagebrush lava rim habitat of the Tulelake and Lower Klamath Basins of northeastern California (Table 3).

Epizootic plague occurred each year during 1990-1993 in two counties (El Dorado and Mono), in three of the four years in two counties (Nevada and Placer), in two of the four years in six counties (Alpine, Kern, Plumas, Riverside, San Bernardino, and Tulare), and in one of the four years in eight counties (Fresno, Inyo, Madera, Monterey, San Diego, Sierra, Siskiyou, and Ventura).

Table 2. Number of counties in California with positive plague evidence during 1990-1993. Rodent epizootics documented by laboratory confirmation, wild carnivores tested positive by passive or indirect hemagglutination test, and domestic pets tested positive by fluorescent antibody and culture tests or hemagglutination test.

Plague Positive	Number of Counties			
	1990	1991	1992	1993
Specimens	20	13	18	22
Positive Human Cases	0	0	1	0
Rodent Epizootics	9	5	6	13
Wild Carnivores	14	7	10	9
Domestic Pets	2	2	1	3

Epizootics were sustained among a complex of ground squirrels, chipmunks, and their fleas in Montane Forest, among Beechey ground squirrels and fleas in oak woodland and Yellow Pine forest of Central and Southern California, and among woodrats and fleas in juniper/sagebrush lava rim habitat of northeastern California.

The highest percentage plague-positive samples (14.4%) in California during 1990-1993 have been from wild carnivores (Table 1). Wild carnivores serve as excellent serological sentinels of plague activity among rodent populations which they feed upon in plague endemic regions (Smith et al. 1984). Carnivores are infected by feeding upon both plague reservoir rodent species, as well as plague susceptible rodent species which sustain epizootics. The majority of samples in the California surveillance system are submitted through a contractual agreement with the U.S. Department of Agriculture, APHIS, Animal Damage Control program. Additional samples are submitted by wildlife researchers, and private trappers.

The testing of suspect rodents submitted through the intelligence network in endemic regions yielded the second highest percentage positives (12.2%) in California during 1990-1993 (Table 1). An intelligence network of trained observers, an informed public, and trained vector control program personnel reacting to suspect reports of potential epizootic die-offs among wild rodents provides for a sensitive surveillance network of disease detection.

The testing of rodent sera collected by direct sampling by state and local vector-borne disease surveillance programs yielded a 4.4% overall positive rate in California during 1990-1993 (Table 1). Selective rodent species within specific endemic regions of California are used as serological sentinels of plague activity. An emphasis is placed on early season sampling to provide for an early warning detection and early response to prevent epizootic amplification.

The testing of flea pools collected from live rodents and by swabbing rodent burrows during epizootic investigations yielded a 4.8% positive rate in California during 1990-1993 (Table 1). The flea species, *Diamanus montanus*, found on ground squirrels, and *Monopsyllus eumolpi* and *M. ciliatus*, found on chipmunks, were the fleas most frequently found plague positive. All the above species are known plague vectors in California (Eskey and Haas 1940), and readily bite

Table 3. Occurrence of plague in California by geographic region during 1990-1993. Enzootics are defined by serological evidence of plague among rodents or wild carnivores. Epizootics are defined by positive amplifying rodents and fleas confirmed by laboratory bacterial isolation and/or clusters of high antibody titers by hemagglutination test.

Geographic Region	Occurrence of Plague			
	1990	1991	1992	1993
Modoc Plateau	Enzootic	-	Enzootic	-
North Coast Ranges	Enzootic	-	Enzootic	Enzootic
Inter-Mountain Valleys	Epizootic	-	Enzootic	Enzootic
Cascade Mountains	-	-	-	Enzootic
Sierra Nevada Mountains	Epizootic	Epizootic	Epizootic	Epizootic
Kern Plateau	Enzootic	-	-	Enzootic
Transverse Ranges	Epizootic	Enzootic	Enzootic	Epizootic
Central Coast Ranges	Epizootic	Enzootic	Enzootic	Enzootic
San Gabriel Mountains	Enzootic	-	Enzootic	-
San Bernardino Mountains	Epizootic	-	-	Epizootic
San Jacinto Mountains	Enzootic	Epizootic	Enzootic	Epizootic
Palomar and Cuyamaca Mountains	-	-	Enzootic	Enzootic

humans (Barnes 1982) in the absence of their normal hosts.

CONTROL AND PREVENTION

In areas where epizootics were confirmed and a high risk of exposure to humans was identified, plague suppression was recommended and initiated to control vector fleas and protect human health. Recreation sites with highest risk were temporarily closed during control, and reopened following evaluation of successful control operations. Control was achieved utilizing insecticidal dusts to kill vector fleas, applied by dusting of rodent burrow systems and through the use of bait stations. Materials currently available for wild rodent flea control in California include Diazinon 2%, and Sevin 10-50 insecticidal dusts.

DISCUSSION

High densities of plague amplifying host rodent species which harbor proven vector fleas capable of infecting man exist in many disease endemic regions of California. High densities of these plague susceptible rodent hosts and vector fleas are pre-requisites to epizootics of bubonic plague in endemic areas (Smith and Lusk 1990). Many of these same regions are being developed for homesites, or are heavily used for recreational activities. California experienced a four fold increase in the number of human plague cases in the twenty year period, 1973-1993, as compared to the previous twenty year period, 1953-1973 (California Department of Health Services records). All cases have been directly or indirectly associated with epizootics among wild rodent populations.

CONCLUSIONS

It is prudent that health and landuse agencies in plague endemic regions of California maintain an awareness of sylvatic plague and work together to prevent human cases. Within the framework of a geographical disease foci, plague will seem to disappear during inter-epizootic periods. The disease again becomes noticeable when as a part of it's cyclic pattern it suddenly reappears causing epizootic mortality among populations of susceptible rodents. In many instances, plague epizootics reappear at precisely the same locations where epizootic mortality was observed in the past. If we become complacent in surveillance and disease management, and epizootics flourish, the first indication received by health authorities announcing a reoccurrence of the disease may be the travelling human pneumonic case with epidemic potential.

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PLAGUE SURVEILLANCE IN SAN BERNARDINO COUNTY DURING 1993

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ABSTRACT

In 1993, the San Bernardino County Vector Control Program carried out 17 plague surveys at various sites in San Bernardino County. Of the 148 live trap collected rodents, 89% were ground squirrels, including 123 *Spermophilus beecheyi*, 8 *Spermophilus lateralis*, and 1 *Ammospermophilus leucurus*. The remaining rodents consisted of 2 *Tamias merriami*, 2 *Dipodomys merriami*, 5 *Neotoma lepida*, 3 *Neotoma fuscipes*, and 4 *Peromyscus californicus*. None of the 468 fleas collected from these animals tested positive for plague. In addition, sera collected from all rodents through July 1993 also tested negative for plague. However, in mid-August, 12 out of 21 sera from a single campground tested plague-positive, showing antibody titer as high as 1:4096. This resulted in immediate closure of the campground and posting of "Plague Warning" signs within one mile of the campground. Almost all visible rodent burrows were treated with 3% diazinon dust to control fleas.

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 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 542 AD, which involved Arabia, Europe, and North Africa. The second pandemic, the "Black Death", of the Middle Ages (1300s) 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 1992).

Following the introduction of plague in North American, 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 Nevadas from Lassen Peak to the Kern Plateau, and the Tehachapi, San Gabriel, San Bernardino, and San Jacinto Mountains 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

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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 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 at plague enzootic areas during the season (April through October). The data generated in routine plague surveillance during 1993 are presented here in this paper.

MATERIALS AND METHODS

In routine plague surveys, the general method described by Lang and Wills (1991) was used as follows:

In a typical daily survey, 20-25 Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, WI) baited with peanut butter and rolled oats were set at appropriate shaded locations close to rodent burrows in the survey area. In a campground situation, traps were also set near picnic tables that attract wild rodents, especially ground squirrels and chipmunks. For location purposes, each trap site was flagged with orange color nylon ribbon hung from an adjacent bush or tree. The traps were usually set by 10:00 a.m. and picked up in the early afternoon the same day. For smaller rodents, such as wood rats, Sherman live traps were used overnight around rodent nesting sites.

All traps with live animals were brought to a shady site for processing. Each trap with an animal inside was transferred into an 18" x 36" clear polyethylene bag (3 mil.). A ball of cotton drenched in ethyl ether was introduced into the bag and the bag was kept tightly closed with a rubber band until the animal was anesthetized (usually in 5-15 min). The animal was taken out of the bag and cage and transferred to a white enamel pan (12" x 7.5" x 2" deep) where the fleas were combed out using a stiff bristled brush. The fleas from each animal were collected in labelled 2 ml polypropylene 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 needle. Before the animal regained consciousness, all pertinent data such as species, sex, and reproductive stage were recorded after which it was released back into its habitat. All necessary information on the survey site was also recorded before leaving the area.

All collected blood samples were brought to the laboratory where they were centrifuged for 20 minutes at 2000 rpm before the serum from each sample was transferred to labelled 2 ml polypropylene screw cap tubes. All 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 in Sacramento would immediately inform us via telephone of any plague-positive samples. In the event of plague-positive sample confirmation, the standard plague epizootic protocol would be followed. The protocol includes posting the area with "Plague Warning" signs, public education, press releases (if warranted), immediate evacuation (if a campground), pre-treatment flea index evaluation depending on the date of original survey, burrow dusting for fleas followed by rodent control, if necessary, and finally post-treatment evaluation flea index prior to re-opening the area for public use, especially a campground or public park.

RESULTS AND DISCUSSION

During 1993, the SBCVCP carried out routine plague surveys at 17 locations in San Bernardino County. Except for one desert site, all others were situated in plague enzootic mountain and foothill areas (Table 1).

Of the 148 live trap collected rodents, 132 (89%) were ground squirrels species, namely, 123 *Spermophilus beecheyi*, 8 *Spermophilus lateralis* and 1 *Ammospermophilus leucurus*. Additional specific collection records for the sites include exclusively *S. becheyi* (except for 1 *Tamias merriami* each) from El Rancho Country Club and Hanna Flats Campground; eight golden-mantled ground squirrels, *S. lateralis*, from the Heart Bear Campground; one white-tailed antelope squirrel, *A. leucurus*, one desert wood rat, *Neotoma lepida*, and two (1♂ and 1♀) Merriam's kangaroo rats, *Dipodomys merriami*, at the Hole in the Wall Campground; and four (2♂ and 2♀) California mice, *Peromyscus californicus*, four (2♂ and 2♀) wood rats, *N. lepida* and 3 male dusky-footed wood rats, *Neotoma fuscipes*, at the Big Bear Lake (North Shore) site.

All 468 fleas combed out from the rodents (except wood rats) tested negative for the plague bacterium. Initial examination of ectoparasites recovered from the rodent samples include 37 (13♂ and 24♀) *Oropsylla (Dimanus) montanus* from Yucaipa Park; and two male *Oropsylla idahoensis* and 218 (83♂ and 137♀) *O. montanus* from the Hanna Flats Campground..

In addition, all blood sera samples collected from squirrels and chipmunks during April through July were negative for plague. However, 12 out of 20 *S. beecheyi* sera taken in early August at one site - Hanna Flats

Table 1. Summary of plague surveys carried out in San Bernardino County during 1993.

Survey Date	Site	Altitude (Ft)	SERA SAMPLES Tested (♂,♀)	SERA SAMPLES Positive		ECTOPARASITES Tested (♂,♀,?)	ECTOPARASITES Positive
4-7-93	Serrano Campground	6800	10 (6,4)	0		55 (?)	0
4-28-93	Devore Foothill Little League	2000	3 (1,2)	0		7 (?)	0
5-7-93	Oso-Lobo Group Camp	7400	2 (1,1)	0		10 (7,3)	0
5-11-93	Yucaipa Regional Park	2700	5 (1,4)	0		10 (7,3)	0
5-12-93	Yucaipa Regional Park	2700	6 (0,6)	0		27 (6,21)	0
5-25-93	Barton Flats Campground	7000	12 (5,7)	0		35 (?)	0
6-3-93	El Rancho Country Club, Rialto	2000	5 (1,4)	0		18 (?)	0
6-7-93	East Flats Campground	7000	8 (3,5)	0		0	0
6-9-93	Rio-Barranca Group Camp	4700	4 (1,3)	0		9 (?)	0
6-10-93	Silverwood Lake	4700	21 (5,16)	0		22 (?)	0
6-21-93	Heart Bar Campground	8000	8 (3,5)	0		0	0
6-28-93	Apple White Campground	2700	10 (5,5)	0		41 (?)	0
7-7-93	Hanna Flats Campground	7000	18 (5,13)	0		44 (8,22,4)	0
7-21-93	Hole in the Wall Campground	4000	4 (1,3)	0		0	0
7-23-93	Big Bear Lake Ranger Station	6800	11 (3,8)	Not Tested		0	0
8-9-93	Hanna Flats Campground	7000	21 (10,11)	12		190 (73,117)	0
			148 (51,97)	12	Totals	468 (101,166,201)	0

Campground in the San Bernardino Mountains - were diagnosed plague-positive, showing titers ranging from 1:16 to 1:4096 for plague antibodies. Generally, an antibody titer of 1:16 or higher in the serum is considered plague-positive. Moreover, the flea index in the August survey was very high (4 to 36 fleas/animal). A flea index of ≥ 1.0 is considered high enough to require flea control.

Upon receiving confirmation of the plague-positive samples, the Hanna Flats campground was closed and posted with "Plague Warning" signs, thus prompting immediate evacuation of the campers. Two other sites within one mile of the closed campground were posted with plague warning signs. At the same time, press releases were issued advising people to take the necessary precautions in the plague affected areas. The following week, rodent burrows at the closed campground were dusted with 3% diazinon dust. Pending post-treatment evaluation due to Hantavirus concerns and the onset of cold weather, the campground remained closed during the winter. A post-treatment evaluation of both the rodent and ectoparasite (flea) populations will be made prior to reopening the campground for public use in late spring, 1994.

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HANTAVIRUS (BUNYAVIRIDAE) IN DEER MICE (*PEROMYSCUS MANICULATUS*) IN ORANGE COUNTY DURING 1993

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Nearly all of the Hantavirus related forms have been found in Europe and Asia. Only two were known here in the United States, the most notable being Prospect Hill Virus (PHV) associated with *Microtus pennsylvanicus*, a vole species from the eastern United States. This virus has not been demonstrated to cause disease. The other known strain was found in Baltimore in rodents and also has no known pathogenicity.

In June of 1993, the Centers for Disease Control and Prevention (CDC) in Atlanta identified a new Hantavirus associated with a cluster of unusual fatal illnesses in the Four Corners area of the southwestern United States (the area surrounding the common corner of the states of Utah, Colorado, New Mexico and Arizona). Soon afterward, the CDC confirmed the link between deer mice (*Peromyscus maniculatus*) and the human cases caused by this new Hantavirus.

Because etiologic or disease-causing agents (e.g., bacteria, viruses, etc.) are often associated with a specific reservoir host species (or species group) and/or vector species throughout their distributional range, our laboratory decided to initiate a Hantavirus Serosurveillance Program. This decision was predicated on the fact that large numbers of deer mice (*P. maniculatus*) had been collected from many areas of Orange County during the District's Lyme Disease Serosurveillance Program.

The Hantavirus Serosurveillance Program (HSP) officially began on June 25, 1993 at Crystal Cove State Park. On August 3, 1993, Dr. Richard Jackson of the California State Department of Health Services (CDHS) notified the state's public health agencies about two confirmed cases (both fatal) of Hantavirus in California; one in Santa Barbara County, the other in Mono County. Shortly thereafter, Mr. Charles Myers (CDHS, Ontario office) recommended that we postpone our HSP until a safe set of procedures could be developed. We followed his recommendation immediately and also began developing a trapping and bleeding protocol that would meet suggested safety standards and protect the members of the surveillance team. Mr. Al Blevans (Employee Health Services) and Mr. Gary Zimmerman (Environmental Health), both from the Orange County Health Care Agency, supervised the development of a workable and safe rodent trapping, handling, and bleeding protocol.

During the time of surveillance protocol development, banked, frozen (-80°C) rodent sera (277 samples), the result of the District's Lyme Disease and Bubonic Plague Serosurveillance Programs, were sent on August 24, 1993 to Dr. Thomas Ksiazek, CDC, Atlanta. Preliminary results were received from Dr. Ksiazek on September 3, 1993 and confirmed positive results were faxed to us on September 13, 1993. Of the 277 rodent sera samples sent to CDC for testing, 57 were samples from *Peromyscus* spp. trapped in 1992 at the TRW Test Site near the city of San Clemente (Table 1). Thirty-four of these 57 samples were from *P. maniculatus* and five of those specimens tested positive for Hantavirus (Table 2). Positive-testing *P. maniculatus* were collected in February, April, and July from several collection localities within the TRW test facility (Fig. 1).

A meeting was held on September 17, 1993 with TRW representatives to discuss our findings and to make recommendations for problem resolution. Dr. Richard Evans (Orange County Animal Control) and Ms. Barbara Peck (Orange County Health Care Agency) were also in attendance.

The Hantavirus Serosurveillance Program was recommenced at and nearby the TRW Test Site with further trapping and blood sampling on October 17 and November 4, 1993. The sera from these two sampling periods were sent to the CDC laboratory (Atlanta) on November 8, 1993. Results (all negative) were received from the CDC on December 9, 1993.

ACKNOWLEDGEMENTS

Our gratitude is given to Mr. Minoo B. Madon (California State Department of Health Services), Ms. Tina J. Smith, and Mr. Jeffrey Dean (both formerly with the Orange County Vector Control District) for assistance with trapping rodents in the field. Appreciation is also extended to Mr. Gil Challet (Orange County Vector Control District) for his support of these field studies. Special thanks are extended to Drs. James Childs and Thomas Ksiazek (CDC) for their rapid response to our information requests and for test results.

Table 1. Total number of *Peromyscus* spp. trapped, blood sampled, and tested for Hantavirus from the TRW Test Site Facility near San Clemente, California during 1992.

Peromyscus Host Species	# Sampled	Test Facility Collection Site	Collection Date
P. boylii	3	Blind Canyon	AUG 5
P. californicus	3	Blind Canyon	AUG 5
P. californicus	8	Near entrance	FEB 25
P. californicus	6	Talega Canyon	OCT 28
P. eremicus	1	Blind Canyon	AUG 5
P. eremicus	2	Talega Canyon	APR 7
P. maniculatus	5	Blind Canyon	AUG 5
P. maniculatus	8	Near entrance	FEB 25
P. maniculatus	21	Talega Canyon	OCT 28
Total	57		

Table 2. Temporal and geographic distribution of Hantavirus-tested *Peromyscus maniculatus* from the TRW Test Site Facility near San Clemente, California during 1992.

Test Site Locality	Collection Date	# Trapped	Positive #	Positive %
Avenida PicoEntrance	FEB 4	6	0	0
Avenida Pico Entrance	FEB 25	2	1	50
Blind Canyon	FEB 25	4	2	50
Blind Canyon	AUG 5	1	0	0
Talega Canyon	APR 7	7	1	14
Talega Canyon	MAY 28	5	0	0
Talega Canyon	JUL 9	2	1	50
Talega Canyon	OCT 28	7	0	0
	Totals	34	5	15

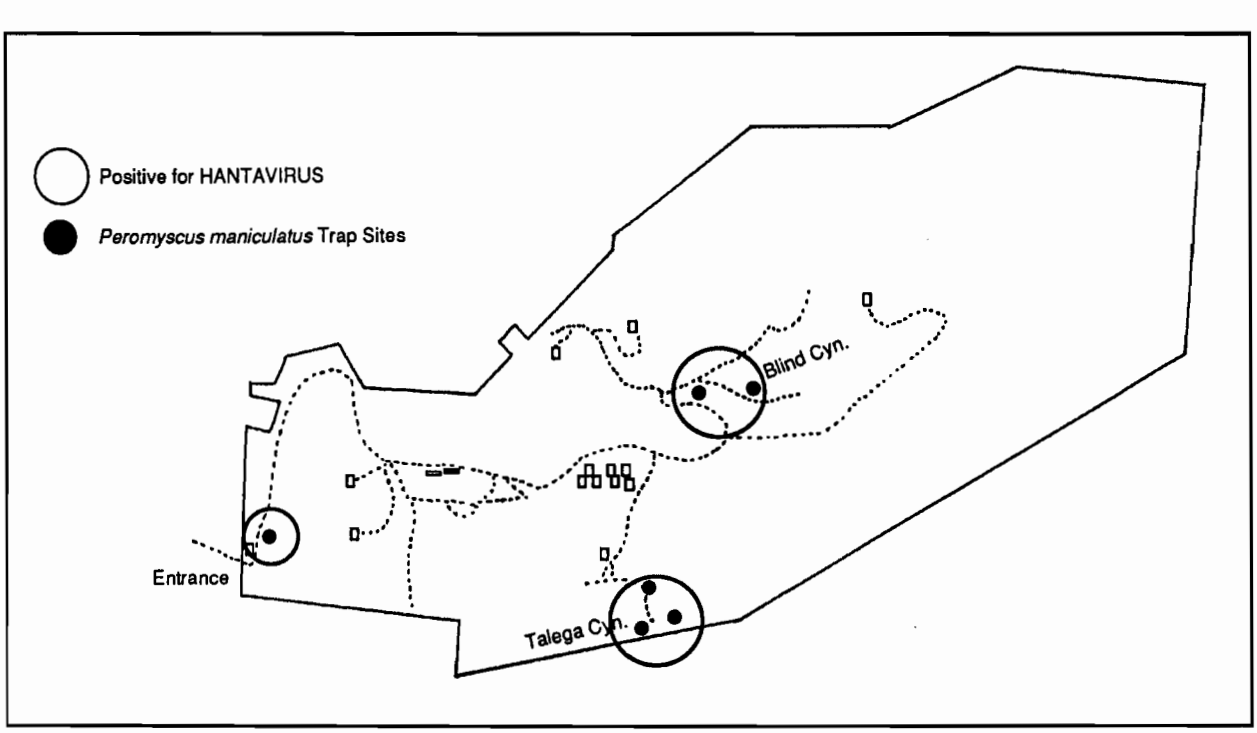

Figure 1. Collection sites and Hantavirus-positive (antibody) records for *Peromyscus maniculatus* at the TRW Test Site Facility near San Clemente, California during 1992.

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THE EFFECTIVENESS AND ENVIRONMENTAL IMPACTS OF RUNNELLING, A MOSQUITO CONTROL TECHNIQUE

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ABSTRACT

The use of shallow channels (runnels) is potentially important in integrated mosquito control in Western Australia. This study addresses the impacts of runnelling on saltmarsh flora and fauna. The four experimental sites ranged from saline to relatively fresh water. Runnelling was found to reduce larval mosquito numbers, but impacts on aquatic flora were also recorded.

This presentation explains the technique of runnelling, discusses the study undertaken in Western Australia, and presents findings from that study. The study is funded by the Health Department of Western Australia.

Runnelling.

Runnelling is a mosquito control technique which has been successfully tried in Queensland and New South Wales (Dale et al. 1993) and is presently being tested in the south of Western Australia. Mosquitoes controlled by runnels are saltmarsh species which lay their eggs on damp mud and hatch when inundated by the tide or rain.

Runnels are very shallow spoon-shaped channels which connect pannes in saltmarshes to estuaries. The width is three times greater than the depth, and they follow natural drainage lines along a very low gradient. The aim of runnelling is to drain surface water accumulating from tidal action and rainfall. The depth of runnels and pannes is adjusted such that water is drained from the lowest panne on an ebb tide.

Mosquito breeding is discouraged by reducing the time that water remains on the marsh and in pannes by drainage; by increasing the access of estuarine fish to larvae; and by flushing larvae off the marsh into the more hazardous estuarine environment. It also appears to affect the oviposition characteristics of these sites making them less attractive to gravid females.

This study evaluates the effectiveness of runnelling as a mosquito control option and examines the ecological impacts of this technique.

METHODS AND RESULTS

Study Area.

The study area was the Peel-Harvey Estuary which is located 75 km south of Perth, in Western Australia (Fig. 1). The town of Mandurah is situated on this estuary and it is an important commercial fishing and tourist center. Studies have shown that Ross River virus is endemic in the Peel region, and during the last six months a small outbreak of Barmah Forest virus occurred in this area. Both viruses produce the disease epidemic poly arthritis (Lindsay, unpublished data)

Within this area, four saltmarsh sites have been chosen. The sites are of different sizes and contain various numbers of pannes. Site 1, which is the largest of the four sites, was modified with runnels prior to the commencement of the study. Sites 2 and 4, which are of comparable area, were modified after one year of monitoring, to enable a pre- and post-modification comparison. All three of these sites have control pannes. Site 3 is not runnelled, it contains a large control panne. The sites range from saline, Sites 1, 2, and 3, which are in the Peel Inlet, to relatively fresh water, Site 4 on the Serpentine River flood plain.

Mosquito Study.

The two major coastal and estuarine pest species in the southwest of Western Australia are *Aedes camptorhynchus* (Thomson) and *Aedes vigilax* (Skuse). *Aedes camptorhynchus* is present all year, while *Ae.*

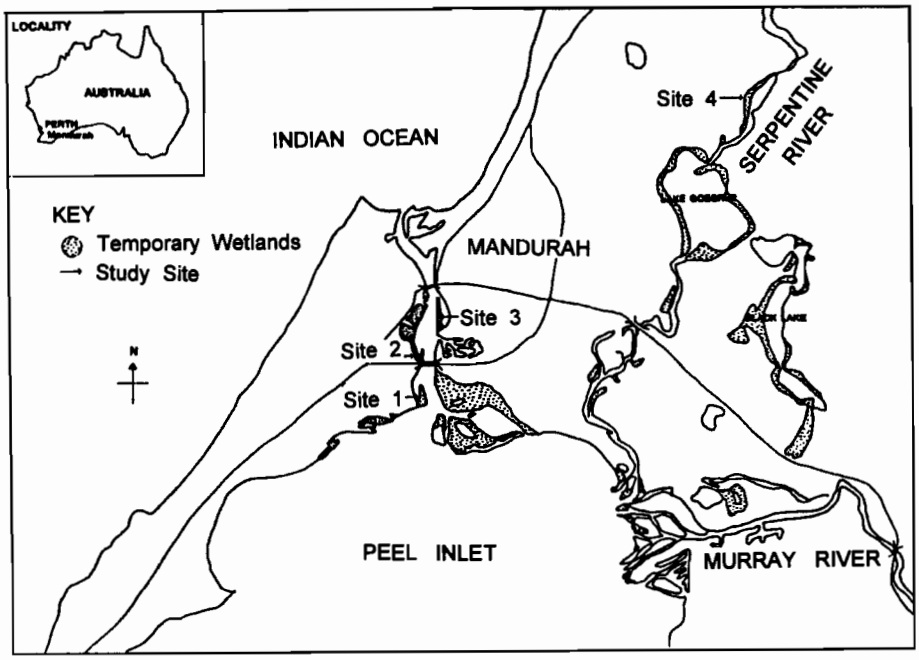

Figure 1. Location of the four study sites; Site 1 consists of 2 control and 3 runnelled pannes, Site 2 of 1 control and 1 runnelled panne, Site 3 of 1 control panne, and Site 4 of 2 control and 1 runnelled panne.

vigilax is a proven vector in the north of the state (Lindsay et al. 1989).

Larval mosquito numbers have been monitored fortnightly or more frequently during occasions of high tides and/or rain, at the four sites since April 1991.

The larval mosquito numbers in the runnelled pannes at Site 1, have been lower on most occasions than in the control pannes over the three year period (Fig. 2). Chemical control of larvae was required on sixteen occasions in the control area, compared to six in the runnelled pannes. Due to a nutrient enrichment problem in the Peel-Harvey estuary, large wracks of algae occasionally blocked the runnel entrance at this site. It is felt that this prevented the larvae from being flushed into the estuary resulting in the occasional high numbers.

Monitoring began in April 1991 and during September 1992 runnels were installed in two areas at both Sites 2 and 4. Mosquito larvae monitoring continued until April 1994. The runnels at these sites have very effectively reduced mosquito larval numbers to less than one-half of that in the control areas (Fig. 3).

Chemical treatment of larvae at Site 2 was required five times prior to runnelling, and only once after runnelling. At Site 4, however, treatment was only required before runnelling on four occasions.

Ecological Impacts.

In order to detect ecological effects, the following aims were identified. The first was to assess whether runnelling alters the hydrology of the saltmarsh. This was done by installing transects of piezometers at each panne extending ten meters outwards from the center. Monthly measurements of water depth, salinity and pH were taken from July 1991 to July 1993.

The second aim was to identify the primary algal groups and aquatic invertebrates in the saltmarsh pannes, and to compare the composition and abundance between runnelled and natural pannes. Seasonal sampling of the nektonic and benthic invertebrates and phytoplankton was performed from July 1991 to July 1993. Within season variation of phytoplankton and benthic algae was examined in December 1992 and July 1993. The invertebrates found in the pannes range from the larger Diptera and Chironmid larvae to the very small ostracod and copepod crustaceans.

The third aim was to monitor the effect of runnelling on *Sarcocornia quinqueflora*, which, in temperate Western Australia, is the dominant low marsh plant. Monitoring was done by harvesting above-ground biomass at the beginning and end of the growing season, during September 1991 to March 1993. An initial examination of the below-ground biomass was performed and a ratio of above- to below-ground established. Below-ground biomass was not monitored as sampling was difficult and destructive.

A large number of saltmarshes and adjacent mudflats in the Peel-Harvey Estuary are recognized as rich feeding areas for trans-equatorial migratory shorebirds. A number of these birds are protected by

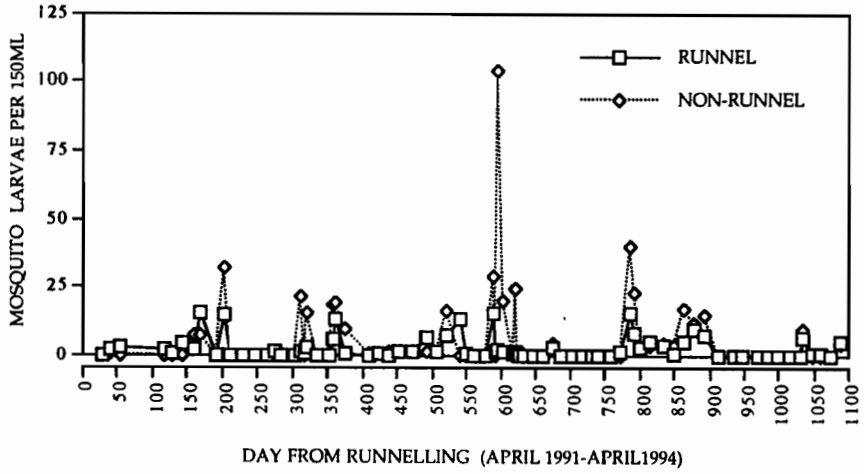

Figure 2. Mosquito larval numbers at Site 1 during April 1991-April 1994.

international agreements. Thus, the final aim was to add to information available on bird usage in the saltmarshes and to examine any large differences in bird usage in the runnelled areas. To achieve this, bird surveys were performed at dawn and dusk on a season basis at all sites.

The impacts of the runnels on the saltmarsh are presently being analyzed. Preliminary results indicate that no obvious impacts from runnelling on S. *quinqueflora* were noted during the first year of monitoring. Seasonal monitoring of phytoplankton during 1991-1993 revealed that blooms occurred in runnelled pools, when control pools were dry. Benthic algal monitoring in December 1992 and July 1993 showed that runnelled pannes have similar concentrations of chlorophyll *a* to estuary mudflats, while control pannes were different. Bird species and abundance was lower in the second year of monitoring, but evidence suggests that this occurred throughout the estuary and was not restricted to the saltmarshes.

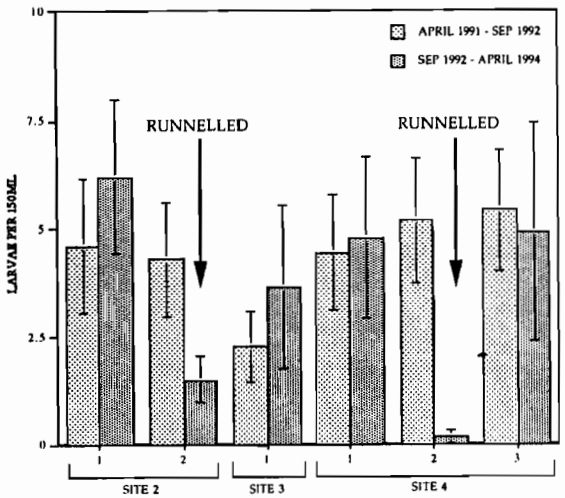

Figure 3. Average number of mosquito larvae per sample at Sites 2, 3 and 4.

CONCLUSIONS

Runnelling reduces larval mosquito numbers to a level only requiring occasional chemical control. However, runnels do appear to have an impact on the phytoplankton and benthic algal composition of the saltmarsh pannes. Preliminary analysis indicates that the algal biomass of the pannes that have been runnelled is similar to that of the adjacent mudflat. Examination of the composition of the algal populations is still to be completed, along with the analysis of the nektonic and benthic invertebrates. It is hoped that these results will provide a clearer picture of the extent of the impacts, which will then allow authorities to decide if runnelling is an acceptable method of mosquito control.

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COMPARATIVE FLIGHT CHARACTERISTICS OF THREE SALT MARSH *AEDES* SPECIES IN NORTHERN CALIFORNIA[1]

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ABSTRACT

Flight potentials of three salt marsh mosquitoes, *Aedes squamiger* (Coquillett), *Aedes dorsalis* (Meigen), and *Aedes washinoi* Lanzaro and Eldridge, were compared using standard flight mills to measure performance. A total of 221 female and 94 male *Ae. squamiger*, 94 female *Ae. washinoi*, and 72 female *Ae. dorsalis* were flown on flight mills.

Mosquitoes were attached to mills and allowed to fly for up to 22 hours. Data for each flight on each mill was accumulated on a microcomputer. Average total and maximum distances and flight durations were compared between species. In addition, within species comparisons were made between different age groups of *Ae. squamiger*.

No significant correlations were found between distance or duration flown and percent weight loss. Female *Ae. dorsalis* averaged over twice the distance flown by *Ae. squamiger* (16.7 vs. 8.1 km) and over four times the distance flown by *Ae. washinoi* (3.8 km). Unfed, newly emerged *Ae. dorsalis*, *Ae. squamiger*, and *Ae. washinoi* flew 1.9, 1.2, and 1.1 km, respectively, indicating that all species emerged with sufficient reserves to find nectar sources prior to longer distance flights. *Aedes squamiger* less than four days old flew less than 1/3 the distances flown by older individuals. Flight potential did not decline over seven weeks in captivity. Field collected 1- and 2-parous females also showed no decline in flight potential. Male *Ae. squamiger* flew less than one-half the distances flown by females.

Based on field collections, *Ae. squamiger* is more likely to be taken at greater distances from breeding sources than *Ae. dorsalis*. The possible reasons for the disparity between laboratory and field results are related to the landscape ecologies of the three species.

[1] A manuscript summarizing this study will be submitted to the Journal of the American Mosquito Control Association.

MOSQUITO SEASONALITY IN NEWLY CREATED WETLANDS DESIGNED FOR WASTEWATER TREATMENT WITH AQUATIC MACROPHYTES

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ABSTRACT

In 1993 the Sacramento Regional Wastewater Treatment Plant started construction of a series of experimental wetland cells designed to polish secondary effluent. Their objective is to remove heavy metals and organics by passing the effluent over bulrush and cattail rhizomes to sufficiently clear it for release into natural waterways, increasing local wetlands and saving water.

The layout for the wetlands consists of a series of shallow cells, each measuring 1,260 feet long, 50 feet wide and 2 feet deep. Each cell receives 69 gallons of effluent per minute, equalling approximately 1 million gallons per day. During the first year of operation, groundwater was used to establish the macrophyte population. Secondary effluent is scheduled to be added to the cells in January, 1994.

Mosquito larvae were sampled each week by taking 528 dips and showed a seasonal trend similar to that found in local rice fields. Four species of mosquito larvae were collected in the wetland cells; *Culex tarsalis* Coquillett, *Culex erythrothorax* Dyar, *Anopheles freeborni* Aitken, and *Anopheles franciscanus* McCracken. *Culex tarsalis* predominated throughout the summer from June to August, and *An. freeborni* predominated in the fall from late August through October. *Anopheles franciscanus* occurred in greatest numbers (0.22/dip) in late September and *Cx. erythrothorax* occurred in very small numbers and only in the fall. *Culex tarsalis* had a population peak in July (0.70/dip) and *An. freeborni* peaked in September (0.42/dip). Overall, average mosquito numbers remained below 1.0/dip each week except for the week of July 29th when individual cells were as high as 2.64/dip, but the week averaged 1.02/dip. Certain cells with low vegetation consistently remained low throughout the season, pulling down the weekly average.

The *Cx. tarsalis* peak was necessarily reduced by a single Bti treatment in early August due to collections of WEE positive *Cx. tarsalis* at the demonstration wetlands. After the treatment there was a natural decline through the end of the season.

Adult mosquito populations corresponded closely with larval peaks. On-site CO_2-baited traps collected larger numbers than did the off-site control traps. *Culex erythrothorax* were collected abundantly on-site and were not found in the control traps off-site.

Sampling for mosquito larvae and adults will be duplicated next season after the secondary effluent in applied, for a comparison to this year's data. The result of both seasons will be presented together at next year's meeting.

1994 Proc. Calif. Mosq. Vector Control Assoc. 62:54-57

MARK-RELEASE-RECAPTURE STUDIES WITH *CULEX TARSALIS* IN THE COACHELLA VALLEY[1]

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ABSTRACT

The population ecology of *Culex tarsalis* was studied along the Salton Sea in the southern Coachella Valley using mark-release-recapture methods during February, May, July, September, and November, 1993. A total of 22,500 females emerging from field-collected immatures and 54,400 females collected at CO_2-baited traps were marked and released, of which 0.2% and 6.9% were recaptured, respectively. Females were most dispersive during spring and fall with flights as far as 6.3 km recorded. Estimated population densities ranged from 8,000 in July to 1,800,000 females per km^2 in February. Population density was lowest and turnover rates highest during midsummer.

Studies on the temporal and spatial distribution of *Culex tarsalis* Coquillett and western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses in the southern portion of the Coachella Valley indicated that mosquito and arbovirus activity was initiated each year at salt marshes adjacent to the shore of the Salton Sea and then spread to the northwest within the flood plain of the Whitewater Channel (Reisen et al. 1992a, 1995a,b). The abundance of host-seeking *Cx. tarsalis* was greatest near the Salton Sea and then decreased as a linear function of elevation and the distances of collection stations from the Salton Sea (Reisen et al. 1994a). WEE and SLE virus activity also was greatest near the Salton Sea and then decreased gradually towards the northwest along the Whitewater Channel. These data indicated that arboviruses may be dispersed, in part, by infected *Cx. tarsalis*. However, the rate and direction of *Cx. tarsalis* dispersal has not been studied by mark-release-recapture methods in the Coachella Valley

The objectives of the present research was to determine seasonal changes in the direction and rate of dispersal and estimates of size, loss and addition rates for *Cx. tarsalis* populations in the southern Coachella Valley, a habitat quite different from the Central Valley where previous release studies with *Cx. tarsalis* have been conducted (Reisen and Reeves 1990, Reisen et al. 1992b).

MATERIALS AND METHODS

Culex tarsalis immatures were collected by dipping at productive surface water sources near the Salton Sea and then transported to the Coachella Valley Mosquito Abatement District where they were allowed to emerge at room temperature. Host-seeking females were collected by 10-20 dry ice-baited CDC traps (CO_2 traps) operated at productive sites as near to the release site as possible.

Adults were counted by the strip method (Dow et al. 1965), marked with cohort-specific colored fluorescent dust and released in late afternoon near a natural resting site at a Tamarisk wind break in a managed marsh near the Salton Sea (Fig. 1). Adults collected as immatures were <24 hrs old at release, whereas adults collected host-seeking were of unknown age. Recapture was attempted using CO_2 traps operated

[1] A manuscript summarizing this study has been submitted for publication in the Journal of Medical Entomology.

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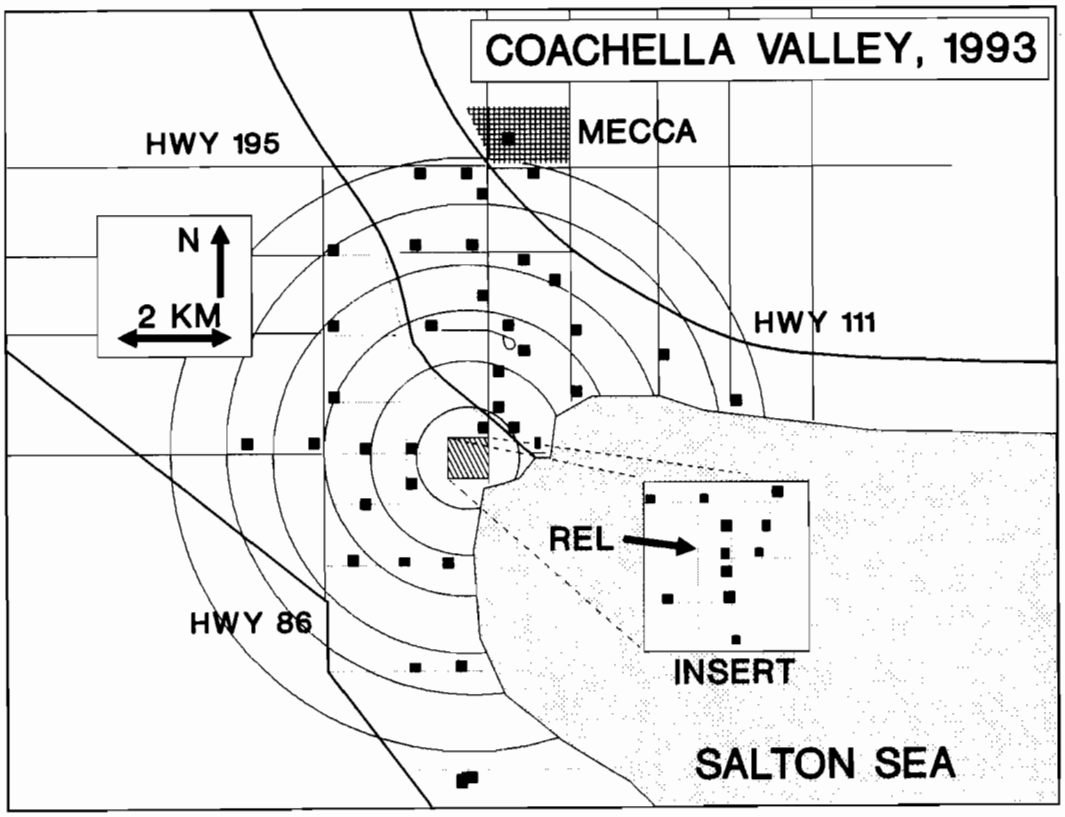

Figure 1. Map of the southern Coachella Valley showing the locations of the release and recapture sites.

on fixed standards at 49 sites distributed over a 6 km radius study area (Fig. 1). Traps were operated for 8 to 12 successive nights after release depending upon recapture success. Based on previous seasonal abundance trends, experiments during 1993 were planned for the late winter period of population increase (February), the vernal peak (May), the mid-summer decrease (July), the autumnal peak (September), and late fall decline (November).

Mean dispersal distance per day was calculated from the recapture of marked females corrected for the decrease in sampling effort (i.e., trap density) with distance from the release point (Brenner et al. 1984). Population size, loss and addition rates were estimated for the 0.5 km^2 area sampled by the central 11 traps (Fig. 1) using the methods described in Reisen et al. (1991).

RESULTS AND DISCUSSION

Overall, 22,500 females collected as pupae were marked and released, of which only 37 (0.2%) were recaptured - too few for further analysis. This low recapture rate was unexpected, because a similar protocol in Kern County resulted in a 2-4% recapture rate (Reisen et al. 1992b). Although elevated loss rates due to emigration and autogeny may have been contributing factors, we currently have no explanation for this unexpected low recapture rate.

In contrast, 3,758 (6.9%) of 54,400 host-seeking females released concurrently with the adults emerging from field-caught pupae were recaptured. Overall, 1,973 (52%) recaptures were made on the night of release at the 11 traps positioned within 0.5 km of the release site. However, the corrected mean distance at which females were recaptured increased as a linear function of days after release from about 1.0 km on day 1 to about 2.5 km on day 8. A few highly dispersive females were collected at the most distant traps to the south (6.3 km) within 2 nights after release.

The degree and direction of dispersal varied seasonally, although the wind was from the NW to SE during all experiments. During February, females were recaptured at 42 of the 49 CO_2 traps, including traps located 6.2 km south of the release site. Marked females did not disperse to the north into the town of Mecca, but

rather moved to the northwest in the Whitewater Channel flood plain, the same pattern exhibited by WEE and SLE virus dispersal during previous years. In May, dispersal was limited to the southwest portion of the recapture grid. Although the same number of females were recaptured in July as May, hot dry weather seemed to reduce movement and recaptures were restricted to traps within a 4 km radius of the release site. In September, dispersal was generally similar to February with most traps positive for marked females with the exception of those in the Mecca area. In November, when temperatures were cool and several rain storms occurred during the recapture period, most females were recaptured near the release site and few dispersed to the north or west.

An attempt was made to relate female dispersiveness to wing length, parity status and fructose content. A comparison among females at release with those recaptured at traps within the 0.5 km radius central area, between 0.5 and 1 km, and at distances >1 km revealed no discernible pattern. Interestingly, some marked females were still nulliparous when recaptured host-seeking more than 8 days after release. The inability of host-seeking females to effectively locate and obtain a blood meal in this managed marsh was unexpected and may indicate low host availability within our study area leading to increased dispersiveness in this portion of the Coachella Valley.

The relative abundance of *Cx. tarsalis* was monitored biweekly by three CO_2 traps positioned near the release site and was characteristically bimodal with peaks in March and October (Fig. 2). The March peak followed extraordinary flooding along the Whitewater Channel and occurred earlier than anticipated, whereas the autumnal peak was associated with flooding of the marshes for water fowl and occurred later than anticipated. *Cx. tarsalis* density in the central 11 traps was estimated to range from ca. 8,000 females/km^2 in July to 1.8 million females/km^2 in February. These estimates were made before and after the early and late season peaks.

Population addition rates (immigration + emergence) ranged from 0.3 females per female per day in November to 0.6 in September at the onset of the fall peak. Loss rates (emigration + death) ranged from 0.6 in July to 0.3 in November. Highest addition and loss rates occurred during July when temperatures were hot, survival low and generation times minimal.

In summary, host-seeking *Cx. tarsalis* were extremely dispersive in Coachella Valley, but most movement occurred along the Salton Sea and within the flood plain of the Whitewater Channel. A similar spatial pattern was observed previously for WEE and SLE virus dissemination (Reisen et al. 1995b). Temporally arbovirus activity is greatest during July to September, a

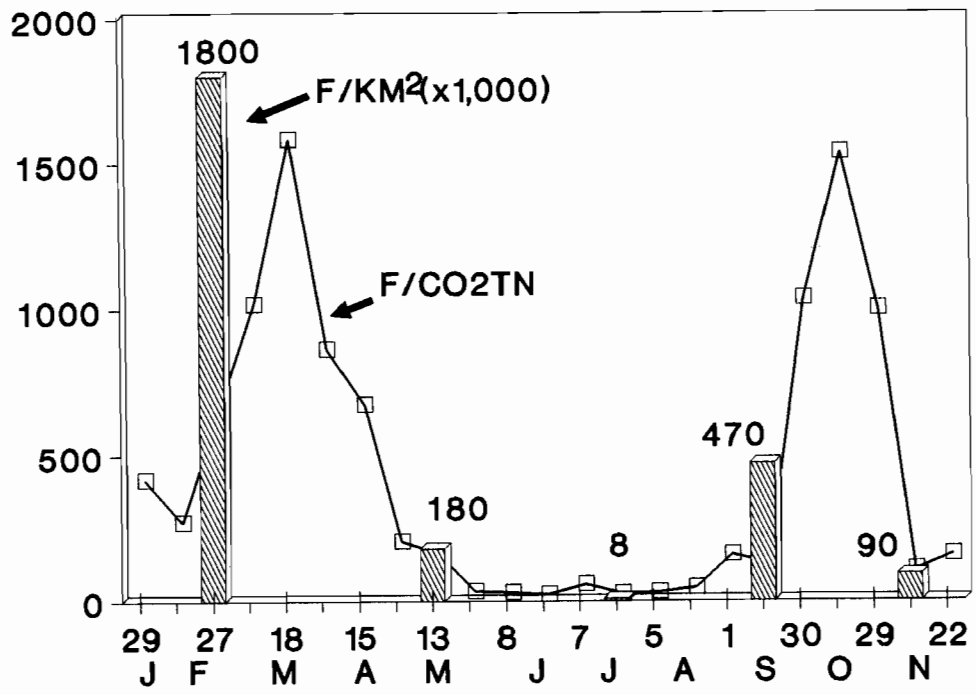

Figure 2. Temporal changes in numbers of host-seeking *Cx. tarsalis* females at 3 CO_2 traps (F/CO_2TN) near the release site and female population density [F/KM^2 (x1,000)] at 11 traps within 0.5 km of the release site.

period when *Cx. tarsalis* density was lowest and population turnover rates greatest. Apparently, low density and survival are compensated by a marked reduction in the length of the extrinsic incubation and gonotrophic cycle periods due to elevated temperatures.

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RE-COLONIZATION OF BACTERIA ISOLATED FROM THE GUTS OF FIELD COLLECTED MOSQUITO LARVAE IN *AEDES AEGYPTI*

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ABSTRACT

A total of 22 types of bacteria were isolated from the gut contents of field collected mosquito larvae with 21 of them identified as *Bacillus* and the other as *Pseudomonas*. From these, three *Bacillus* species were selected for re-colonization; one was from the gut of laboratory-reared *Aedes aegypti* larvae and the other two from guts of field collected *Culex* larvae. Three mixtures of three selected *Bacillus* species and *Escherichia coli* Kl-14 were re-introduced into the guts of *Ae. aegypti* larvae. Results revealed that *E. coli* Kl-14 was unable to survive in the larval gut environment. Meanwhile, the vegetative cells of the three *Bacillus* species could survive for at least eight days after feeding and develop into spore forming cells. Thus, the three selected *Bacillus* species were able to re-colonize in the guts of *Ae. aegypti* larvae.

Microbial insecticides (i.e., *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus*) have been widely used and intensively studied. Several of their mosquitocidal crystal protein genes have been expressed in *Escherichia coli* (Chungjatupornchai et al. 1988, Hofte and Whiteley 1989), *Bacillus subtilis* (Hofte and Whiteley, Baumann and Baumann 1989), *Caulobacter crescentus* (Thanubula et al. 1992), and cyanobacteria (Chungjatupornchai 1990, Angsuthanasombat and Panyim 1989, and Tandeau de Marsac et al. 1987). However, all these micro-organisms used as hosts for the gene expression are laboratory strains. The relationship of these micro-organisms with mosquito larvae in the natural breeding habitats is unknown.

In this work, bacteria found in the guts of field collected mosquito larvae were isolated and identified. The dominant bacteria were re-introduced into the guts of *Aedes aegypti* (L.) larvae, in order to examine their ability to re-colonize. The appropriate bacteria, which are able to re-colonize in the guts of mosquito larvae, will be used as vectors for prolonged delivery of the mosquitocidal crystal proteins in the guts of mosquito larvae.

MATERIALS AND METHODS

Isolation and Identification of Bacteria.

Twenty-three larval and breeding water samples of six different mosquito species were collected from natural breeding habitats located throughout five provinces of Thailand. The mosquito larvae were identified and washed several times with sterile distilled water to eliminate surface micro-organisms before the guts were dissected out. The contents from each gut and water sample collected from the same habitat were spread separately on LB agar plates. After incubation at 30°C for 24 hours, bacterial colonies on the agar plates were counted. The isolated bacteria were differentiated into types by morphology and further identified using gram stain and biochemical tests.

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Re-Introduction of the Isolated Bacteria into the Guts of Mosquito Larvae.

The selected bacteria, isolated from the guts of mosquito larvae, were re-introduced into the guts of *Ae. aegypti* larvae as follows. Selected bacteria and *E. coli* Kl-14 were grown separately in LB broth at 30°C until cell concentrations reached ~1 X 10^8 cells/ml. Bacterial cells were then washed and resuspended with sterile distilled water before a 20 ml mixture (at a final concentration of ~2 X 10^8 cells/ml), consisting of equal amounts (10 ml each) of the selected bacteria and *E. coli* Kl-14 , were fed to 50 *Ae. aegypti* 3rd instar larvae for 16 hours. The mosquito larvae were afterwards washed many times with sterile distilled water and fasted. After a period of fasting consisting of 1, 2, or 8 days, the mosquito larval guts were dissected out. The contents of each gut was divided into two halves. One half was spread directly onto an LB agar plate. The other half was treated at 78°C for ten minutes before being spread out on a similar plate. All plates were then incubated at 30°C for 24 hours before the bacterial colonies were counted and identified.

RESULTS AND DISCUSSION

Isolation of Bacteria from Mosquito Larval Guts and Their Natural Breeding Water.

Mosquito larvae, collected from different natural breeding habitats in Thailand, were identified and their guts dissected out (Table 1). All gut contents and water samples collected from the same breeding habitat were incubated on media plates before the bacterial colonies growing on the plates were counted and compared (data not shown). Comparison of the number of bacterial colonies on each plate indicated that all seven samples of laboratory-reared larvae had lower numbers of bacterial colonies in their gut contents than found in their larval breeding water. On the other hand, 18 samples of field collected larvae had lower numbers of bacterial colonies in their gut contents than found in their larval breeding water, while the remaining 5 samples of field collected larvae had slightly higher numbers of bacterial colonies in their gut contents than found in their larval breeding water. These results indicate that the bacteria in the gut contents obtained by the above described method were not contaminated by the bacteria in the larval breeding water.

Table 1. Number of laboratory-reared and field collected larval samples of each species used in the experiments.

Species	Number of Samples	
	Laboratory Reared	Field Collected
Aedes aegypti	3	9
Aedes albopictus	-	1
Anopheles bengalensis	-	1
Anopheles dirus	1	-
Culex sp.	-	6
Culex vishnui	-	1
Culex quinquefasciatus	2	5
Culex tritaeniorhynchus	1	-
Totals	7	23

Identification of Bacteria Isolated from the Guts of Mosquito Larvae.

The bacteria isolated from the larval guts were differentiated by morphology into 22 types. Further identification revealed that 21 of these types were in the genus *Bacillus* and the other in the genus *Pseudomonas*. Thus, *Bacillus* were the dominant bacterial species in the guts of mosquito larvae. The *Bacillus* bacteria were further characterized using antibiotic sensitivity tests (data not shown) before the appropriate three *Bacillus* species were selected for the re-colonization experiments.

Re-Colonization of Selected *Bacillus* Species into the Guts of *Ae. aegypti* Larvae.

The three *Bacillus* species selected for re-colonization were designated Ae10/2G1, from the guts of laboratory-reared *Ae. aegypti* larvae, Cx227/5G2 and Cx527/5G1, from the guts of field collected *Culex* larvae found in different habitats.

Third instar laboratory-reared *Ae. aegypti* larvae were fed cell mixtures of each selected *Bacillus* species (vegetative cells) and *E. coli* Kl-14 for 16 hours before they were fasted 1,2 or 8 days. After the larvae had fasted, they were dissected and their guts removed. The gut contents were divided into two halves with one half immediately spread onto LB agar plates to obtain the total number of bacterial cells per gut and the other half heat-treated to dispose of vegetative cells, leaving only the spore forming cells. Both halves were spread onto LB agar plates with and without tetracycline. Tetracycline was used as a marker to distinguish between the selected *Bacillus* and *E. coli* Kl-14. The selected *Bacillus* were sensitive to tetracycline whereas *E. coli* Kl-14, which harbors a tetracycline resistance gene on the plasmid, was resistant.

As expected, no *E. coli* Kl-14 grew on tetracyclilne treated plates, indicating that since day 1, *E. coli* was not able to survive in the gut of *Ae. aegypti* larvae. On those plates with non-treated gut contents, the number of *Bacillus* viable cells decreased dramatically from day 1 to

day 2 and then slightly from day 2 to day 8 (Fig. 1). The number of *Bacillus* viable cells from heat-treated gut contents at day 1, and between day 2 and day 8, was less than 15 % and 1 % of those from non-treated gut contents, respectively. These results indicate that the vegetative cells of the selected *Bacillus* species can survive and develop to spore forming cells in the gut of mosquito larvae for at least 8 days after feeding. Thus, the three selected *Bacillus* species were able to re-colonize in the gut of *Ae. aegypti* larvae.

Several other selected *Bacillus* species isolated from the guts of mosquito larvae are now under investigation for re-colonization into the gut of *Aedes*, *Culex*, and *Anopheles* larvae. Eventually, suitable *Bacillus* species will be found and used as hosts for cloning and expression of genes which encode mosquitocidal crystal proteins. The *Bacillus* clones expressing the highest levels of mosquitocidal activity will be further studied, in order to used as a vector for prolonged delivery of mosquitocidal proteins into the guts of mosquito larvae.

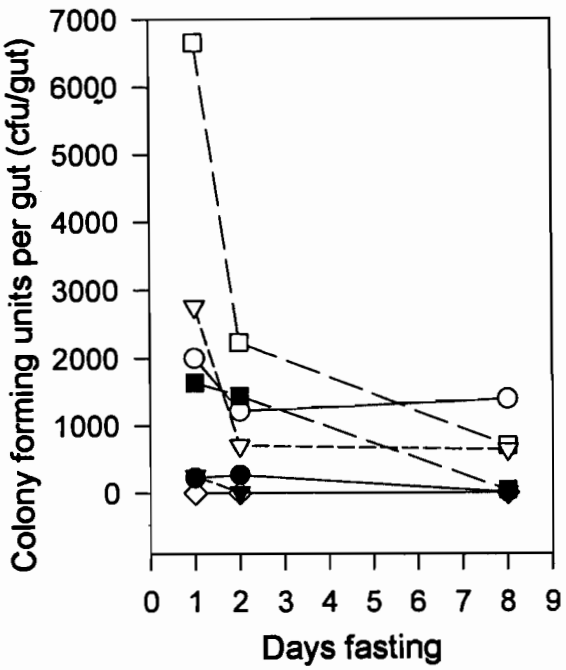

Figure 1. Re-colonization of 3 selected *Bacillus* species in the guts of *Ae. aegypti* larvae (○,□, ▽ are gut content *Bacillus*; ●,■,▼ are heat-treated *Bacillus* and *E. coli* mixtures; ◇ are gut content *Bacillus* used as control).

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POPULATION GENETICS STUDIES ON *AEDES VEXANS* BY CELLULOSE ACETATE ELECTROPHORESIS

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ABSTRACT

Aedes vexans specimens were collected as larvae or adults from eight locations throughout the Upper Rhine Valley in Germany with about a hundred adult mosquitoes from each location submitted to cellulose acetate electrophoresis. Their genotypes were scored for five polymorphic loci; the alleles from each location were then compared to each other as well as two laboratory colonies originating from the same area. Additionally, differences between the German mosquitoes and two populations from North America were examined using the same methods.

A wide range of *Aedes* mosquito species have been genetically evaluated by different methods of electrophoresis, yet specific data on *Aedes vexans* (Meigen) is not available. In order to research the genetic structure of *Ae. vexans* populations from different parts of the world, cellulose acetate electrophoresis was applied to a broad sampling of *Ae. vexans* populations from Germany and two populations from North America. Cellulose acetate electrophoresis has several advantages compared to the more common starch gel or polyacrylamide electrophoresis; e.g., preparation, run, and stain times are short; only a little amount of sample is required; and handling is extremely easy.

The main purposes of this study were to gain information on *Ae. vexans* population formation and structure, to compare close and distant populations to one another, and to observe differences between laboratory populations and their wild counterparts.

MATERIALS AND METHODS

Aedes vexans specimens were collected either as larvae or adults during the summer of 1993 from eight different locations throughout the Upper Rhine Valley in Germany (Fig. 1). Larvae collected from the following locations: Bingen (Bg), Mombach (Mom), Mönchbruch (Mö), Hamm (H), and Queich (Q) were raised in plastic tubs containing 24 liters of a 50:50 mixture of aquarium and aerated tap water. The larvae in each tub were daily fed two tablespoons of ground Tetramin® fish food, passed through a strainer and rinsed with 100 ml of water. After reaching the fourth instar, the larvae were identified by the known criteria (Mohrig 1969).

Since the summer of 1993 was very dry, it was not easy to find larvae at all of the sampling locations. Yet, surprisingly, a few adult female *Ae. vexans* were caught in Oppenheim (Opp), Germersheim (Ger), and Kühkopf (Kü), even though their breeding places could not be found. These female mosquitoes were transferred to four-liter cages and fed with apple slices and sponges soaked with a 10% sugar solution in water. Additionally, they were given a daily blood meal. After a few days, the females began laying eggs on a flat dish covered with moss. This procedure was continued for three weeks, after which the moss was removed and stored in an incubator at 28°C for ten days. Larvae obtained as a result of eggs hatched from the flooding of this moss were raised under the same conditions as described above.

Pupae were collected in dishes and placed inside cages for eventual emergence. After emergence, the adult mosquitoes were collected and frozen alive in liquid nitrogen to preserve the enzyme functions.

Two laboratory populations of German *Ae. vexans*, one (Kül) an F_{118} generation originating from the Kü population, the other (Ql) an F_{12} generation originating from the Q population, were also sampled. To compare these wild and laboratory German *Ae. vexans* populations

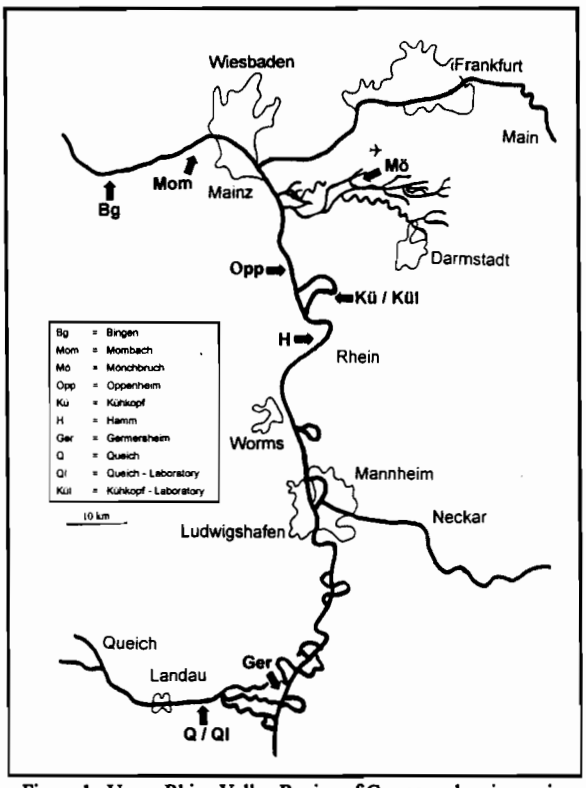

Figure 1. Upper Rhine Valley Region of Germany showing major cities and towns along the Rhine River and indicating sample sites for the German *Ae. vexans* populations used in this study.

to very distant ones, eggs of field collected *Ae. vexans* (Wpg) from Winnipeg, Manitoba, Canada, were sent to us by Dr. Brust and specimens from an F_{15} generation laboratory colony (Mnl) originating from Minnesota, United States and maintained by Dr. Kuhn, were prepared and sampled as above described. Hybrids in the F_1 generation between Mnl females and Kül males served as controls.

Frozen adult mosquitoes were ground in Eppendorf caps along with 10 μl of distilled water. The homogenate was then transferred into wells and stamped onto the presoaked plates. The soaking solution consisted of a trisglycine buffer at a pH of 8.5, which was identical to the run buffer. The plates were then placed into the electrophoresis chamber for a run time of 45 minutes at 150 Volts.

Staining was accomplished by mixing enzyme-specific stain chemicals with agar and pouring the mixture into the plates as described by Herbert and Beaton (1989).

Of the 29 enzymes tested, only the following five were polymorphic and interpretable (and therefore, will be the focus of the rest of this presentation):

- PGI	(phosphoglucose isomerase EC 5.3.1)
- GOT 1	(glutamate oxaloacetate transferase EC 2.6.1.1) cathodal form
- GOT 2	(glutamate oxaloacetate transferase EC 2.6.1.1) anodal form
- PEP	(peptidase EC 3.4.11)
- IDH 2	(isocitrate dehydrogenase EC 1.1.1.42)

In scoring the cellulose acetate results, the mobility of the most common band of each locus was determined as 100 with the mobility of all other bands measured relative to this. To simplify matters, the different bands were designated numbers according to their mobility.

RESULTS

All of the tested enzymes were dimeric indicating that one stained band (allele) represents homozygotes for that locus and three stained bands represents heterozygotes.

Allele Frequencies.

A locus was considered polymorphic, when the most abundant allele had a frequency ≤ 0.99. Examination of the observed allele frequency for each *Ae. vexans* population and locus demonstrates that, apparently, not every allele appears in each population (Table 1). The allele distribution varies, especially between the North American and German populations, but there are also differences between the two North American populations. When comparing these particular two populations, it must be kept in mind that the Minnesota specimens stem from a laboratory strain and are therefore not necessarily representative for their wild counterparts.

An interesting observation is allele No. 4 of the locus IDH 2; it is only found in the Wpg and Mö populations and in the latter only in the homozygotic state.

Heterozygosity.

Among the twelve sampled populations, observed heterozygosity ranged from 9-15% in the Upper Rhine Valley populations and 22-24% in the North American populations (Table 2). Interestingly, the widest observed difference in heterozygosity occurred in the neighboring Upper Rhine Valley populations of Ger (15%) and Q (9%). The two German laboratory populations of *Ae. vexans* derived from Upper Rhine Valley populations, Ql and Kül, had relatively low observed heterozygosities of 8.2 and 10.4%, respectively.

Hardy-Weinberg Equilibrium.

Using a G-test, significant differences between

Table 1. Allele frequencies and number of individuals tested (N) for each *Ae. vexans* population.

Locus	Allele	Allele frequency											
		Bg	Mom	Mö	Opp	Kil	H	Q	Ger	Ql	Kil	Wpg	Mnl	Mnl x Kil
PGI	1	0	0	0	0	0	0	0	0	0	0	0.089	0	0
	2	0.015	0	0.096	0	0	0	0	0.034	0	0	0.044	0	0
	3	0.903	0.888	0.836	0.977	0.898	0.913	0.935	0.865	1	0.79	0.856	1	0.894
	4	0.083	0.112	0.068	0.023	0.102	0.087	0.065	0.101	0	0.21	0.011	0	0.106
	N	103	125	73	107	118	98	107	104	91	112	45	125	90
GOT 1	1	0	0	0	0	0	0	0	0	0	0	0.023	0	0
	2	0	0	0	0	0	0	0	0	0	0	0.841	1	0.5
	3	0.005	0	0.008	0	0.017	0.011	0.005	0	0	0	0	0	0
	4	0.938	0.994	0.861	0.958	0.983	0.984	0.991	0.989	1	1	0.102	0	0.5
	5	0.01	0	0.008	0	0	0	0	0.011	0	0	0	0	0
	6	0.043	0.006	0.057	0	0	0	0.005	0	0	0	0.034	0	0
	7	0.005	0	0.066	0.042	0	0.005	0	0	0	0	0	0	0
	N	105	89	61	107	116	92	108	93	84	114	44	128	90
GOT 2	1	0.005	0.014	0	0	0	0	0	0.005	0	0	0	0	0
	2	0.014	0.06	0.036	0.051	0.011	0.036	0.005	0.01	0	0	0.011	0	0
	3	0	0.051	0.007	0.02	0.011	0.006	0	0.005	0	0	0	0	0
	4	0.957	0.87	0.949	0.918	0.924	0.94	0.947	0.956	1	0.994	0.915	0.771	0.904
	5	0.005	0	0	0	0.011	0	0.01	0	0	0.006	0	0.229	0.096
	6	0.01	0.005	0.007	0.01	0.033	0.012	0.029	0.025	0	0	0.032	0	0
	7	0.01	0	0	0	0.011	0.006	0.01	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0	0.043	0	0
	N	104	108	69	98	92	83	104	102	85	83	47	109	73
PEP	1	0.01	0.008	0.007	0	0	0.011	0	0	0	0	0	0	0
	2	0.01	0.04	0.021	0.049	0.008	0.028	0.024	0.011	0	0	0.043	0.863	0.402
	3	0.934	0.884	0.852	0.876	0.925	0.893	0.857	0.872	0.753	0.942	0.755	0.137	0.591
	4	0.046	0.048	0.113	0.075	0.067	0.062	0.114	0.112	0.247	0.058	0.202	0	0.006
	5	0	0.02	0.007	0	0	0.006	0.005	0.005	0	0	0	0	0
	N	98	125	71	113	126	89	105	94	73	104	47	117	82
IDH 2	1	0	0	0	0	0	0	0	0	0	0	0.11	0	0
	2	0	0	0	0	0	0	0	0	0	0	0.26	0.268	0.012
	3	0	0	0	0	0	0	0	0	0	0	0.3	0.732	0.488
	4	0	0	0.21	0	0	0	0	0	0	0	0.23	0	0
	5	0.05	0.035	0.081	0.089	0.061	0.012	0.021	0.015	0	0.03	0.1	0	0.048
	6	0.939	0.911	0.629	0.846	0.939	0.971	0.891	0.985	1	0.97	0	0	0.452
	7	0	0.025	0.081	0.042	0	0.012	0.052	0	0	0	0	0	0
	8	0.011	0.03	0	0.023	0	0.006	0.036	0	0	0	0	0	0
	N	90	101	62	107	98	85	96	99	83	101	50	112	84

Table 2. Observed heterozygosity (Ho) for each locus and overall mean heterozygosity in each *Ae. vexans* population.

Population	Observed heterozygosity (Ho)					Mean Ho
	PGI	GOT 1	GOT 2	PEP	IDH 2	
Bg	0.18	0.05	0.09	0.09	0.1	0.102
Mom	0.22	0.01	0.19	0.22	0.1	0.148
Mö	0.23	0.08	0.07	0.1	0.19	0.134
Opp	0.05	0.08	0.1	0.19	0.21	0.126
Kü	0.17	0.03	0.13	0.15	0.1	0.116
H	0.17	0.03	0.12	0.18	0.04	0.108
Ger	0.23	0	0.11	0.26	0.15	0.15
Q	0.11	0.02	0.09	0.2	0.03	0.09
Ql	0	0	0	0.41	0	0.082
Kül	0.33	0	0.01	0.12	0.06	0.104
Mnl	0	0	0.35	0.21	0.54	0.22
Wpg	0.16	0.14	0.17	0.23	0.52	0.244
Mnl x Kül	0.21	1	0.19	0.8	1	0.64

observed and expected genotype frequencies can be detected (Table 3). It is conspicuous, that of all the Rhine Valley populations sampled only the Mö population is not in a general state of equilibrium since there is an excess of homozygotes. There is also an excess of homozygotes in the North American Wpg population at the PGI locus, whereas the Mnl x Kül hybrids are mostly heterozygotes due to the fact that their parents were monomorphic homozygotic for certain alleles, thus producing an overproportion of heterozygotic offspring.

Genetic Distances.

Genetic distances were calculated as given by Nei (1972); these measure the genetic relatedness between pairs of populations. On the basis of these calculations and using the UPGMA-Cluster Analysis method, a dendrogram was constructed clearly showing that genetic distances between the German populations are very small (Fig. 2). In fact, within this group only the two laboratory populations (Ql and Kül) and the Mö population show any substantial distance to the others.

Table 3. G-test for deviation from Hardy-Weinberg equilibrium.

Population	PGI	GOT 1	GOT 2	PEP	IDH 2	ΣG
Bg	2.97	27.83	0.41	6.55	1.93	39.69
Mom	3.54	0.01	14.53	0.35	19.79	38.22
Mö	7.35	33.04*	3.91	26.32*	74.27*	144.89*
Opp	0.12	0.4	21.19	1.2	17.04	39.95
Kü	0.54	0.07	11.88	0.84	0.89	14.22
H	1.62	0.05	0.64	1.2	10.93	14.44
Ger	11.67	11.05	0.61	0.01	11.76	35.1
Q	0.57	0.02	0.42	1.85	0.05	2.91
Ql	0	0	0	0.82	0	0.82
Kül	0	0	0.01	0.39	0.18	0.49
Wpg	13.68*	21.78	0.75	6.32	41.30	83.83
Mnl	0	0	0.02	2.01	22.47	24.50
Mnl x Kül	2.25	124.77*	1.49	33.61*	118.67*	280.79*

* Significant deviation from Hardy-Weinberg equilibrium (expected heterozygosity) at the 5% level.

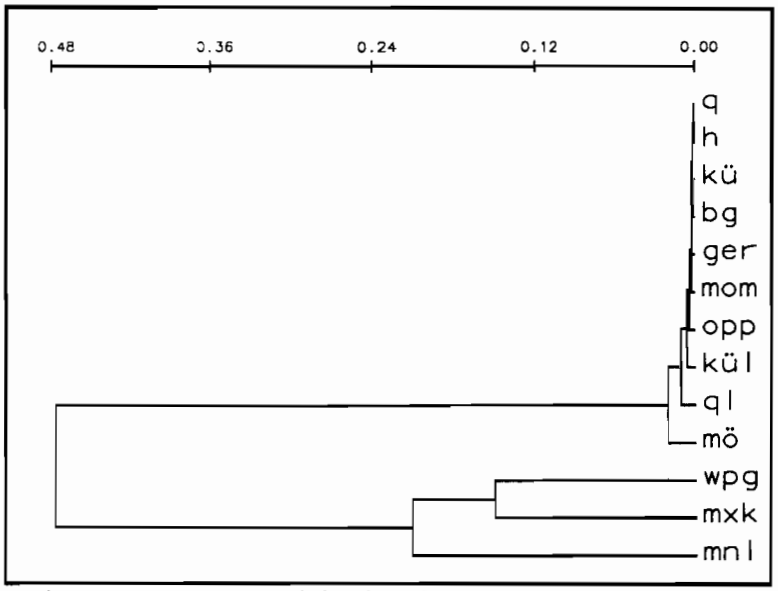

Figure 2. UPGMA Cluster Analysis (using Nei's genetic distances) of the ten German and two North American *Aedes vexans* populations used in this study.

The North American populations, however, not only show considerable genetic differences from one another, but both are greatly different from their German counterparts. In addition, the genetic distance between the Mnl population and the Rhine Valley population group is even greater than that between the Wpg population and the Rhine Valley population group.

Wright's F-Statistics.

The population structure of any sample can be described by using Wright's F-Statistics (Wright 1969) where the parameter F_{IT} represents the correlation between gametes that unite to produce the individuals (I) relative to the gametes of the total population, F_{IS} is the factor for inbreeding and F_{ST} shows the proportion of genetic differentiation between subpopulations in relation to the total population:

$$(1-F_{IT}) = (1-F_{IS})\,(1-F_{ST})$$

These values were calculated for the Upper Rhine Valley populations only (Table 4) using the jack-knife procedure of Weir and Cockerham (1984). The F_{ST} value for all loci shows, that on the average, about 97% of the total genetic differences are found in mosquitoes within any given population ($1-F_{ST}$) and 3% of the total variety is due to the genetic differences of mosquitoes from the populations, the mean value over all loci shows a rather homogenous total population. The observed F_{IS} values are due to the Mö population, which is not in Hardy-Weinberg equilibrium. In general, this statistic confirms the results of the calculations for genetic distances and Hardy-Weinberg equilibrium.

Differences in Allele Frequencies.

To determine if the total *Ae. vexans* population of the Rhine Valley region is as homogenous as the small

Table 4. Wright's F-statistics (± standard deviation) for the Upper Rhine Valley populations of *Ae. vexans*.

Locus	F_{IT} ± s.d.	F_{IS} ± s.d.	F_{ST} ± s.d.
PGI	0.033 ± 0.048	0.020 ± 0.044	0.013 ± 0.010
GOT 1	0.456 ± 0.173	0.435 ± 0.171	0.033 ± 0.017
GOT 2	0.145 ± 0.060	0.136 ± 0.057	0.010 ± 0.008
PEP	0.126 ± 0.072	0.122 ± 0.073	0.004 ± 0.002
IDH 2	0.424 ± 0.141	0.367 ± 0.110	0.080 ± 0.056
All loci	0.205 ± 0.083	0.182 ± 0.071	0.027 ± 0.002

genetic distances and F_{ST} value suggest, a likelihood ratio test for independence of allele distribution was applied. Here, the populations were tested against each other to find significant differences in allele frequencies at each locus and locate possible homogenous regions. This was done only for the Rhine Valley populations, since the North American populations are so obviously different that it is trivial to test these. The result of this test was that the distribution of allele frequencies in all populations differ significantly from one another except for Q and H. In examining neighboring Rhine Valley populations against one another, alleles were pooled to an expected minimum of three or no test was carried out when this was not possible (Table 5).

Gene Flow and Migration Rate.

The method for evaluating gene flow (Nm), migration rate (m) and the effective population size (N) as described by Larson et al. (1983) uses the formula

$$F_{ST} = 1/(4Nm + 1)$$

to calculate gene flow where m is derived from the following sequence of formulae according to Avise (1975)

$$I = \exp(-D) \rightarrow m/(m + v) \rightarrow m = (I \times v)/(1-I)$$

using the variables I = genetic identity, v = mutation rate or 10^{-6} (Sperlich 1988), and D= genetic distance.

A minimum estimate of effective population size (N) is given by the formula

$$N = Nm/m$$

Gene flow and migration rate figures for the Upper Rhine Valley *Ae. vexans* populations, calculated from the current study using the above method, indicate a gene flow (Nm) of 9.01, a migration rate (m) of 0.000157, and an effective population size (N) of approximately 57,000 individuals. This indicates that an average of nine individuals per generation immigrate into each subpopulation. The actual gene flow and migration rates are probably much larger, since generations are overlapping and actual population sizes are assumably larger than the calculated effective population size (N).

Differences Between Wild and Laboratory Populations.

The allele frequencies of the two lab-strains Kül (118 generations in the lab) and Ql (12 laboratory generations) were compared to the wild populations Kü

Table 5. Significant differences in allele frequencies in neighboring *Ae. vexans* populations.

Locus	Stat	Bg-Mom	Opp-H	Opp-Kü	Kü-H	Ger-Q
PGI	G	1.12	8.45*	12.49*	0.28	12.08*
	d.f.	1	1	1	1	2
	P	0.2901	0.0036	0.0004	0.5964	0.0024
GOT 1	G	6.24*	6.35*	13.42*	0.3	-
	d.f.	1	1	1	1	-
	P	0.0125	0.0118	0.0002	0.5835	-
GOT 2	G	22.26*	0.48	8.32*	4.23	0.07
	d.f.	2	1	3	2	1
	P	0	0.4897	0.0399	0.1206	0.7847
PEP	G	4.18	1.49	8.29*	2.66	1.05
	d.f.	2	2	2	2	2
	P	0.1235	0.4744	0.0158	0.2648	0.591
IDH 2	G	2.19	19.32*	13.29*	6.8*	25.19*
	d.f.	2	3	2	1	3
	P	0.3339	0.0002	0.0013	0.0091	0
All Loci	ΣG	35.99*	36.09*	55.81*	14.27*	38.39*
	Σd.f.	8	8	9	7	8
	P	0.000	0	0	0.048	0

* Significant difference at the 5% level.

and Q from which they originated (Table 1). In one comparison, there were relatively little differences in allele frequencies between the laboratory population Kül and its origin population Kü, despite the 118 generations of laboratory-rearing.

The general theory towards the loss of rare alleles and the fixation of more frequent ones after only a few laboratory-reared generations is supported by the observations that after 12 generations in the lab, Ql shows a fixation for PGI allele No. 3, whereas No. 4 has been lost, even though this was not a rare allele. The opposite has also happened in this Ql population where colonization has resulted in an increased frequency of PEP allele No. 4 and corresponding decreased frequency of the prevailing allele (No. 3).

DISCUSSION

International Variation.

The results of this study show quite clearly that there is a great difference within the species *Aedes vexans* when looking at distant populations. The North American populations differ from their German counterparts in heterozygosity, allele distribution, and genetic distances. The values for mean heterozygosity range from 9-15% for the Upper Rhine Valley populations to 24% for the Canadian Wpg population. The mean effective number of alleles per locus are 1.2 in the Germany populations and 1.97 in the Canadian Wpg population.

Bullini and Coluzzi (1973) state that in several species, particularly *Ae. aegypti, Aedes mariae* (Sergent and Sergent), and *Culex pipiens* Linnaeus, the prevalence of a single electrophoretic allele, generally one with an intermediate mobility, can be observed in populations (whose numbers are strongly fluctuating) originating from very distant locations. They conclude that genetic polymorphism is not primarily influenced by random genetic drift acting on a number of neutral isoalleles. Since only one locus (PGM) was studied, no statement is made if this also applies to other loci.

The above described observation can be confirmed for all loci studied in the German populations, yet the North American mosquitoes show a different pattern at two loci: GOT 1 and IDH 2. The allele with the highest frequency at locus PEP in the Mnl population does not concord with either the Wpg or the German populations. In the case of IDH 2 in the Wpg population, no allele prevails as strongly as in the other loci although allele No. 3 has the highest frequency, (which is the prevailing allele in the other North American population, Mnl). Unfortunately, no mosquitoes from the Minnesota wild population were available, so no comparison between these and the Wpg mosquitoes were possible.

Genetic distances between the German and North American populations are high (0.4 for Wpg-Germany and even 0.8 for Mnl-Germany). Ayala et al. (1974) present values of genetic distances at several levels of evolutionary divergence for *Drosophila willistoni* (Table 6), but Cianchi et al. (1985) found the genetic distances between sibling species in the genus *Aedes* to range from to 0.04 to 0.48. Considering these values, the genetic distance between the German and Wpg populations lie well within this range; an interpretation of the Mnl value would be precipitate, since laboratory strains usually differ to some extent from the original wild population.

Table 6. Genetic distance between taxa of the *D. willistoni* group at different levels of evolutionary divergence (by Ayala et al. 1974).

Taxonomic level	D ± s.d.
Local populations	0.003 ± 0.006
Subspecies	0.228 ± 0.026
Semispecies	0.226 ± 0.033
Sibling species	0.538 ± 0.049
Non-sibling species	1.214 ± 0.064

In conclusion, further experiments need to be carried out, especially regarding mating behavior and possible reproductive barriers between the discussed *Ae. vexans* populations. Mating experiments between Mnl-females and Kül-males were successful by Kuhn in 1993, but the reciprocal mating was not. This indicates different behavior - it must be kept in mind though that these mosquitoes were selected to copulate in minimum space (cages measuring 50 x 47.5 x 80 cm) and are therefore not necessarily representative for wild populations.

Genetic studies of *Aedes vexans* need to be carried out on different populations throughout the world with more loci, to make clearer statements about different levels of variance.

National Variation.

In general, the local populations of the Upper Rhine Valley are very similar to one another, as indicated by the low genetic distance (D_{mean}= 0.0023 excluding Mö and 0.0064 including Mö) and F_{ST} - values. As mentioned above, the same allele prevails at each locus in high frequency at each population. The overall similarity of populations concerning the frequency of the most abundant allele should rule out a determination by random processes. As an example, though some of the localities are more or less regularly sprayed with the insecticide Bti (no exact data available), none of these

show any form of recent genetic drift.

Although in the Rhine Valley populations, heterozygosity ranges from 9-15%; there is a medium range of variation from locus to locus within any given population (e.g., Ger: Ho_{mean} GOT 1 = 0%, PEP = 26%). The variation between populations within a given locus is also medium for most loci (PGI, IDH 2, PEP, GOT 2) and small for others (GOT 1). Even though the populations are very similar, significant differences in allele frequencies do exist, as the likelihood ratio test for independence of allele distribution demonstrates. These differences are mostly comprised by the occurrence or absence of rarer alleles.

Generally, the frequencies of many of the rarer alleles are very low, usually ranging from 0.5-4% (percentages ≥5% were not considered rare). Since only about 100 individuals per location were sampled, a certain amount of error must be considered inevitable; even if a rare allele exists within a population, chances are it is not sampled. The observed allele frequency values are probably underestimated. Nevertheless, there are populations that carry alleles with a higher frequency and allow statements about gene flow and migration. An example is in the Mö population in which allele No. 4 on the IDH 2 locus (frequency of 21%) only appears at this location, even though there are no geographical migration barriers throughout the sampled region and mosquitoes are able to fly long distances. Another example is in the Q and Ger populations in which allele No. 7 (frequency of 5%) at the IDH 2 locus is found in the Ger population but not in neighboring Q population. Although the average migration rate is relatively high, some populations experience higher gene flow than others (e.g., Kü-H compared to Q-H).

An explanation for similarities between populations could be that *Ae. vexans* is able to fly far (according to Mohrig 1969 up to 48 km, and 22 km in 24 hrs) and at high altitude. Different authors (see Mohrig 1969) report, that mass swarming from breeding places occurs, in order to find a blood meal in distant locations. There is no indication in the population structure of the Upper Rhine Valley for mass swarming to even neighboring locations. If this were the case, total homogeneity should prevail in at least neighboring populations. This could be explained by two reasons:

1) Female mosquitoes find enough hosts for a blood meal in their immediate surroundings and do not swarm.
2) Mass swarming might occur, but the females return to their own breeding site to lay their eggs, after finding a blood meal.

An observation pointing in this direction has been made by Hamlyn-Harris (1934) concerning *Aedes vigilax* (Skuse). A lot of literature about mosquito dispersal is available, but there are no studies about the behavior of the females after dispersal in search of a blood meal.

An indication for behavioral differences between populations is the copulation rate of wild mosquitoes in the laboratory (Friederich 1983). In these experiments, wild mosquitoes were transfered to large cages (1,400 liters) and the copulation rates examined; they ranged from 8-19% in populations that were only about 20 km away from each other. Mosquitoes from the Q population mate at a rate of about 40% in small cages (see above - personal observation). One conclusion that can be drawn is that the lower the copulation rate in the laboratory, the higher the mosquitoes fly in order to mate in their natural habitat. The high rate for the Q mosquitoes indicates that these individuals mate at a much lower altitude than individuals from other populations. Since the Q population is in a very constant environment with grass always cut low and the pastures flooded artificially on the same two days each year, adaptation towards these conditions could have taken place. Individuals stemming from single immigrated females possibly cannot mate with mosquitoes that show different mating behavior.

Mating experiments with mosquitoes from the Mom and Kü populations (Friederich 1983), which have a similar copulation rate, were successful. Yet, experiments with individuals showing totally different rates have not been carried out yet. Again, a larger study of more loci and an extended sampling region is necessary to determine certain population structures.

Mönchbruch.

It is conspicuous that of all the Rhine Valley populations, only the Mö population is not in a general state of Hardy-Weinberg equilibrium since there is an excess of homozygotes in this population. A possible explanation for this could be the coexistence of two subpopulations that do not mix, within what was originally sampled as one. It is also the only population, where allele No. 4 on the IDH locus is found; an electromorph with the same mobility also appears in Wpg. Since the Frankfurt International Airport lies close by, it is not absurd to assume that mosquitoes were accidentally imported via airplane to establish a population of their own alongside the native population. Reports of mosquitoes on airplanes date as far back as 1931 (Griffitts and Griffitts). An indication for this theory is, that allele No. 4 only occurs in the homozygotic state at a frequency of 21%. It is possible that individuals carrying this genotype are not able to mate with the native individuals due to premating isolation.

Laboratory Populations.

When comparing laboratory populations to the wild ones, the tendency of loss of rare alleles and the fixation of more frequent ones can often be observed after only a few generations. This happened with both lab samples: very rare alleles were lost while the prevailing alleles increased in frequency. In the case of PGI in the Kül population and PEP in the Ql population, however, alleles of an intermediate frequency increased, while the most abundant allele decreased. This is explainable through the small population size and random picking of alleles for each following generation (only a small proportion of eggs laid are actually flooded and hatched).

The inevitable fate of alleles in such populations is either loss or fixation, purely by chance.

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PRELIMINARY EVALUATION OF THE AFFECT OF ALTOSID® (METHOPRENE) ON CRUSTACEANS ASSOCIATED WITH WATERFOWL HOLDING PONDS AT THE KERN NATIONAL WILDLIFE REFUGE[1]

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Application of the juvenile hormone mimic methoprene (Altosid® - Zoëcon Corporation, Dallas, Texas) represents a principal option available to vector control agencies for controlling *Aedes* and *Culex* mosquitoes associated with wetlands environs in the state of California. However, methoprene has recently come under increased scrutiny regarding its potential deleterious impact on crustaceans which represent a significant portion of the aquatic food chain and biodiversity component of wetlands. The consequences of this potential impact have been the focus of many discussions between vector control agencies and the United States Fish and Wildlife Service (USFWS). Open discussions eventually resulted in the draft proposal of a five-year program to evaluate various integrated mosquito control strategies designed to minimize the perceived environmental impact of mosquito control activities on wetlands in California. This cooperative study was undertaken to begin the process of evaluating control options for wetlands mosquito management.

This preliminary study was designed to evaluate the potential impact of a single or double application of aerially applied methoprene (Altosid Liquid Larvicide) on crustaceans (specifically Copepoda, Cladocera, and Ostracoda) associated with waterfowl holding ponds within Unit 4 of the Kern National Wildlife Refuge, Kern County, California. Crustaceans, particularly Cladocera, constitute a major food resource available to wildlife predators during the winter months.

MATERIALS AND METHODS

Study Site.

Methoprene evaluations were conducted along the extreme southern edge of Unit 4 bordering the Poso Creek Channel on the Kern National Wildlife Refuge. Three sections of contiguous shoreline were selected and randomly designated as Treatment 1, Control and Treatment 2 (Fig. 1). Emergent and shoreline vegetation consisted of a mixed aggregate of salt grass, bullhead rush, sedge, cattail, tule, and an assortment of dried annual weeds and grasses.

Treatment Regimes.

Two sections, Treatment 1 and 2, respectively, were treated aerially at the standard rate of four ounces of methoprene/acre as follows: Treatment 1 section received only one insecticide treatment (October 22) and Treatment 2 section received two applications (October 22 and October 29). Aerial applications were conducted within operational limits between 0800 and 0900 hours on the dates indicated. A total of three swaths were applied parallel to and overlapping the shoreline per treatment section. The overall 200' wide treatment zone paralleling the shoreline was created to significantly reduce the potential masking effects from peripheral non-target crustacean immigration.

Crustacean Sampling.

Both the control and treatment sections were sampled using a standard 1-pint (0.47 liter) dipper at weekly intervals beginning on October 22 (Week 1) and

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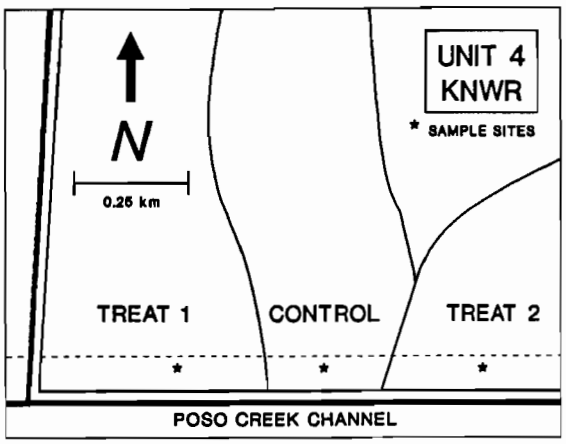

Figure 1. Map of the study area locating the sample sites within the control and two treatment sections of Unit 4 at the KNWR.

concluding on December 7, 1993 (Week 8). Crustacean abundances within each section were determined by sampling populations along three replicated linear transects within one meter and parallel to the shoreline, and at water depths ranging from 10-30 cm (Fig. 1). A total of 20 dips (ca. 10 liters) were taken for each transect. Individual transects (10 m in length) were separated by a minimum of 10-15 meters. Water temperature was measured by a single maximum-minimum thermometer placed in the center transect (C2) of the control section at a depth of 20 centimeters. Each 20-dip transect sample was concentrated in a standard one gallon larval concentrator fitted with organdy netting (200 micron mesh) to trap all macroinvertebrates sampled.

Transect Vegetation Profiles.

In the Control section, the vegetation in Transect 1 (C1) consisted principally of bullhead rush, Transect 2 (C2) was composed of dried annual grasses, and bullhead rush, and Transect 3 (C3) consisted of bullhead rush, dried annual grasses and salt grass. In the Treatment 1 section, Transects 1 (T1A) and 2 (T1B) consisted of bullhead rush while the Control section (T1C) contained bullhead rush and sedge. In the Treatment 2 section, the primary vegetation in Transects 1 (T2A) and 3 (T2C) were salt grasses and bullhead rush, while Transect 2 (T2B) consisted solely of salt grass.

Sample Processing.

Dipper samples were transferred to individual one liter plastic sample bottles and returned to the Kern Mosquito and Vector Control District for processing. Samples were grossly screened to remove debris and aquatic vegetation and subsequently run through the standard sieve series to assure that no crustaceans were lost during the sieving process. All samples were preserved in 70% isopropyl alcohol. The crustaceans present in each sample were identified to class for this preliminary study and quantified by grid subsample (100 cm^2 plastic dish) under a standard binocular microscope (25-50X).

Data Evaluation.

All numerical data were transformed to (Log N)+1 and tested by 2-way ANOVA to determine statistical differences in the overall abundances of Copepoda, Cladocera, and Ostracoda within and between the Control, Treatment 1, and Treatment 2 sections. Differences in the overall abundances between the control and two treatment sections were tested by a Least Significant Difference (LSD) test. Logistic means were back transformed and all data are presented as Williams means (Mw).

RESULTS

Water Depth and Temperature.

Water depth remained constant throughout the study. Any marked changes in depth would have certainly influenced sampling by significantly altering the distribution patterns of the crustaceans within the fixed transects. Water temperatures did not change appreciably from week to week, but oscillated over a wide range between a maximum of 20°C and a minimum of 6°C.

Within Treatment Affects.

There were no significant differences in the mean numbers (Mw) of copepods, cladocerans, and ostracods between individual transects (replicates) within either the control or treatment sections (Table 1).

Between Treatment Affects.

On the first date of sampling (Week 1 - October 22), there were no significant differences in the abundances of copepods, cladocerans, or ostracods between the control and two treatment sections (Fig. 2).

Subsequently, copepods were overall significantly more abundant in the Control section (160.8) than in either the Treatment 1 section (63.2) or the Treatment 2 section (81.4), while there were no significant differences in copepod abundances between the Treatment 1 and Treatment 2 sections (Table 2). Inthe Control section, copepod numbers increased dramatically between Weeks 3 (115/liter of water column) and 6 (300/liter of water

Table 1. Mean numbers of crustaceans sampled per liter of water column (N=8) for each transect within the 3 study sections of the KNWR during 1993.

Transect	Control	Treatment 1	Treatment 2
COPEPODA			
1/A	140	67	94
2/B	166	52	67
3/C	181	73	87
CLADOCERA			
1/A	65	89	53
2/B	113	81	40
3/C	53	59	57
OSTRACODA			
1/A	23	19	7
2/B	34	12	9
3/C	32	29	6

column) and then decreased slightly thereafter. By comparison, copepod populations remained relatively constant at less than 100/liter of water column in both the Treatment 1 and Treatment 2 sections (Fig. 2).

There were no significant differences in the overall abundances of cladocerans between the Control, Treatment 1, and Treatment 2 sections (Table 2). Cladoceran abundance increased in the Control section up to Week 5 (175/liter of water column), peaked and remained relatively constant throughout the remainder of sampling (Fig. 2). In the Treatment 1 and 2 sections (particularly Treatment 1), cladoceran abundance increased rapidly from Week 4 to Week 5, eventually surpassing that of the Control section (Treatment 1 section - 410/liter of water column and Treatment 2 section - 210/liter of water column) before remaining either constant (Treatment 1 section) or increasing further (Treatment 2 section- Week 8).

Overall ostracod numbers were not significantly different between the Control section and Treatment 1 section, but were significantly higher in the Control and Treatment 1 sections compared to the Treatment 2 section (Table 2). Ostracods increased in abundance in the Control section from Week 2 to Week 3 and remained unchanged to Week 8 with the exception of a marked decrease at Week 6 (Fig. 2). Within the Treatment 1 and Treatment 2 sections, ostracods did not increase in abundance until the later weeks of the study. By Week 7, their abundance in the Treatment 1 section exceeded that of the Control section and suddenly increased by 3-fold during Week 8 (50/liter of water column).

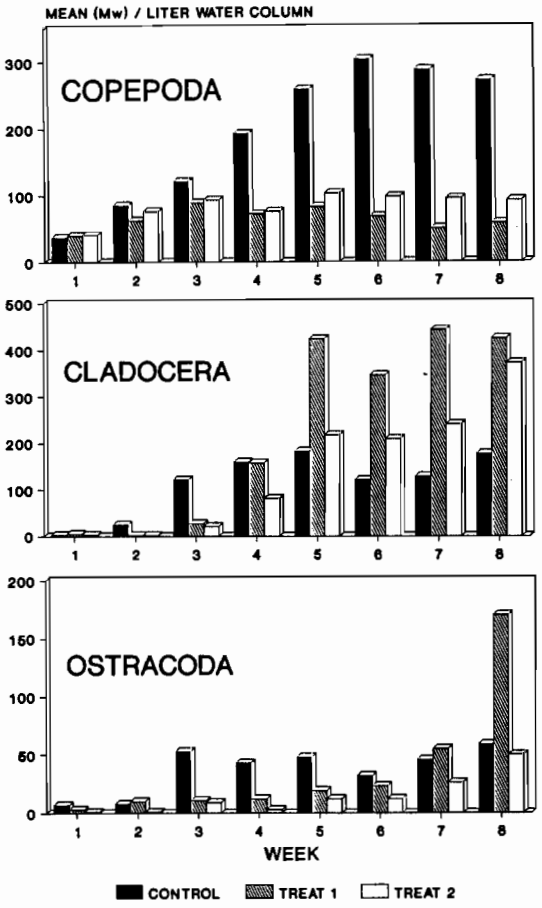

Figure 2. Weekly mean number of crustaceans sampled per liter of water column (N=24) in the 3 study sections of the KNWR.

Composition of Crustacean "Communities".

Compositions of the crustacean "community" was similar between the Control and Treatment 2 sections, but distinctly different than the Treatment 1 section (Fig. 3). Copepods comprised approximately 60% of the total number of crustaceans sampled in both the Control and Treatment 2 sections, but only 40% of the sampling in the

Table 2. Mean numbers of crustaceans sampled per liter of water column (N=24) in the 3 study sections of the KNWR.

Group	Control		Treatment 1		Treatment 2	
Copepoda	160.8	a*	63.2	b	81.4	b
Cladocera	73.3	a	75.1	a	49.4	a
Ostracoda	29.1	a	18.6	a	7.1	b

* Means within rows followed by different letters are significantly different in a Least Significant Difference Test (P>0.05).

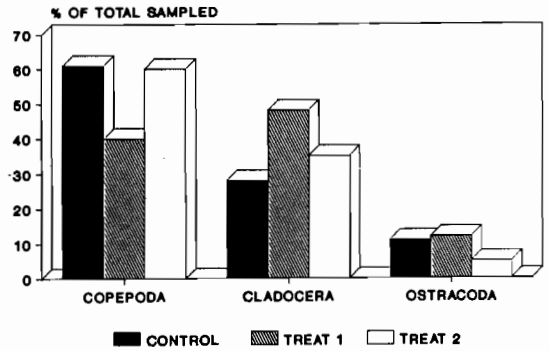

Figure 3. Percentage composition of crustacean "communities" associated with the 3 study sections at the KNWR. Percentages were calculated from the overall mean (N=24) for each crustacean group in each treatment section.

Treatment 1 section. Cladocerans, by comparison, constituted approximately one-half (48%) of the crustaceans in the Treatment 1 section compared to only 28% in the Control and 35% in the Treatment 1 sections, respectively. Ostracods contributed to 12% or less of the sample crustaceans in the Control (11%), Treatment 1 (12%), and Treatment 2 sections (5%).

SUMMARY

This study examined the effects of two different methoprene application regimes on crustacean populations associated with waterfowl holding ponds at the Kern National Wildlife Refuge in Kern County. The data presented herein should not be interpreted as definitive because this initial evaluation was completed in the absence of historical population data for the one control and two treatment sections. The preliminary data does, however, indicate significant differences in population growth patterns as a potential consequence of the methoprene treatments. Overall, the effects of the treatments were variably expressed: 1) there was a distinct retardation of crustacean growth rates in both treatments, 2) copepod productivity may have been significantly impacted as indicated by the absences of a measurable recovery by Week 8 in both treatment sections, and 3) retardation of copepod productivity consequently may have resulted in the uncontested and eventual disproportionate increase in cladoceran productivity in both treatments. Potentially enhanced cladoceran productivity is of particular interest because this crustacean group usually comprises the greatest portion of invertebrate biomass associated with seasonal wetlands in California.

Future evaluations will be required to resolve the potential conflicts resulting from the lack of historical baseline crustacean population data. More critical and in-depth studies will necessarily include the addition of more controls and treatments that are randomly distributed among more than a single holding pond.

ACKNOWLEDGEMENTS

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BIOASSAYS FOR THE DETECTION OF MOSQUITOCIDAL COMPONENTS IN NATURAL PRODUCTS

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ABSTRACT

Development of bioassays for screening botanical materials for the presence of mosquitocidal components allowed extracts of 291 botanical species to be tested with three new larvicides and two new adulticides found. In addition, smaller-scale bioassays were also developed for directing chemical fractionation of botanical specimens. Using these, the active ingredients from each of the five species exhibiting mosquitocidal activity were isolated in pure form.

During the past five years there has been a steady decline in the availability of new candidate mosquito control agents (both larvicides and adulticides). Industrial sources of new synthetic control agents appear to be depleted. However, the need for new, safe and effective mosquito control agents has not declined. Thus, there became a need to initiate a program for generating novel leads for such agents. After reviewing possible directions for this new program, it was concluded that the old approach of screening natural products for biological activity offered as much potential as any other.

A preliminary assessment of the past work on the insecticidal components present in natural products might cause one to conclude that the field has already received ample attention. For example, Willaman and Schubert (1961) list approximately 3,700 plants that contain alkaloids and Jacobson (1958, 1975) reviews the literature on insecticides derived from plants. A major survey of the insecticidal properties of plants was conducted by the Boyce Thompson Institute and summarized by Hartzell and Wilcoxon (1941) and Hartzell (1944, 1948). Another large study was conducted in a joint effort between Merck and Company and Rutgers University (Heal et al. 1950) which unsuccessfully screened approximately 2,500 plant species. In several studies, plant extracts were initially tested against mosquito larvae; Supavarn et al. (1974) screened 36 plants against larvae of *Aedes aegypti* (Linnaeus) as did Patterson et al. (1975) using 325 different plants. Sukumar et al. (1991) reviewed the tests of numerous botanicals that have biological activity against mosquitoes. Unfortunately, no practical leads have resulted, except for pyrethrin which has been known as a mosquito adulticide for over a century and still is in use today.

The large volume of the older literature is difficult to evaluate, but careful study allows some important conclusions concerning why so few discoveries have resulted from the extensive efforts that have been made. Roark (1942), a prominent earlier authority on the insecticidal components in plants, reviewed this area, stating "most of the tests for insecticidal value that have been made on plants and plant extracts are of little value". He bases his statement on several problems he found in the studies, including: 1) failure to test the material upon the proper insect, 2) failure to test proper plant material, and 3) failure to use the proper solvent for extraction of insecticidal principles. It is clear that the entire concept of finding biologically active components from natural products is bioassay-directed; thus, unless the bioassay has been carefully designed and understood, efforts to find novel chemical components will likely be unsuccessful. As will be discussed later, this factor alone probably explains the lack of success in most of the former studies cited above.

With this historical perspective in mind, bioassays for: 1) the detection of active compounds and 2) guiding the fractionation of botanical samples for mosquito

larvicides and adulticides were then developed to direct a new research program for finding mosquitocidal components.

MATERIALS AND METHODS

Larvicide Screening Assays.

Previous experience in the evaluation of mosquito toxicants led to an initial decision that a much larger volume of test solution was required than had been used in past studies, e.g., Patterson et al. (1975) and Supavarn et al. (1974) had used only 10 ml for each individual larvicide test. Since many components in plants are present in small amounts and since some of these are not water soluble, it was necessary to include some organic solvent. Acetone was chosen to aid in dissolving the residue, but was limited to a maximum concentration of 1 ml per liter of water.

Samples of plants and lichens were extracted with 95% ethanol, concentrated in a rotating evaporator, desiccated and sub-samples were weighed out (0.5 gm each) and transferred into a one-liter volumetric flask, using first 1 ml acetone and then tap water to make 1 liter. The flask was vigorously shaken and two 250 ml aliquots were removed and placed into duplicate 80 x 100 mm Pyrex® containers (Pyrex no. 3250) to give the initial test concentration of 500 ppm of the ethanol extract. The 500 ml remaining in the volumetric flask was then diluted with tap water, successively, to give 1:1 dilutions which provided duplicate test containers treated at 250, 125 and 62.5 ppm. The test containers were then fitted with a collar, made by removing the bottom of a 325 ml Styrofoam cup (Styrocup no. 12FC), and covered with a clear plastic lid. Control containers, prepared in the same manner with the same amount of acetone, and check containers (no solvent) were also used in duplicate.

Twenty-five early, fourth-instar *Culex quinquefasciatus* Say larvae were added to each container, a small amount of powdered larval food added and the test containers were held at approximately 27°C under a 14:10 h (L:D) photoperiod. After completion of adult emergence, the mortality of larvae, pupae, and adults was recorded. For samples showing activity, additional tests with further dilutions were made and the LC_{50}s and LC_{95}s were determined using probit analysis (SAS Institute 1985).

Assays of Larvicidal Fractions.

For extracts which showed significant biological activity and therefore justified fractionation, a bioassay was required which consumed a much smaller mass of material and yet would be sensitive enough to direct the fractionation sequence. Fractions were taken to dryness, weighed and then dissolved in a suitable solvent to produce a concentration of 5 mg/5 ml. Aliquots of 1.25 ml were then pipetted into each of two wide-mouth glass jars (5 cm diam x 4 cm high) and the solvent was removed under a stream of dry nitrogen; twenty-five ml of tap water was added to each to give an initial concentration of 50 ppm and the sample was swirled. A series of 1:1 dilutions of the initial 5 mg/5 ml solution were also prepared and pipetted as above to provide a series of decreasing concentrations. Ten early fourth-instar *Cx. quinquefasciatus* larvae were added to each jar and they were fed and held as for the screening bioassay. Controls and checks were also run in duplicate.

Adulticide Screening Assays.

Adulticide tests were conducted by exposing adult females to treated filter paper. Solutions were prepared by dissolving 250 mg amounts of test extract in acetone, plus water as required, into a final volume of 25 ml. Whatman #2 filter papers (15 x 18 cm) were treated with 4 ml of this 1% (wt/vol) solution and each paper was treated three times to give a 3% initial treatment. All treatments were in duplicate. After air drying, the treated paper was rolled treated side in and placed inside a 4.5 x 18 cm long polyvinylchloride tube. The tube ends were covered with screen and 25 unblooded 3-6 day old female *Cx. quinquefasciatus* adults were aspirated into each. Then each tube was placed inside a plastic bag, containing wetted paper toweling and each was inflated with air to prevent collapse of the plastic over the tube ends. The bags were held in a dark incubator at 20°C and mortalities were determined at 24 hours. Mortality data (in percent concentration) was analyzed by probit analysis as previously mentioned.

Plants have long been known to contain sources of hydrocyanic acid (HCN) (Whitmore 1951) and sometimes when the tissues are macerated, enzymes cause release of this toxic gas. Some plants, e.g. those in the genus *Prunus*, have such large amounts that their leaves can be used to make insect killing jars (Hall et al. 1969). Unless one is interested in detecting HCN, an assay is needed so that this "false lead" is readily apparent. Therefore when positive results were obtained in the initial 3% exposure, a simple test for HCN was made by repeating the test but adding an HCN sensitive indicator (HCN Passive Dositube, 10-200 ppm/hr, Lab Safety Supply Co., Jamesville, WI) in the plastic holding bag. If a positive result for HCN was found, the lead was then discounted.

Assays of Adulticidal Fractions.

As in the case for larvicides, when sufficient

biological activity justified fractionation studies, a bioassay is required which uses a lower mass than that required for the screening bioassay. In this test, glass shell vials (13 x 84 mm) were used to hold treated papers. Circular (11 cm) Whatman #2 papers were trimmed using a paper cutter to fit into the shell vials. One ml of a 1% (wt/vol) solution, as above, was used to treat each paper for the initial (highest) 1% concentration. Twenty female *Cx. quinquefasciatus*, as those above, were aspirated into each vial and the open tube ends were covered with nylon mesh. The vials were bagged as above under the same holding conditions. Duplication and analysis of data were also the same.

Validation of Bioassays.

Known larvicides and adulticides were put through the four bioassays to assess their validity. In addition, 291 species of a wide variety of plants and lichens were tested in the larval and adult screening assays; five plants were fractionated, with adulticide fractionation assays being used on two and larvicide assays being used on three.

RESULTS AND DISCUSSION

Larvicide Screening Bioassays.

Of 291 species of plants and lichens, several showed larvicidal activity which had already been reported in the literature. In addition, one plant showed very high larvicidal action, LC_{50} of less than 0.01 ppm; this species had been tested in the study of Heal et al. (1950) and was found not to have sufficient insecticidal activity to justify secondary testing (note: these authors used two species of cockroaches and a milkweed bug for their primary bioassays). Another fraction from the same plant lacked direct toxicity but exhibited a strong juvenile hormone-type effect, i.e., no larval mortality but pupal and adult mortality. Thus the bioassay does detect several types of biological activity. Another species showed positive larvicidal action and no previous reports in the literature could be found relative to this species. Thus three of the 291 species tested showed novel larvicide leads.

Results of screening bioassays for larvicidal activity are summarized in Tables 1-3; Plants with LC_{50}s less than 100 ppm are in Table 1, plants with LC_{50}s greater than 100 but less than 500 ppm are in Table 2, and plants with LC_{50}s equal to or greater than 500, or no significant activity, are listed in Table 3. Overall, nearly one-half of the screened plant species showed little or no larvicidal activity ($LC_{50} \geq 500$ ppm) while one-sixth of them showed moderate activity ($LC_{50} \leq 100$ ppm) (Fig. 1).

Adulticide Screening Bioassays.

Most of the bioassays for adulticidal activity were negative. The first of several positive results in this screen had to be discounted as the cyanide test in each case was positive. However, in the case of two others, the cyanide tests were negative and the leads were therefore considered as positive. Thus, two of 291 species evaluated gave positive results; these were different species than the larvicides and neither lead could be found in the literature.

Bioassays of Larvicide and Adulticide Fractions.

Each of the five positive leads, three larvicides and two adulticides, justified fractionation attempts. The five attempts were successful, i.e. for each one the combination of multiple chemical procedures used, in sequences directed by the bioassays, resulted in the isolation of pure compounds. The chemical structures of four of the five compounds were determined and will be published elsewhere. The fifth compound is very complex and while most of the chemical structure was elucidated, the complete structure is yet to be determined.

CONCLUSIONS

The usefulness of the screening bioassays was validated by the positive results obtained. These assays allow the use of mosquitoes for finding different types of insecticidal components in extracts of botanical materials. The fractionation bioassays also were proven practical.

The number of positive leads obtained, five from 291 plants and lichens, plus at least two other species which merit further fractionation, is higher than one would expect based on past reports; this is assumably due to the improved bioassays that were developed. Using these bioassays, other variables also need to be explored, e.g., the manner in which natural products are extracted.

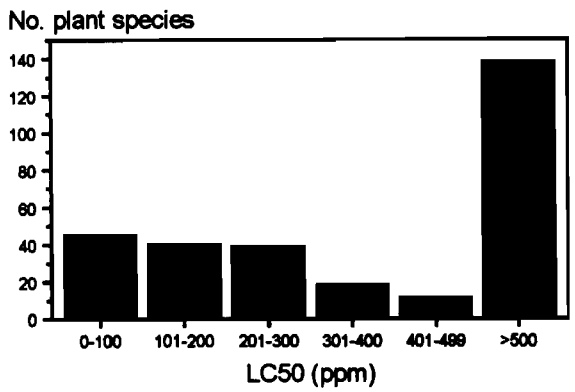

Figure 1. Number of plant species tested in relation to LC_{50}s.

Table 1. Plants with ethanol extracts having $LC_{50} < 100$ ppm.

FAMILY	NAME	PART(S)	LC_{50} (ppm)	95% LIMITS	PROB $>\chi^2$
Acanthaceae *	*Beloperone guttata*	leaves, stems, flowers	12	none	0.0731
Alectoriaceae	*Alectoria sarmentosa*	lichen	14	none	0.0163
Amaranthaceae	*Amaranthus albus*	large stems	90	none	0.0008
Amaranthaceae	*Amaranthus albus*	whole plant	33	28-38	0.8505
Amaranthaceae	*Amaranthus albus*	leaves, small stems	68	56-87	0.6995
Apiaceae	*Heracleum lanatum*	leaves, stems, flowers, fruits	78	none	0.0124
Araliaceae	*Hedera helix*	whole plant	37	33-42	0.3407
Asteraceae	*Artemisia absinthium x arborescens*	stems	6	4-7	0.9688
Asteraceae	*Artemisia absinthium x arborescens*	leaves	27	22-33	0.1396
Asteraceae	*Artemisia absinthium x arborescens*	leaves, stems, roots	7	none	1.0000
Asteraceae	*Artemisia absinthium x arborescens*	roots	9	8-11	0.9153
Asteraceae	*Echinacea purpurea*	whole plant	28	26-32	0.3948
Asteraceae	*Gnaphalium californicum*	leaves	92	none	0.0550
Asteraceae	*Helianthus annuus*	leaves, stems	79	71-89	0.9909
Asteraceae	*Helianthus annuus*	flowers	89	77-101	0.4782
Asteraceae	*Hemizonia pungens*	whole plant	52	35-62	0.9346
Boraginaceae	*Amsinckia intermedia*	whole plant	16	none	0.8854
Calycanthaceae	*Calycanthus occidentalis*	stems	63	52-77	0.3305
Chenopodiaceae	*Atriplex polycarpa*	leaves, stems	76	63-87	0.8794
Chenopodiaceae	*Bassia hyssopifolia*	whole plant	68	53-62	0.1204
Chenopodiaceae	*Chenopodium botrys*	whole plant	16	none	0.6911
Convolvulaceae	*Convolvulus arvensis*	leaves, stems, flowers	47	40-53	0.4982
Convolvulaceae	*Cressa truxillensis*	leaves, stems, flowers, fruits	33	none	
Cuscutaceae	*Cuscuta subinclusa*	whole plant	25	22-28	0.1464
Davalliaceae	*Nephrolepis cordifolia*	whole plant	18	none	0.0038
Dryopteridaceae	*Athyrium filix-femina*	roots	62	none	0.0004
Euphorbiaceae	*Eremocarpus setigerus*	whole plant	31	9-52	0.1824
Fagaceae	*Quercus douglasii*	leaves, stems, seeds, flowers	92	65-116	0.1234
Hydrophyllaceae	*Phacelia quickii*	whole plant	56	46-70	0.4760
Liliaceae	*Chlorogalum pomeridianum*	roots	75	67-85	0.9014
Liliaceae	*Chlorogalum pomeridianum*	leaves	64	57-72	0.3672
Liliaceae	*Veratrum californicum*	whole plant	75	66-85	0.9684
Liliaceae	*Yucca whipplei*	roots	84	none	0.0000
Magnoliaceae	*Magnolia grandiflora*	leaves, stems	96	50-139	0.9550
Myrtaceae *	*Myrtus comminis*	leaves, stems, flowers	88	67-108	0.1132
Papaveraceae	*Dendromecon rigida var. rhamnoides*	brown stems	22	19-24	0.6159
Papaveraceae	*Dendromecon rigida var. rhamnoides*	green stems	27	11-202	0.0471
Papaveraceae	*Romneya coulteri*	seeds	33	0.01-77	0.1663
Papaveraceae	*Romneya coulteri*	stems	87	72-102	0.1402
Parmeliaceae	*Tuckermonopsis platyphilla*	lichen	83	36-113	0.3177
Plantanaceae	*Plantanus recemosa*	leaves, stems	98	none	0.0011
Pteridaceae	*Pellaea mucronata*	roots	57	0.26-84	0.7905
Pteridaceae	*Pellaea mucronata*	whole plant	40	34-47	0.1010
Rosaceae	*Prunus subcordata*	stems	50	none	0.0376
Saxifragaceae	*Carpenteria californica*	leaves	39		
Scrophulariaceae	*Mimulus puniceus*	leaves, stems	36	33-40	0.6457
Simarubaceae	*Ailanthus altissima*		33	6-48	0.8914
Solanaceae	*Solanum americanum*	whole plant	10		
Verbenaceae	*Lantana montevidensis*	stems	43	33-53	0.1480
Verbenaceae	*Lippia nodiflora*	leaves, stems, roots	72	none	0.0000
Vitaceae	*Vitis californica*	leaves, stems	88	46-123	0.5219
Zygnemataceae	*Spirogyra sp.*	filaments	80	62-97	0.1431
Zygophyllaceae	*Tribulus terrestris*	leaves, stems	44	33-63	0.3780
Zygophyllaceae	*Tribulus terrestris*	roots	96	77-134	0.1966
Zygophyllaceae	*Tribulus terrestris*	whole plant	17	14-17	0.1601

* Ornamental plant.

Table 2. Plants with ethanol extracts having LC_{50} > 100 and < 500 ppm.

FAMILY	NAME	PART(S)	LC_{50} (ppm)	95 % LIMITS	PROB $>\chi^2$
Aceraceae	*Acer saccharinum*	leaves, stems	242	none	0.0003
Apiaceae	*Foeniculum vulgare*	leaves, stems, flowers	148	none	0.0039
Asclepiadaceae	*Asclepias vestita*	flowers	156	137-177	0.1073
Asclepiadaceae	*Asclepias vestita*	stems	438	49-3910	0.0000
Asclepiadaceae	*Asclepias vestita*	fruits	346	none	0.0000
Asclepiadaceae	*Asclepias vestita*	roots	187	72-834	0.0010
Asteraceae	*Achillea millefolium*	whole plant	139	127-154	0.9946
Asteraceae	*Anaphalis margaritacea*	whole plant	237	none	0.0518
Asteraceae	*Antennaria corymbosa*	whole plant	164	none	0.0002
Asteraceae	*Anthemis cotula*	whole plant	480	381-698	0.1301
Asteraceae	*Artemisia californica*	leaves, stems, fruits	252	227-281	0.1649
Asteraceae	*Brickellia californica*	leaves, stems	280	none	1.0000
Asteraceae	*Centaurea repens*	leaves, stems	200	162-249	0.5652
Asteraceae	*Centaurea solstitialis*	leaves, stems, flowers	168	none	0.0398
Asteraceae	*Chamomilla suaveolens*	whole plant	245	205-291	0.1968
Asteraceae	*Chrysothamnus nauseosus*	leaves, stems	383	none	0.0005
Asteraceae	*Cirsium coulteri*	leaves	297	none	0.0000
Asteraceae	*Coreopsis lanceolata*	whole plant	274	244-308	0.4544
Asteraceae	*Franseria acanthicarpa*	whole plant	222	176-275	0.5005
Asteraceae	*Gnaphalium californicum*	seeds	259	228-294	0.2699
Asteraceae	*Gnaphalium palustre*	whole plant	143	none	0.0041
Asteraceae	*Gnaphalium purpureum*	whole plant	210	45-375	0.6159
Asteraceae	*Haplopappus arborescens*	brown stems	256	159-435	0.7756
Asteraceae	*Haplopappus arborescens*	leaves, green stems	302	238-404	0.9222
Asteraceae	*Hemizonia kelloggii*	whole plant	464	none	0.0161
Asteraceae	*Layia gaillardioides*	whole plant	443	none	0.0629
Asteraceae	*Madia elegans*	whole plant	238	210-271	0.4055
Asteraceae	*Senecio douglassii*	stems	214	none	0.0825
Asteraceae	*Senecio douglassii*	flowers	102	80-160	0.7570
Asteraceae	*Xanthium strumarium*	leaves, stems	192	162-229	0.6844
Betulaceae	*Corylus rostrata*	leaves, stems	279	201-402	0.3165
Boraginaceae	*Hackelia mundula*	leaves, stems, flowers	452	383-580	0.9059
Boraginaceae	*Heliotropium curassavicum*	whole plant	231	none	0.0000
Boraginaceae	*Plagiobothrys canescens*	leaves, stems, flowers	197	58-318	0.3262
Boraginaceae	*Plagiobothrys nothofulvus*	leaves, stems, flowers	129	108-152	0.2126
Brassicaceae	*Brassica geniculata*	whole plant	103	9.4-167	0.5204
Brassicaceae	*Capsella bursa-pastoris*	leaves, stems, roots	138	none	0.0385
Brassicaceae	*Cardamine breweri*	whole plant	452	none	0.0018
Brassicaceae	*Erysimum capitatum*	leaves, stems	259	none	0.0526
Brassicaceae	*Erysimum capitatum*	flowers	420	none	0.0003
Brassicaceae	*Sisymbrium altissimum*	whole plant	137	none	0.0532
Brassicaceae	*Sisymbrium irio*	whole plant	205	2-2400	0.0468
Calycanthaceae	*Calycanthus occidentalis*	leaves	430	362-547	0.9079
Cannaceae	*Canna orchiodes*	whole plant	486	none	0.0304
Caprifoliaceae	*Lonicera interrupta*	leaves	243	115-599	0.0023
Caprifoliaceae *	*Viburnum odoratisimum*	leaves, stems	245	none	0.0017
Caryophyllaceae	*Spergula villosa*	whole plant	271	none	0.0052
Chenopodiaceae	*Atriplex serenana*	leaves, stems, flowers, fruits	173	none	0.0409
Chenopodiaceae	*Salicornia subterminalis*	leaves, stems, seeds	309	none	0.0280
Chenopodiaceae	*Suaeda californica*	leaves, stems, roots	219	180-272	0.1177
Dryopteridaceae	*Athyrium filix-femina*	leaves	257	55-1137	0.0001

Table 2. Plants with ethanol extracts having LC_{50} > 100 and < 500 ppm.

FAMILY	NAME	PART(S)	LC_{50} (ppm)	95 % LIMITS	PROB $>\chi^2$
Equisetaceae	*Equisetum arvense*	whole plant (no roots)	346	109-5384	0.0001
Ericaceae	*Leucothoe davisiae*	leaves, stems	288	none	0.0001
Fabaceae	*Astragalus symmetricus*	leaves, stems, flowers, fruits	111	none	0.0003
Fabaceae	*Cercis occidentalis*	stems	117	96-140	0.8460
Fabaceae	*Cercis occidentalis*	leaves	314	229-514	0.2931
Fabaceae	*Medicago polymorpha*	whole plant	171	151-192	0.7733
Fabaceae	*Melilotus indica*	whole plant	367	none	0.0017
Fabaceae	*Psoralea sp.*	leaves, stems, flowers	151	none	0.0000
Fabaceae	*Trifolium variegatum*	whole plant	382	none	0.0270
Frankeniaceae	*Frankenia sp.*	leaves, stems	266	198-411	0.5949
Grossulariaceae	*Ribes nevadense*	green stems	102	39-145	0.7706
Grossulariaceae	*Ribes nevadense*	brown stems	461	none	0.0975
Hippocastanaceae	*Aesculus californica*	fruits	231	161-381	0.8930
Hippocastanaceae	*Aesculus californica*	flowers	106	12-170	0.2131
Hippocastanaceae	*Aesculus californica*	leaves, stems	135	105-167	0.2344
Hydrophyllaceae	*Erodictyon californicum*	stems	361	337-460	0.8018
Hydrophyllaceae	*Erodictyon californicum*	roots	260	219-311	0.5679
Hypericaceae	*Hypericum perforatum*	whole plant	256	none	
Juncaceae	*Juncus bufonius*	whole plant	152	none	0.0000
Lamiaceae	*Salvia clevelandii*	leaves, stems, flowers	442	none	0.0303
Lamiaceae	*Salvia mellifera*	leaves, stems	486	394-671	0.1726
Lamiaceae	*Stachys albens*	leaves, stems	390	335-473	0.9372
Lauraceae *	*Cinnamomum camphorum*	leaves, stems	263	none	0.0016
Liliaceae	*Agave deserti*	leaves	111	97-125	0.7388
Liliaceae	*Asparagus officinalis*	leaves, stems	332	263-462	0.9564
Liliaceae	*Chlorogalum pomeridianum*	stems	134	122-139	0.1406
Loasaceacae	*Mentzelia lindleyi*	leaves, stems	361	none	0.0322
Lythraceae *	*Lagerstroemia indica*	leaves, stems	420	299-770	0.1414
Malvaceae	*Malva parviflora*	whole plant	116	101-133	0.6594
Malvaceae	*Sidalcea hartwegii*	leaves, stems, flowers	179	none	0.0070
Moraceae	*Ficus carica*	leaves, stems, fruits	225	none	0.0518
Nymphaeaceae	*Nuphar luteum polysepalum*	leaves, stems	117	none	1.0000
Oleaceae	*Olea europaea*	leaves, stems, flowers	326	307-376	0.8402
Onagraceae	*Clarkia williamsonii*	whole plant	449	398-524	0.8023
Onagraceae	*Oenothera contorta*	leaves, stems	215	188-247	0.2114
Oxalidaceae	*Oxalis pes-caprae*	leaves, stems	165	143-191	0.8585
Papaveraceae	*Romneya coulteri*	flowers	243	none	0.0236
Papaveraceae	*Romneya coulteri*	leaves	204	181-230	0.4993
Parmeliaceae	*Bryoria fremontii*	lichen	293	none	0.0574
Pittosporaceae *	*Pittosporium tobira*	whole plant	294	none	0.0104
Plantaginaceae	*Plantago lanceolata*	whole plant	368	none	0.0001
Poaceae	*Holcus lanatus*	leaves, stems, seeds	315	none	0.0000
Poaceae	*Poa sp.*	flowers, leaves, roots	368	288-545	0.4739
Poaceae	*Polypogon monspeliensis*	whole plant	293	none	0.0832
Polemoniaceae	*Linanthus montanus*	leaves, stems	123	none	0.0000
Polygonaceae	*Eriogonum fasiculatum polifolium*	leaves, stems, flowers, fruits	354	306-420	
Portulacaceae	*Calandrinia ciliatum*	whole plant	254	none	0.0183
Portulacaceae	*Montia perfoliata*	leaves, stems, flowers	219	69-819	0.0623
Pteridaceae	*Adiantum capillus venerus*	whole plant	288	none	0.0697
Pteridaceae	*Pellaea mucronata*	leaves, stems	101	84-132	0.2877
Ranunculaceae	*Ranunculus aquatilus*	whole plant	177	none	0.0056

Table 2. Plants with ethanol extracts having LC_{50} > 100 and < 500 ppm.

FAMILY	NAME	PART(S)	LC_{50} (ppm)	95 % LIMITS	PROB $>\chi^2$
Rhamnaceae	*Ceanothus integerrimus var. californicus*	leaves, stems	207	none	0.0008
Rhamnaceae	*Ceanothus leucodermis*	leaves	387	254-631	0.0310
Rhamnaceae	*Rhamnus californica*	leaves, stems, fruits	130	none	0.0000
Rhamnaceae	*Rhamnus rubra*	stems	184	116-243	0.2730
Rhamnaceae	*Rhamnus rubra*	leaves	119	76-161	0.6943
Rosaceae	*Chamaebatia foliolosa*	stems	463	370-659	0.7771
Rosaceae	*Chamaebatia foliolosa*	roots	187	166-211	0.4940
Rosaceae	*Heteromeles arbutifolia*	leaves, stems	168	102-283	0.0358
Rosaceae	*Heteromeles arbutifolia*	leaves, fruits	289	none	0.0000
Rosaceae	*Potentilla glandulosa*	whole plant	196	none	0.0221
Rosaceae	*Prunus emarginata*	leaves	201	178-226	0.2668
Rosaceae	*Prunus emarginata*	stems	145	none	0.0262
Rosaceae	*Prunus virginiana demissa*	stems	354	297-441	0.1758
Rosaceae	*Prunus virginiana demissa*	leaves	225	none	0.0000
Rutaceae	*Ptelea crenulata*	leaves, stems, flowers	113	101-125	0.7502
Saururaceae	*Anemopsis californica*	whole plant	204	184-225	0.7393
Saxifragaceae	*Carpenteria californica*	small stems	152	128-181	0.4689
Saxifragaceae	*Carpenteria californica*	fruits	139	77-604	0.0000
Saxifragaceae	*Carpenteria californica*	large stems	125	none	0.0389
Scrophulariaceae	*Castelleja applegatei*	leaves, stems, flowers	169	154-185	0.9821
Scrophulariaceae	*Collinsqia heterophylla*	leaves, stems	369	none	
Scrophulariaceae	*Digitalus purparea*	leaves, stems	326	230-608	0.6900
Scrophulariaceae	*Mimulus longiflorus*	stems	269	none	0.0008
Scrophulariaceae	*Mimulus longiflorus*	leaves	425	none	0.0106
Scrophulariaceae	*Mimulus longiflorus*	flowers	141	116-207	0.5042
Scrophulariaceae	*Mimulus puniceus*	flower, fruits	129	5.6-504	0.0322
Scrophulariaceae	*Penstemon breviflorus*	brown stems	137	109-161	0.1853
Scrophulariaceae	*Penstemon breviflorus*	large green stems	123	56-171	0.8168
Scrophulariaceae	*Penstemon heterophyllus*	flowers	348	144-1186	0.0004
Scrophulariaceae	*Verbascum thapsus*	leaves, stems	499	367-1019	0.1029
Solanaceae	*Solanum xantii*	flower, fruits	237	213-263	0.3105
Solanaceae	*Solanum xantii*	leaves, stems	144	64-352	0.0007
Teloschistaceae	*Xanthoria polycarpa*	whole plant	288	260-321	0.2119

* Ornamental Plant.

Table 3. Plants with ethanol extracts having $LC_{50} \geq 500$ ppm.

FAMILY	NAME	PART(S)
Aizoaceae	*Mesembryanthemum nodiflorum*	whole plant
Aizoaceae	*Sesuvium verrucosum sessile*	whole plant
Alismataceae	*Echinodorus cordifolius*	leaves, flowers
Anacardiaceae	*Rhus trilobata*	leaves, stems, flowers
Apocynaceae *	*Trachelospermum jasmoides*	leaves, stems, flowers
Asclepiadaceae	*Asclepias vestita*	leaves
Asteraceae	*Adenocaulon bicolor*	whole plant
Asteraceae	*Artemisia douglasiana*	leaves, stems
Asteraceae	*Baccharis pilularis*	leaves, stems
Asteraceae	*Balsamorhiza deltoidea*	whole plant
Asteraceae	*Cichorium intybus*	whole plant
Asteraceae	*Cirsium coulteri*	stems
Asteraceae	*Cirsium coulteri*	fruits
Asteraceae	*Conyza bonariensis*	whole plant
Asteraceae	*Conyza canadensis*	whole plant
Asteraceae	*Cotula australis*	whole plant
Asteraceae	*Eriophyllum confertiflorum*	whole plant
Asteraceae	*Grindelia camporum*	whole plant
Asteraceae	*Lactuca serriola*	leaves, stems
Asteraceae	*Lygodesmia exigua*	leaves
Asteraceae	*Madia sp.*	whole plant
Asteraceae	*Picris echioides*	whole plant
Asteraceae	*Senecio canus*	leaves, stems
Asteraceae	*Senecio vulgaris*	whole plant
Asteraceae	*Silybum marianum*	leaves, stems
Asteraceae	*Sonchus oleraceus*	whole plant
Berberidaceae *	*Nandina domestica*	leaves, stems, fruits
Betulaceae	*Alnus rhombifolia*	leaves, stems
Bignoniaceae *	*Campsis radicans*	leaves, stems, roots
Brassicaceae	*Brassica nigra*	whole plant
Brassicaceae	*Cardaria draba*	whole plant
Brassicaceae	*Raphanus sativus*	leaves, stems
Brassicaceae	*Thysanocarpus curvipes elegans*	whole plant, seeds
Capparidaceae	*Wislizenia refractor*	whole plant
Caprifoliaceae	*Lonicera interrupta*	seeds
Caprifoliaceae	*Lonicera interrupta*	stems
Caprifoliaceae	*Sambucus caerulea*	leaves
Casuarinaceae *	*Casuarina equisetafolia*	stems
Celastraceae *	*Euonymus japonica*	leaves
Chenopodiaceae	*Allenrolfea occidentalis*	leaves, stems
Chenopodiaceae	*Atriplex phyllostegia*	whole plant
Chenopodiaceae	*Salsola pestifer*	leaves, stems, roots
Cistaceae *	*Cistus purpareus*	leaves, stem, flowers
Commelinaceae	*Tradescantia andersoniana*	leaves, stems
Cornaceae	*Cornus nutallii*	flowers
Cornaceae	*Cornus nutallii*	leaves
Crassulaceae	*Tillaea erecta*	whole plant
Cucurbitaceae	*Cucurbita foetidissima*	leaves, stems
Cucurbitaceae	*Marah horridus*	fruits
Cucurbitaceae	*Marah horridus*	leaves, stems
Cyperaceae	*Carex dristatus*	leaves, flowers

Table 3. Plants with ethanol extracts having $LC_{50} \geq 500$ ppm.

FAMILY	NAME	PART(S)
Cyperaceae	*Cyperus esculentus*	leaves, stems, flowers
Datiscaceae	*Datisca glomerata*	whole plant
Dennstaedtiaceae	*Pteridium aquilinum*	whole plant
Equisetaceae	*Equisetum arvense*	roots
Ericaceae	*Arbutus undeo*	leaves, stems, buds
Ericaceae	*Arctostaphylos patula*	leaves, stems
Ericaceae	*Arctostaphylos viscida*	leaves, stems
Ericaceae	*Rhododendron indicum*	stems
Ericaceae	*Rhododendron indicum*	leaves
Ericaceae	*Rhododendron occidentale*	leaves, stems
Euphorbiaceae	*Euphorbia supina*	whole plant
Euphorbiaceae *	*Sapium sebiferum*	leaves, stems, flowers
Fabaceae	*Albizia julibrissum*	leaves, stems, flowers
Fabaceae	*Cercis occidentalis*	fruits
Fabaceae	*Cytisus scoparius*	leaves, stems, flowers
Fabaceae	*Lathrys sulphureus*	leaves, stems, flowers
Fabaceae	*Lotus scoparius*	stems
Fabaceae	*Lotus scoparius*	flowers, seeds
Fabaceae	*Lupinus albifrons*	flowers
Fabaceae	*Lupinus albifrons*	leaves, stems
Fabaceae	*Lupinus benthamii*	leaves, stems, flowers
Fabaceae	*Lupinus bicolor*	whole plant
Fabaceae	*Melilotus alba*	whole plant
Fabaceae	*Trifolium hirtum*	whole plant
Fabaceae	*Trifolium repens*	leaves, stems, flowers
Fabaceae	*Vicia sativa*	whole plant
Fagaceae	*Castanopsis sempervirens*	leaves, stems
Fagaceae	*Quercus wislizenii*	leaves, stems
Flacorteaceae *	*Xylosma congestum*	leaves, stems
Geraniaceae	*Erodium botrys*	whole plant
Ginkgoaceae *	*Ginkgo biloba*	stems, leaves, flowers
Hamamelidaceae *	*Liquidambar styraciflua*	leaves, stems
Hydrophyllaceae	*Eriodictyon tomentosum*	leaves, stems, flowers
Hydrophyllaceae	*Erodictyon californicum*	flowers
Hydrophyllaceae	*Phacelia cicutaria*	stems
Hydrophyllaceae	*Pholistoma racemosum*	whole plant
Iridaceae	*Iris germanica*	leaves
Juglandaceae	*Juglans californica*	leaves, stems, flowers
Juncaceae	*Juncus balticus*	leaves, flowers, fruits
Juncaceae	*Juncus dubius*	whole plant
Lamiaceae	*Marrubium vulgare*	leaves, stems
Lamiaceae	*Monardella odoratissima*	whole plant
Lamiaceae	*Salvia columbariae*	whole plant
Lamiaceae	*Salvia elegans*	leaves, stems
Lamiaceae	*Salvia mellifera*	flowers
Lamiaceae	*Teucrium chamaedrys*	leaves, stems
Lamiaceae	*Trichostema lanceolatum*	leaves, stems
Liliaceae	*Ruscus aculeatus*	leaves, stems
Liliaceae	*Yucca whipplei*	leaves, stems
Malvaceae	*Sida hederacea*	whole plant
Oleaceae	*Fraxinus dipetala*	leaves, stems, flowers

Table 3. Plants with ethanol extracts having $LC_{50} \geq 500$ ppm.

FAMILY	NAME	PART(S)
Oleaceae	*Ligustrum japonicum*	leaves, stems, flowers
Onagraceae	*Epilobium brachycarpum*	leaves, stems, roots
Onagraceae	*Oenothera hookeri*	whole plant
Onagraceae	*Oenothera laciniata*	whole plant
Poaceae	*Arundo donax*	leaves, stems
Poaceae	*Avena fatua*	whole plant
Poaceae	*Bromus mollis*	whole plant
Poaceae	*Distichlis spicata*	leaves, stems, seeds
Poaceae	*Echinochloa crusgalli*	leaves, stems, fruits, flowers
Poaceae	*Hordeum marinum gussoneanum*	leaves, stems, seeds
Poaceae	*Lolium multiflorum*	whole plant
Poaceae	*Paspalum dilatatum*	whole plant
Poaceae	*Phalaris minor*	leaves, stems
Polemoniaceae	*Collomia grandiflora*	whole plant
Polemoniaceae	*Gilia capitala*	whole plant
Polygonaceae	*Eriogonum fasciculatum*	leaves, stems
Polygonaceae	*Polygonum bistortoides*	leaves, flowers
Polygonaceae	*Rumex crispus*	whole plant
Polygonaceae *	*Polygonum aubertii*	leaves, stems
Pottiaceae	*Tortula puralis+Desmatodon sp*	whole plant
Primulaceae	*Dodecatheon jeffreyi*	leaves, stems
Pyrolaceae	*Sarcodes sanguine*	whole plant
Ranunculaceae	*Ranunculus occidentalis*	leaves, stems
Rhamnaceae	*Adolphia californica*	leaves, stems
Rhamnaceae	*Ceanothus integerrimus v. californicus*	flowers
Rhamnaceae	*Ceanothus leucodermis*	stems, fruits
Rhamnaceae	*Ceanothus leucodermis*	stems
Rhamnaceae *	*Zizyphus jujuba*	leaves, stems, flowers
Rosaceae	*Adenostoma fasiculatum*	flowers
Rosaceae	*Adenostoma fasiculatum*	leaves, stems
Rosaceae	*Cercocarpus betuloides*	leaves
Rosaceae	*Cercocarpus betuloides*	brown stems
Rosaceae	*Chamaebatia foliolosa*	leaves
Rosaceae	*Prunus subcordata*	leaves
Rosaceae	*Prunus virginiana demissa*	immature fruit
Rosaceae	*Rosa gymnocarpa*	leaves, stems, flower buds
Rosaceae	*Rubus parviflorus*	leaves, stems
Rosaceae	*Rubus ursinus*	leaves, stems, flowers
Rubiaceae	*Cephalanthus occidentalis californicus*	stems
Rubiaceae	*Cephalanthus occidentalis californicus*	leaves
Rubiaceae	*Galium aparine*	leaves, stems
Salicaceae	*Populus tremuloides*	leaves, stems
Salicaceae	*Salix laevigata*	leaves, stems, flowers
Saxifragaceae	*Peltiphyllum peltatum*	leaves, stems, flowers
Scrohpulariaceae	*Veronica serpyllifolia*	leaves, stems, flowers, fruits
Scrophulariaceae	*Mimulus guttatus*	leaves, stems
Scrophulariaceae	*Orthocarpus linearilobus*	leaves, stems
Scrophulariaceae	*Penstemon breviflorus*	leaves, stems
Scrophulariaceae	*Penstemon breviflorus*	flowers
Scrophulariaceae	*Penstemon heterophyllus*	leaves, stems
Scrophulariaceae	*Scrophularia californica*	leaves, stems, fruits

Table 3. Plants with ethanol extracts having LC_{50} ≥500 ppm.

FAMILY	NAME	PART(S)
Scrophulariaceae	*Verbascum thapsus*	stems
Scrophulariaceae	*Verbascum thapsus*	roots
Selaginellaceae	*Selaginella hanseni*	whole plant
Solanaceae	*Nicotiana glauca*	leaves, stems
Sterculiaceae	*Fremontia californica*	leaves, stems
Sterculiaceae	*Fremontia californica*	flowers
Theaceae *	*Camelia japonica var. Buddy*	leaves, stems, buds
Urticaceae	*Urtica holosericea*	leaves, stems, fruits
Usneaceae	*Evernia prunastri*	lichen
Verbenaceae	*Lantana montevidensis*	flowers
Verbenaceae	*Verbena hastata*	stems, flowers
Viscaceae	*Phoradendron flavescens*	leaves, stems, flowers

* Ornamental Plant.

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EFFICACY AND FIELD EVALUATION OF FENDONA® AND ICON® AGAINST DENGUE VECTORS IN MALAYSIA

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ABSTRACT

Both Fendona® (alphacypermethrin) and Icon® (lambdacyhalothrin) were evaluated against bloodfed adult and 4th-instar larval *Aedes aegypti* (Linnaeus) in a housing estate in Malaysia. The housing estate is composed of single-story brick-walled houses which are linked together, sharing a common wall with their neighbors on either side. The houses were divided into three sectors with ten study houses randomly chosen from each sector. Homes chosen from the first two sectors were individually sprayed (inside and outside) with a specific pyrethroid and houses in the third sector were designated as control. Each pyrethroid was sprayed using a Pulsfog fogging machine at an insecticide dosage rate of 0.01 gm a.i./m^2.

Mosquito mortality and knockdown were monitored using caged, bloodfed, 4-day old *Ae. aegypti* adults. During testing, each cage (26 cm long and 18 cm in diameter) contained 25 bloodfed *Ae. aegypti* females. Cages were hung inside and outside of each house, along with bottle containers (5.7 cm long and 6.5 cm wide), each containing 25 4th-instar *Ae. aegypti* larvae. Knock-down of adult and larval mosquitoes were recorded one hour after spraying and mortalities were recorded 24 hours post-spraying. Bottle containers, each with water and an oviposition paddle, were placed inside and outside of each house weekly during the study period to monitor natural populations of oviposited eggs and larvae. The numbers of *Ae. aegypti* and *Aedes albopictus* (Skuse) in the field were pooled together for statistical purposes. Analysis of variance was performed using the ANOVA and LSD test.

There was no significant difference between Fendona and Icon in causing larval knockdown either inside or outside of houses (LSD, $P > 0.05$). However, both pyrethroids significantly differed from the control (LSD, $P < 0.001$). There also was no significant difference between Fendona and Icon in causing larval mortalities either inside or outside of houses (LSD, $P > 0.05$). But, once again, both pyrethroids significantly differed from the controls (LSD, $P < 0.001$) when sprayed at a dose of 0.01 gm a.i./m^2. Similarly, there was no significant difference on knockdown and mortality of adults either inside or outside of houses (LSD, $P > 0.05$) by both pyrethroids but they too significantly differed from the controls (LSD, $P < 0.001$). Thus, both pyrethroids demonstrated significant larvicidal and adulticidal effects but differed little from each other.

In studying the impact of both pyrethroids on natural populations of *Aedes* larvae, zero larval populations was observed for certain periods after spraying of both Fendona and Icon. Similarly, zero egg ovipositions had been shown in those houses and their compounds sprayed by both Fendona and Icon, but not in the control. Thus, both pyrethroids showed residual effects in suppressing field populations of larvae and eggs of *Aedes* mosquitoes.

In conclusion, Fendona and Icon both have larvicidal and adulticidal effects on *Ae. aegypti* and *Ae. albopictus* in the field.

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NICHE SPECIALIZATION OF MIDGE SPECIES (DIPTERA: CHIRONOMIDAE) ASSOCIATED WITH SUBMERGED VEGETATION[1]

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In a study of the chironomid midge faunal composition of freshwater lakes, artificial substrates made of plastic netting were used on a biweekly basis for collecting larvae associated with submerged vegetation. From these collections, vertical and seasonal midge larval abundances were estimated and a mode of chironomid larval existence was defined for each taxa. Samples of chironomid larvae collected from the plastic strips indicated taxa that used submerged vegetation as shelter, substrate for living, or food source. At the same time, five bottom mud samples from the sides of the lake were also taken on a biweekly basis for similar abundance studies and mode of existence determinations.

Most chironomid midge control programs are directed at destroying larvae developing in the bottom mud of lakes. It is important that program planners at these districts be aware that significant emergences of midges may arise from other substrates such as submerged vegetation and algal mats.

Beginning in October, 1991, Lake #12 at Mission Hills Country Club in Rancho Mirage (Riverside County), California and many more similar lakes from throughout this and other golf resorts were sampled with a long handled scoop (2 m handle; 15x15x5 cm scoop) on a biweekly basis as part of a long range study.

In May 1992, the Coachella Valley Mosquito Abatement District began to receive complaints about adult chironomid midges from Mission Hills Country Club residents living around Lake #12. At that time, mud samples from the lake contained only 5-10 midge larvae (mostly *Chironomus* Meigen) per sample. Visual observation confirmed that onshore midge populations were much higher than the larval numbers as indicated by the mud samples. It was obvious that chironomid midges were coming from a substrate other than the bottom mud of the lake.

At the end of April through the beginning of May, submerged vegetation in Lake #12 became visible as filamentous branches of *Najas* pondweed reached the water surface. Part of that vegetation contained algal mats of filamentous algae, *Cladophora* and *Spirogira*, and attached erect algae, *Chara*. The presence of the pondweed not only provided large surface areas for chironomid midge species associated with submerged vegetation but also created certain problems with regard to midge larvae sampling.

In October 1992, we began using an artificial substrate for collecting midge larvae associated with a submerged vegetation and algal mats. Eighteen strips of plastic netting (surface area of 100 cm^2) were suspended in the lake at depths from 1-6 feet, three at each depth. On a biweekly basis, the artificial substrates were changed and midge larval numbers and species composition were recorded according to the different depths.

During our research, 18 midge species within 13 genera were recorded in Lake #12 at Mission Hills Country Club. Categorization of midge larval mode of existence was defined by careful examinations of benthic samples and submerged vegetation in the laboratory. Our findings were compared with those given in Table 22A of Merrit and Cummins (1978). While submerged vegetation was present in the lake, midge larvae with special modes of existence, such as climbers (*Nimbocera* Reiss and *Polypedilum* Kieffer), clingers and miners (*Crycotopus* Wulp), portable send tube builders

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(*Goeldichironomus* Fittkau), and tube builders on the plants or other objects (*Apedilum* Townes) were found in high numbers on our artificial substrates. Burrowers, bottom send tube builders, and sprawlers, such as *Chironomus stigmaterus* Say, *Tanypus neopunctipennis* Sublette, and *Tanypus grodhausi* Sublette, were never found on the artificial substrates.

Using statistical methods of single classification ANOVA with unequal sample size, we found that abundance of midge larvae regardless depth was significantly higher on the artificial substrate during the months when vegetation was present. In June 1993, due to an application of weed and algal control chemicals into Lake #12, most of the submerged vegetation was destroyed and bottom dwellers and sprawlers increased in number, to occasionally be found inhabiting the artificial substrate at depths of 5 or 6 feet. Using the least significant range (LSR), we compared midge larvae abundances among the different depths of artificial substrate. When we compared depths of 1,2, 3 and 4 feet with a 5 and 6 feet artificial substrate, we found that the presence of submerged vegetation provided harborage for significantly higher numbers of midge larvae which are normally associated with submerged vegetation.

During our experiment we witnessed a change in chironomid midge fauna, caused by a changed habitat. While submerged vegetation was present, midge larvae associated with it were in high numbers. After the submerged vegetation was destroyed, bottom dwellers increased in numbers. During the study in Spring Valley Lake, Johnson and Mulla (1983) collected greater numbers of chironomid adults from the portion of the lake kept free of aquatic macrophytes. According to them, reasons for this might include *M. spicatum* acting as a physical barrier or emitting biologically active chemicals inhibiting growth and survival of midge species. One of a possible approaches to this problem is to keep the submerged vegetation, which indirectly according to our research suppressed bottom dwellers, and focus on controlling midges associated with submerged vegetation.

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POPULATION DYNAMICS OF MUSCOID FLIES AT THREE COMMERCIAL POULTRY RANCHES AND ASSOCIATED FLY COMPLAINTS IN SAN BERNARDINO COUNTY DURING 1993

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ABSTRACT

The population dynamics of muscoid flies was sampled using sticky tapes at three commercial poultry ranches in San Bernardino County during 1993. Data generated at the Fontana, Redlands and Yucaipa ranches revealed *Fannia canicularis* as the most abundant species (47.3% to 84.2%), followed by *Musca domestica* (5.0% to 42.6%), *Fannia femoralis* (3.0% to 9.5%), and *Ophyra leucostoma* (0.4 to 4.3). Other species found in small numbers (≤1.0%) included *Muscina stabulans*, and *Phaenicia sericata*. The distribution of these flies on the north, east, south, and west side of the poultry houses varied both within and between ranches. In seasonal distribution, *F. canicularis* prevailed early in the season with population peaks noted in early spring through early summer while *Musca domestica* was prevalent in the summer and early fall. Residents complained more often about *F. canicularis* than *M. domestica* early in the season. Using weekly *F. canicularis* data in relation to residents complaints, the thresholds of fly tolerance by residents and enforcement action, and adulticidal applications differed from ranch to ranch during January through June, 1993.

Muscoid flies constitute an important group of cyclorrhaphan vectors that includes both biting and nonbiting species. Of the nonbiting species, the cosmopolitan house fly, *Musca domestica* L., is known to transmit several diseases such as diarrhea, dysentery, cholera, typhoid, poliomyelitis, yaws, anthrax, and tularemia and the little house fly, *Fannia canicularis* L., is a persistent nuisance pest in many parts of California (West 1950, Ebeling 1978). Among biting muscoid species, the tsetse fly, *Glossina* spp., is an established vector of both animal (nagana disease) and human (sleeping sickness) trypanosomiasis in many parts of Africa. The stable fly, *Stomoxys calcitrans* (L.), and hornfly, *Haematobia irritans* (L.), are known to suck blood from animals and sometimes humans (Harwood and James 1979, Kettle 1984).

The muscoid flies are also referred to as synanthropic flies or filth breeding flies. The phenomenon of synanthropy or zoophilly, an ecological association of flies with humans or animals, along with notes on a number of these flies has been reviewed by Mulla and Mian (1991). As filth breeders, a great majority of flies breed in a variety of animal wastes in dairies, horse stables, dog kennels, poultry ranches and in human excrement (Greenberg 1971). The proximity of breeding sites of these flies to human habitations undoubtedly creates certain health problems, ranging from nuisance to disease transmission.

High population growth and relatively low land prices in southern California during the past decade have led to significant shifts in planning and development policies in favor of urbanization encroachment upon agricultural zoned rural areas. Whereas this policy shift towards urbanization over ruralization might have helped the real estate industry, it certainly has exposed the intolerance of new residents in and adjacent to agricultural biotypes to muscoid flies, a product of many agricultural industry operations.

Owing to the low tolerance of residents to flies in certain municipalities, it became necessary to study and reevaluate adult fly population levels against complaints by residents. In monitoring adult fly populations, several sampling methods have been used by various researchers in their studies. These include fly grids (Scudder 1949), attractant baited jug-traps (Burg and Axtell 1984), sticky fly tapes (Anderson and Poorbaugh 1964, Legner et al. 1973, Meyer et al. 1987) and sticky cards (Hogsette et al. 1993). Using the sticky fly tape method, a study was initiated in 1992 to monitor the population dynamics of muscoid flies around three commercial poultry ranches (one each in Fontana, Redlands, and Yucaipa) and to ascertain and establish tolerance thresholds of residents to fly nuisance utilizing citizen complaint data in each city. The results of the 1992 studies were reported by Mian (1993) and the present paper elucidates the data generated in these studies during the 1993 fly season.

MATERIALS AND METHODS

Using the general methodology outlined by Mian (1993), a study was conducted for the second year to monitor the population dynamics of muscoid flies around commercial poultry ranches in both the east and west ends of the valley floor of San Bernardino County. One ranch each was studied in the cities of Fontana, Redlands, and Yucaipa. At each study site four 4x56 cm sticky fly tapes (Aeroxon Fly Catcher™, Roxide International Inc., New Rochelle, N.Y.) were hung at about eye-level (approx. 1.6 m) at the periphery of each ranch. The location of traps at each ranch was as follows:

Fontana Ranch.

Trap #1 was located inside the old office building at the north side of the ranch. Trap #2 was hung from a fruitless mulberry tree on the east side and trap #3 was placed inside the dead bird bin on the south side. Trap #4 was hung from a *Ligustrum* sp. plant on the west side of the ranch.

Redlands Ranch.

Traps #1, 2 and 3 were hung from the rafters on the east, north and west side of the ranch, respectively. Trap #4 was hung under the eave of a barn on the south side of the ranch. Unlike traps 1, 2 and 3, which were right next to the chicken houses, trap #4 was approximately 100 m away from the nearest chicken house. The ranch which is situated on the outskirts of the city of Redlands, is surrounded to the east and south by citrus trees, to the north by the Santa Ana River (wash) and to the west by a junk yard and an open field. The nearest residential housing track is about 150 m distant on the west side of the ranch.

Yucaipa Ranch.

Trap #1 was hung from a rafter on the west side of the ranch. Traps #2, 3 and 4 were hung on the north, east and south side of the ranch, respectively. This ranch is situated in the foothills and is surrounded by an open field to the north, east, and south. On the west side there are some residences almost across the street from the ranch.

Most of the traps were exchanged with new ones weekly during the 1993 study period with each trap brought in to the laboratory and carefully examined under a lighted binocular microscope to determine total fly numbers and identifications to genera and species. To correlate fly population levels with citizens complaints, in each study area, complaints were retrieved from our daily service request database.

Complaints were examined against weekly fly population levels over the period of January through June, 1993. The thresholds of fly tolerance by residents, enforcement action and adulticidal applications, especially for *F. canicularis*, were calculated accordingly. The threshold of fly tolerance was calculated as the number of flies/tape/week sufficient to result in the first three complaints of the season. The threshold of enforcement action including but not limited to ranch inspections was fly population level just enough to cause 3 to 5 complaints/week. Population levels of flies high enough to result in ≥5 complaints necessitated adulticidal sprays to quell fly nuisance.

All temperature and rainfall data used in these studies were obtained through the courtesy of the San Bernardino County Flood Control Department, Hydrology Section.

RESULTS AND DISCUSSION

Based on the total number of flies collected on sticky tapes, the fly composition by species varied from ranch to ranch (Table 1). The most predominant species at all three ranches was *F. canicularis*, ranging from 47.3 to 84.2% of the total flies collected. It's close relative, *F. femoralis*, accounted for 3.0 to 9.5% of the total fly collection. Except at the Redlands ranch, *M. domestica* was the second most prevalent species, accounting for 41.8% and 42.6% of the total number of flies collected at the Fontana and Yucaipa ranch, respectively. *Ophyra leucostoma* (Wiedemen) represented 2.0% of the flies at the Fontana ranch, 4.3% at the Redlands ranch, and 0.4% at the Yucaipa ranch. Other species such as *Muscina*

Table 1. Percent composition of fly fauna (%) and actual numbers of flies collected (N) on sticky tapes at three commercial poultry ranches in San Bernardino County during 1993.

	Fontana		Redlands		Yucaipa	
Species	%	N	%	N	%	N
Fannia canicularis	53.0	17,713	84.2	336	47.3	7,589
Fannis femoralis	3.0	994	5.8	23	9.5	1,525
Musca domestica	41.8	13,977	5.0	20	42.6	6,843
Muscina stabulans	<0.1	10	0.7	3	<0.1	2
Ophyra leucostoma	2.0	674	4.3	17	0.4	70
Phaenicia sericata	0.1	50	0.0	0	0.1	12
Totals	100	33,420	100	399	100	16,041

stabulans (Fallen) and *Phaenicia sericata* (Meigan) each accounted for <1.0% of the total fly collections.

Apart from flies, other arthropods caught on sticky tapes at the Fontana ranch included Diptera - 1,267 Sphaeroceridae, Hymenoptera - 6 Pteromalidae/ Braconidae, and Lepidoptera - 21 Tineidae. In the Redlands tapes, there were 1,457 sphaerocerids, 5 tineid moths, Coleoptera - 189 Anobiidae, 1 pteromalid, and 3 termites (winged). The Yucaipa tapes had 2,732 sphaerocerids, 8 pteromalids, 21 tineid moths, 6 anobiid beetles and Dermoptera - 2 Furficulidae (earwigs).

Local spatial distribution of flies varied at each of the three ranches (Table 2). At the Fontana ranch, the trap operated at the north side of the poultry house collected the highest number of flies followed by traps located to the south, west, and east of the ranch in that order. Similarly at the Redlands ranch, traps operated on the north side had the highest number of flies followed by traps located on the east, west, and south. At the Yucaipa ranch, however, the trap on the west side had the highest number of flies (7,773; 48.5%) followed by traps operated on the north, east, and south side. This pattern of fly distribution, not much different from previous data (Mian 1993), was expected because each ranch was different by location and surroundings. The pattern also clearly shows that at least one trap on each of the four sides, (north, east, south, and west) be operated in order to have representative samples in fly distribution surveys.

Fannia canicularis and *M. domestica* were the dominant species collected on sticky tapes during the 12 month study. At the Fontana ranch, *F. canicularis* appeared early in the season in February with population peak starting in March and continuing to grow through July (Table 3). This was in part due to prevailing daytime temperatures of 73-86°F and over 23 inches of rainfall during January through June. Also, residents' complaints were higher (5-106) during February through June, necessitating increased ranch inspections and adulticidal sprays in fly affected residential neighborhoods.

Unlike *F. canicularis*, *M. domestica* was more predominant in the summer and early fall with peak populations found in September. At the Redlands ranch, *F. canicularis* also showed a population peak in May, resulting in the highest number of residents' complaints (13) (Table 3). Unlike the Fontana and Redlands ranches, the Yucaipa ranch showed fly population peaks of *F. canicularis* in March, May and July, drawing 6 to 23 complaints during the peak periods (Table 3). Heavy rainfall and optimum temperatures were partly responsible for this heavy population build up of *F. canicularis* that resulted in increased ranch inspections and enforcement activity coupled with adulticidal sprays in affected neighborhoods.

As reported in the earlier study (Mian 1993), *F. canicularis* was the one species which aroused most residents' complaints early in the season. On the other hand, *M. domestica*, although showing a higher population peak than that of *F. canicularis* in the later part of the season, did not result in higher numbers of complaints. This might be due to the fact that residents became more tolerant to flies later in the season. Moreover, the flying movement of *M. domestica* as different from the zigzag dancing and hovering of *F. canicularis* may be another reason that residents became less tolerant to the latter species.

Weekly fly population data in relation to residents' complaints during January through June, 1993 provided different thresholds of fly tolerance, enforcement action and adulticidal sprays at each poultry ranch. At the Fontana ranch, the threshold of tolerance for *F. canicularis* at which enforcement action began on a 3-complaint basis was 12 flies/tape/week (Fig.1). On a weekly 5-complaint basis or higher, the threshold for carrying out community wide adulticidal sprays was 20 flies/tape/week. The threshold figures at the Redlands poultry ranch were higher (Fig. 2) with the threshold of early season tolerance for *F. canicularis* and enforcement

Table 2. Total number of muscoid flies by trap site (N) and percentage each site is of the total (%) at three commercial poultry ranches in San Bernardino County during 1993.

Poultry Ranch	North		East		South		West		X^2 Value
	N	%	N	%	N	%	N	%	
Fontana	13,124	39.3	5,719	17.1	8,332	24.9	6,245	18.7	13.26 *
Redlands	160	40.1	114	28.6	54	13.5	71	17.8	14.81 *
Yucaipa	5,253	32.7	2,069	12.9	946	5.9	7,773	48.5	19.21 *

* Significant at P = 0.05.

Table 3. Monthly fly populations, residents' complaints, and meteorological data at each of three commercial poultry ranches in San Bernardino County during 1993. Fly population numbers indicate mean number of flies from four sticky tapes.

Month	*Fannia canicularis*	*Musca domestica*	Other * species	Total flies	Residents' Complaints	Temperature (°F) Max.	Temperature (°F) Min.	Rainfall (in.)
FONTANA								
Jan	53	3	127	183	3	60	45	13.06
Feb	324	2	20	18	5	62	45	8.00
Mar	2,156	20	23	2,202	61	73	50	1.34
Apr	2,399	158	0	2,558	106	76	52	0.00
May	2,933	620	315	3,868	13	79	56	0.00
Jun	3,211	920	293	4,424	15	86	60	0.85
Jul	3,398	973	609	4,980	1	86	60	0.00
Aug	1,303	3,621	209	5,133	1	91	65	0.00
Sep	1,136	4,284	96	5,516	3	94	65	0.00
Oct	800	3,376	36	4,212	1	79	59	0.13
Nov	-	-	-	-	1	71	53	0.47
Dec	-	-	-	-	0	69	48	0.64
REDLANDS								
Jan	-	-	-	-	0	59	46	11.61
Feb	0	4	0	4	0	60	46	6.65
Mar	3	38	0	41	0	70	53	1.68
Apr	3	36	0	39	1	76	52	0.00
May	8	118	31	157	13	83	59	0.00
Jun	6	106	2	114	4	89	63	1.20
Jul	0	34	10	44	1	86	62	0.00
Aug	-	-	-	-	1	92	65	0.00
Sep	-	-	-	-	0	87	66	0.00
Oct	-	-	-	-	0	76	57	0.18
Nov	-	-	-	-	0	67	50	1.05
Dec	-	-	-	-	0	60	44	0.81
YUCAIPA								
Jan	4	158	10	172	0	59	46	14.71
Feb	72	36	18	126	0	60	46	9.72
Mar	227	27	42	296	6	70	53	2.41
Apr	172	36	8	216	23	76	52	0.08
May	270	28	4	302	21	83	59	0.18
Jun	1,259	246	305	1,810	15	89	63	1.48
Jul	3,928	808	1,220	5,956	2	86	62	0.00
Aug	450	1,448	2	1,900	1	92	65	0.17
Sep	471	1,333	0	1,804	1	87	66	0.00
Oct	547	1,767	0	2,314	1	76	57	0.17
Nov	189	956	0	1,145	1	67	50	1.13
Dec	-	-	-	-	3	60	44	1.03

* Other species includes *Fannia femoralis*, *Muscina stabulans*, *Ophyra leucostoma* and *Phaenicia sericata*.

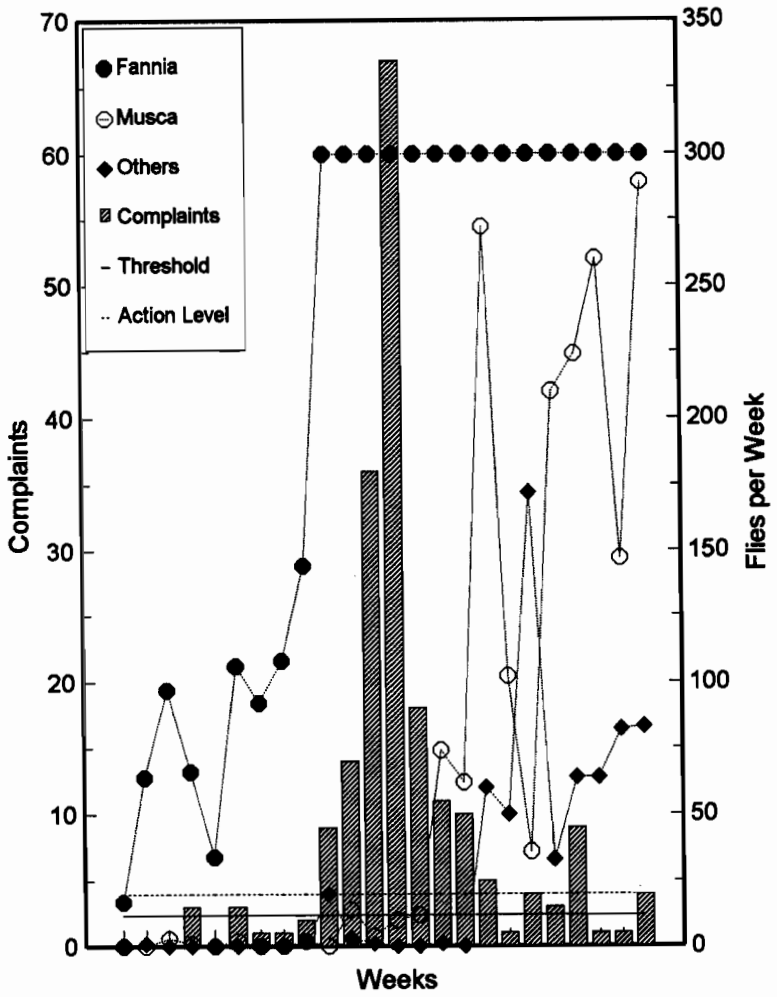

Figure 1. Weekly fly populations at the Fontana poultry ranch site and residents' complaints from the City of Fontana during January through June, 1993.

action estimated at a 3-complaint basis was 23 flies/tape/week. Similarly, the threshold for adulticidal application was 38 flies/week. In the Yucaipa, the two thresholds (fly tolerance and enforcement action for adulticidal activity) were the highest at 42 and 70 flies/tape/week, respectively (Fig. 3).

The data presented and discussed in the foregoing paragraphs are part of a three year study. At the end of the third year, hopefully, these data will provide more meaningful information that will be utilized in developing fly surveillance and control models as part of our routine vector control operational protocol.

Finally, as reported previously (Mian 1993), it is important to point out that the complaints used in establishing various thresholds were assumed to be due to fly breeding at the poultry ranches. In establishing these thresholds, however, other possible fly sources such as domestic garbage, rotten fruit/vegetable matter, compost, pet droppings, or animal manure have to be ruled out before relating a complaint to fly breeding at a commercial poultry ranch. Other factors (e.g., the distance between a complaint source and that of fly breeding, prevailing temperature, humidity, precipitation, sky cover, wind speed and direction) need to be taken into consideration prior to establishing a relationship between fly breeding at poultry ranches and resident complaints. None-the-less, the aim of this study has been to gather factual information on the subject matter and not to incriminate commercial poultry establishments as fly production sources.

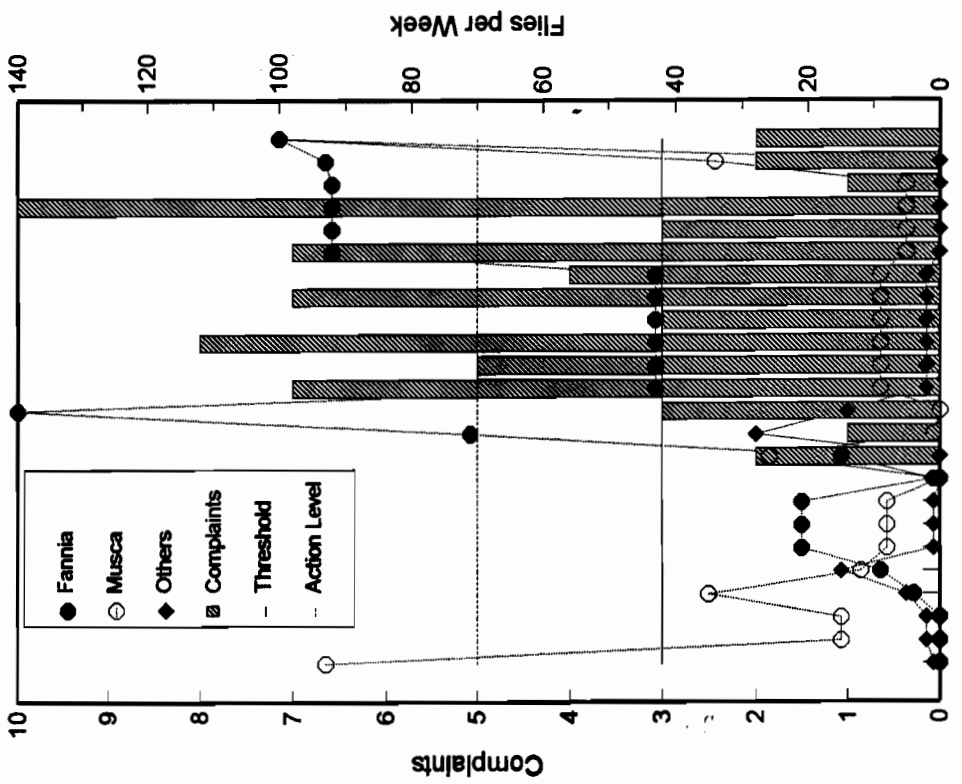

Figure 3. Weekly fly populations at the Yucaipa poultry ranch site and residents' complaints from the City of Yucaipa during January through June, 1993.

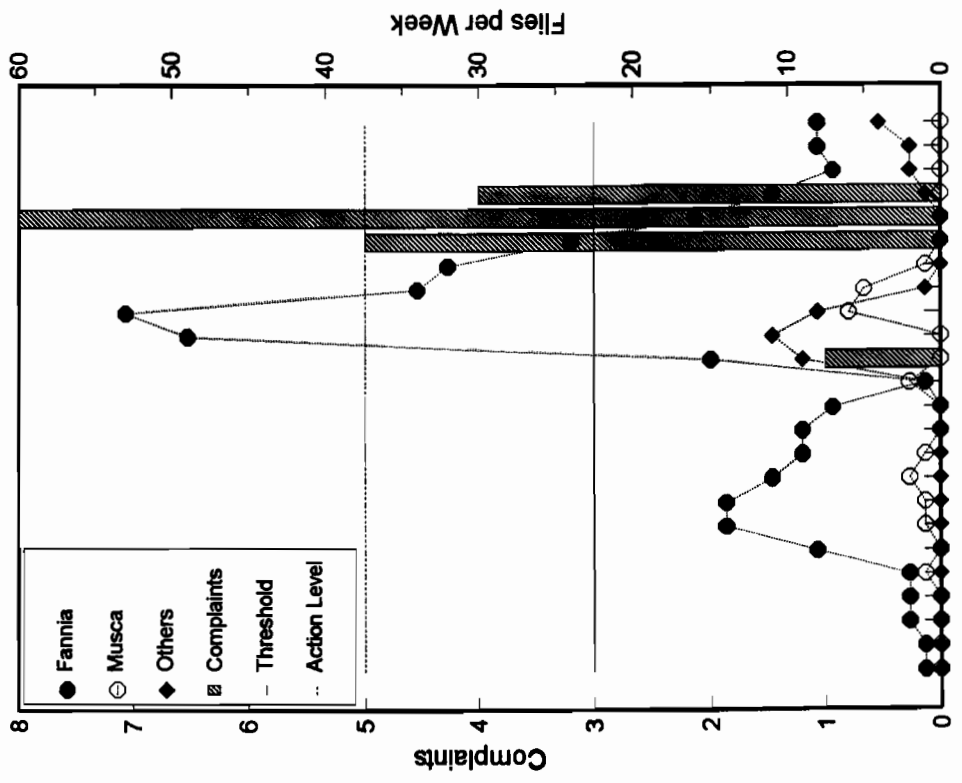

Figure 2. Weekly fly populations at the Redlands poultry ranch site and residents' complaints from the City of Redlands during January through June, 1993.

ACKNOWLEDGEMENTS

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DELUSIONS OF PARASITOSIS: PROGRESS REPORT FROM SANTA CLARA COUNTY VECTOR CONTROL DISTRICT

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Santa Clara County Vector Control District (SCCVCD) became interested in 1993 into going beyond "insect identification" for those individuals who had the obsession they were being invaded by living organisms even though the samples revealed no specimens, just lint, scabs, hair, and other items.

Inspiration and knowledge on these delusions of parasitosis (DOP) started primarily from listening to presentations given by Dr. J.P. Webb. The 1991 Society of Vector Ecologists (SOVE) symposium on DOP (Webb 1993, Poorbaugh 1993, Koblenzer 1993, Kushon et al. 1993) and subsequent conversations with entomologists throughout California and physicians, psychologists, nurse practitioners in Santa Clara County indicated SCCVCD could perform a much needed link between DOP cases and the medical community. Numerous articles have been collected on DOP including Weinstein (1994).

The approach by SCCVCD was to direct all calls to the author if individuals called to obtain identification of small organisms invading their residence in high numbers and biting them. After listening for the first half hour and writing notes, each of the 14 cases was questioned about contact with a physician. The case usually responded that the physician either gave them a prescription for scabies or lice which didn't last long or did not believe them.

Even though the case insisted that a survey of their residence was needed, the individual was told that a powerful microscope in the district lab was needed to look at the samples so they should bring in a few samples on scotch tape. This usually prevented samples of body fluids and numerous articles of clothing from being brought into the lab.

The case was strongly urged during the second phone call or on the first visit to the district lab to provide the name and phone number of the physician. Initial reluctance was common because the case did not trust the author or did not believe the physician would listen to the biologist.

Telephone calls to physicians and follow-up with faxing publications on DOP to them have yielded some positive results. The telephone conversations included how and why the biologist was involved, the expertise of the biologist with DOP, a summary of the conversation between the case and the biologist, and ended with the biologist's offer to fax or mail DOP publications.

Progress has been made in the past 15 months as indicated by the following:

- Educated some primary care physicians and dermatologists about DOP and the role of an entomologist.
- Learned that a tragic event, loss of a loved one to divorce, leaving home, or death had occurred just prior to onset of DOP in each of the five cases where the patient confided in the author.
- Gained satisfaction in this approach due to feedback from appreciative physicians and nurses and call backs from cases under treatment by a physician.
- Learned about statewide interest and expertise with DOP among entomologists and registered environmental health specialists.
- Demonstrated to the cases that someone in government will take the extra time needed to help them. It was the intent to have each case feel more hopeful and ready to see a physician upon departure.
- Learned the limitations and frustrations in dealing with DOP cases.
- Realized that DOP individuals live almost in terror and constantly contact government agencies

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and others trying to solve this problem which may have started a year or more earlier.

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ADVANTAGES OF A STATEWIDE APPROACH TO PUBLIC EDUCATION

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In January, 1993, the California Mosquito and Vector Control Association (CMVCA) created an ad hoc Public Information Committee to identify and develop generic brochures and adopt statewide approaches to the public education needs of individual districts.

The "public" was defined to include residents, elected officials (city councils, Board of Supervisors, State Assembly, and Senate), government agencies (parks, water districts, health departments, and ag commissioners), civic groups (service clubs, garden clubs, and hiking clubs), businesses (utilities, hospitals, and landscapers), and schools (PTA, science teachers, and all grades of students). These target groups were adapted from an earlier list by Costa and Husted (1991).

"Public education" included not only brochures, but encouraging media contact (interviews, newstips, and media days), presentations, and fair exhibits.

Formation of the committee has allowed the following advantages to be realized:

- Saved many districts time and money by reducing brochure printing and storage costs.
- Allowed small districts to have access to the same educational materials as large districts.
- Improved quality of brochures by using collective expertise and interest of CMVCA members.
- Strengthened the image of all districts by providing statewide unity in vector control information.
- Improved statewide political position through public awareness of services.

Since the formation of the committee, the brochure "Lyme Disease in California" has had wide distribution, the "Preparing for the Africanized Honey Bee" and "Bee Smart" children's brochures were prepared by Sacramento-Yolo MVCD with input from the committee, the Africanized Honey Bee color information card was published, and work on a pet brochure and a translation of the "Preparing ..." brochure into Spanish have been started. In addition, two mosquito brochures have been adopted by CMVCA from member districts for statewide distribution: "Are You Raising Mosquitoes in Your Backyard" and "The Western Treehole Mosquito".

In addition, the committee's coordination of the 1994 CMVCA Media Event and work with a public relations consultant further enhanced public awareness (Costa et al. 1994) of mosquito control in California.

Public education has become an integral part in more district programs since early in 1993. More districts have seen the positive side of a proactive attitude when it comes to the media and the public. Only through coordinated statewide efforts, can we expect significant achievement and growth in making the public more aware of the value of our districts and more aware of what they need to do to minimize mosquito/vector problems in their community.

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COMMUNITY RESPONSE TO MOSQUITO CONTROL EDUCATION IN ELEMENTARY SCHOOLS

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The East Volusia Mosquito Control District started a general mosquito education program for elementary school children in 1978, but in 1991 with the spread of *Aedes albopictus* (Skuse) container breeding was emphasized. Currently, in 20% of the 4,000 plus annual telephone service requests, *Aedes albopictus* is the primary mosquito problem. Since education is the best way to reduce breeding areas for this species, our school presentation programs emphasis container breeding.

Annually, approximately 5,000 students, 2nd to 4th grade, are given short outdoor static display instruction or classroom instruction in mosquito container breeding. Classroom mosquito education programs are the best method of teaching container breeding, because approximately one hour can be spent with each class on this subject. Photograph slides showing various mosquito breeding containers around the yard, live mosquitoes and several magic tricks (especially LOT A BOWL trick- a container with hidden water compartment) are used in each presentation. An eight page workbook entitled "Mosquito Control Begins With You" is given to each student. One page of this workbook depicts 21 possible mosquito breeding places and asks the student to find them in the picture and their own yard.

We determined to measure the degree of educational success by the amount of implementation in the student's household. Student's households were examined before and after our classroom instruction in several schools, or a take home survey was distributed.

Homes of 4th graders in six different classrooms were visited when the students weren't home to count dry, wet, and breeding containers in each yard. Care was taken not to discuss the upcoming classroom instruction and containers were left in their original condition at the close of the inspection. Three indices were used - Breteau index: (houses/number of breeding sites) X 100, wet container index: (houses/number of wet containers) X 100 and total containers index: (houses/number of wet containers) X 100. A post-examination, approximately two weeks after classroom instruction, showed less containers breeding, with water or upright, capable of collecting water. Even though we averaged over 50% reduction in breeding containers during this study, we can not determine what effect our unintrusive pre-examination of the yards had on the container reduction system. Since over 80 manhours were spent on this study and no adequate control group could be found, we did not continue with this type of measurement system.

Four, 4th grade classrooms were given the typical mosquito container breeding information and a survey was distributed to take home. The survey "Have You Checked Around Your Home For Mosquito Breeding Sites?" was previously made in the 1960s, during the *Aedes aegypti* (Linnaeus) eradication program. Eighty-seven percent of the students completed and returned this survey. Students marked that they had emptied, put under cover, or otherwise managed containers which could produce mosquitoes. The high number of returned surveys, along with an average of fifty percent stating they had found and/or checked for specific mosquito breeding sites around their yards, shows that the education is being used.

The previously mentioned *Aedes aegypti* survey uses simple pictures to depict common containers that produce mosquitoes and asks students to empty or manage them. However, it does not ask students to observe which containers have immature mosquitoes at the time of the survey. We have made a similar survey which asks students to observe and record which containers are breeding mosquitoes. We hope to distribute this latter survey this year, and hopefully it will not only tell us how well our education is being implemented, but will show us roughly what neighborhoods have the greatest mosquito container breeding problems. Obviously, we are trusting students, or their parents, to accurately fill out the survey. We are optimistic the surveys will be returned promptly, based upon the participation of the *Ae. aegypti* survey.

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HOW MEDIA EVENTS CAN IMPROVE MOSQUITO CONTROL

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A media event can really help improve control of mosquitoes and other vectors in your district. A media event lets the public now what they can do to minimize vectors in their neighborhood. This will reduce the number of vectors in the area, thus reducing the number of complaints or requests for service by the public. More importantly, a media event will help make the public aware of your district and it's services.

Unfortunately, many districts perceive the media as a problem. They see the media people who are always looking to sensationalize and only want to talk to the district if there is something wrong. They also worry that the media will change the facts to make a story newsworthy and interesting.

So, why should you invite the media to your district if you are concerned about possible negative coverage? For three very good reasons. First, by initiating and arranging the media event, you control the message. If well planned and thought out, you send the message you want while developing a good working relationship with the public and the media.

Second, a media event will help educate the public. Not only will the public know what their role is in vector prevention and control, but also how things like budget cuts and pesticide restrictions and bans can and will affect the quality and amount of service they may receive. It also important to remember that public knowledge can help reduce fear and misperceptions. With many districts planning to be involved with the Africanized Honey Bee, a good media event on the subject would help minimize the panic the residents might feel by educating them with the facts.

Thirdly, a successful media event will give your district positive public exposure. Not only will the public understand where their tax dollars are going, but also how the district is protecting their health and comfort by reducing mosquito populations.

The public is more likely to support something they understand. Last year, some districts were looking at possible budget cuts, some up to 40% of their tax revenues. At the California Mosquito and Vector Control Association (CMVCA) quarterly meeting in December, 1993, Assemblyman Paul Woodruff was asked what he thought districts should do about the possible cuts. Assemblyman Woodruff emphasized that districts needed to be proactive when dealing with the public and media. He said districts needed to educate the public through media in a stronger and louder manner because the reality of what we do is lost on the public unless we educate them through the media.

Last year Santa Clara County Vector Control District was involved in two media events. The first event focused on the mosquito and vector control districts of the Bay area. The objective of the event was to make the public aware of the districts and what services they offered. Bay Area residents and schools were invited to come and visit the various displays explaining the services and the importance of vector control. Residents learned what they could do to minimize contact with mosquitoes and other vectors. The response to the media event was very positive. In addition to several schools and residents, five television news teams and two newspapers attended the event. A Bay Area radio news station also reported on the event, which included a telephone interview.

The second media event was a joint effort of the mosquito and vector control districts throughout California and was coordinated by the CMVCA. The

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objective of the media event was public awareness - public awareness of what the districts did in disease prevention and control.

This second media event produced fifteen news stories that day and four follow-up stories later in the week. In addition, there were several radio interviews. But the most important benefit of this media event came a few weeks later when encephalitis virus was found in mosquito pools and sentinel chickens were "positive" in blood samples in Sacramento County. Establishing a good working relationship with the media enabled the district to alert the public to the potential threat of encephalitis without having it sensationalized and causing panic. The public was also able to protect itself by understanding how to minimize contact with mosquitoes.

There are five ingredients in a successful media event:

1. Know your audience and use terms they will understand. Avoid using technical jargon.
2. Make your media event interesting. Focus on the public concerns. Tell them how your district's actions and services affect them and their concerns.
3. Get the word out. Send out newstips and do not forget the non-English speaking stations. These stations are usually very eager for interesting stories. Invite the public, if possible, by putting out public service announcements and listing the media event in your local newspaper's event calendar.
4. Be ready to tell the truth! No matter how hard it might be. Remember, you're trying to build a foundation for a good working relationship.
5. Plan, Plan, Plan. When it comes to a media event, overkill is permitted.

Fortunately, more districts are beginning to realize the importance of a working relationship with the media and are starting to encourage media contact.

The reality is, if you do your homework and coordinate a successful media event, not only will you improve vector control in your district, but you'll see the media as an ally who can help you educate the public and deliver the message you want delivered in an interesting way that leaves the public with a positive attitude towards your district.

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REINVENTING GOVERNMENT: TRANSFORMING THE ALAMEDA COUNTY MOSQUITO ABATEMENT DISTRICT INTO A LEARNING ORGANIZATION

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THE PROBLEM

In the Spring of 1991, the efforts of employees of the Alameda County Mosquito Abatement District seemed to be stalemated. Many unresolved problems had accumulated and had worn employees down. The already complex problem of attempting to deal with 19 species of mosquitoes in 813 square miles of Alameda County had been compounded by a myriad of challenges.

Perhaps the biggest challenge faced by the District was posed by the forces of wetlands creation. In the last two decades, the trend of decreasing mosquito sources in the District had been reversed. Wetlands were being created and enhanced all along the bay front, often in close proximity to residential homes. Although the District involved itself in the planning and management of the wetlands, regulatory agencies, operating from a incoherent tangle of one-dimensional laws, were inexorably eliminating tools of mosquito control and making mosquito control increasingly complex (Roberts 1993).

At the same time the challenges to the District's effectiveness were growing, political and economic forces were operating to reduce the ability of the District to respond effectively. Budget constraints following Proposition 13, exacerbated by the current recession, prevented staffing the District at pre-Proposition 13 levels. Meanwhile, political forces created a disturbing climate of uncertainty. State legislators appeared to have little appreciation for government by special districts and, in the name of regionalization and efficient government, were considering legislation that further threatened the District.

The employees of the district, in the face of these challenges, were being required to acquire ever-increasing levels of technical knowledge with regard to mosquito control technology and the environment. A new "biorational" program was developed to comply with regulations which excluded traditional chemicals. Yet, initial efforts at implementing biorational control met with only partial success. Total employee commitment was needed, perhaps a paradigm shift to a different environmental ethic. At this very critical time, when a new way of thinking about mosquito control was required, employees seemed incapable of such a transformation. Old and new employees alike were stuck in old ways of thinking when new ways were needed.

Employee morale and the ultimate fate of the district seem to be locked in a precipitous decline. Feelings of hopelessness and helplessness were creating a deep malaise in the organization. Employee disillusionments was evidenced by burn-out, cynicism, and withdrawal.

MANAGEMENT COMPLICITY

The District in 1991 was organized in a typical hierarchical structure (Fig. 1). The District was divided into zones which were assigned to technicians. The organization of the district tended to foster independence

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and perhaps even competition between employees. It became more and more apparent, however, that efforts to solve the emerging tidal wave of problems would require employees working together more efficiently. Initial efforts at fostering teamwork, however, using project management techniques, were again only partially successful.

Management, seeing the critical need to change, increased top-down pressure to make changes that seemed appropriate from the management perspective. The efforts produced employee resistance rather than change- A top-down management style was exacerbating the problem rather than solving it. It became apparent to management, after an introduction to the book *The Fifth Discipline* by Peter Senge, that the District was suffering from common maladies of today's organizations - diseases of the hierarchy (Senge 1990). The District hierarchical organization and top-down management style created an organizational defensive pattern (ODP) in the District that distorted information flow (Argyris 1990). The result was that the District remained inflexible and rigid in a rapidly changing world. In the words of Peter Senge, the District was suffering from a "learning dysfunction."

Perhaps most difficult to accept was that the District's problems could be traced to very specific management behavior termed Model I behavior (Table 1). Model I behavior was the engine of ODP (Fig. 2), reinforcing defensive routines and anti-leadership behaviors. It was only when management recognized its own complicity in the problem that the issues began to be addressed effectively. In the Spring of 1991, management asked the employees to read the first chapter of *The Fifth Discipline* to decide whether they wished to use the book as a blueprint for re-organization. On May 5,1991

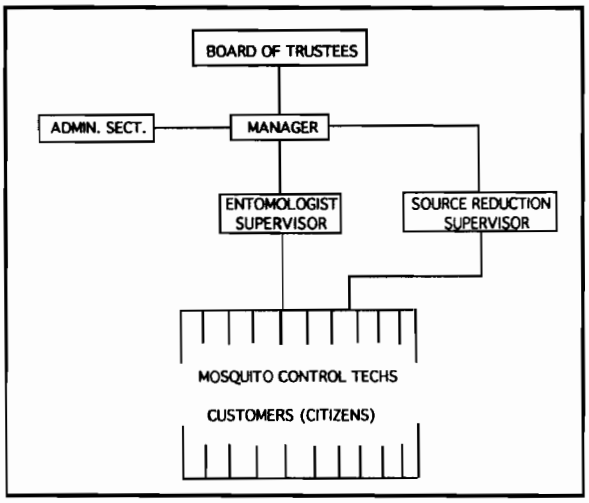

Figure 1. Organizational structure of the Alameda County Mosquito Abatement District prior to re-organization in 1993.

the employees of the Alameda County Mosquito Abatement District committed to re-organize into a "learning organization" as described in the book. A plan was developed to accomplish transformation from within.

A LEARNING ORGANIZATION

A learning organization is a newly emerging type of organization designed to combine adaptive learning, aimed at survival, and generative learning to enhance the capacity to generate innovative solutions.

Employees of a learning organization employ five essential disciplines:

1) Personal Mastery - where employees are deeply committed to learning and actualizing,

Table 1. Comparison of consequences in an organization of action decisions made in Model I and Model II styles.

	MODEL I	MODEL II
GENERATE IDEAS	Own and control ideas (top-down flow).	Collaborate using dialogue, advocacy and inquiry.
SELECTION METHOD	Unilateral decisions (e.g., "Tell me what I want to hear").	Consensus or participation.
ACTIONS	Seek to be in unilateral control, to win, and not to upset people.	Seek valid information, make informed decisions, monitor results, and minimize face saving.
CONSEQUENCES	Variety constrained, options limited. Compliance leads to resentment. Resistance leads to reduced efficiency. Limited learning.	Variety enhanced, options increased. Empowered individuals leads to enrollment leads to greater efficiency. Double-loop learning.

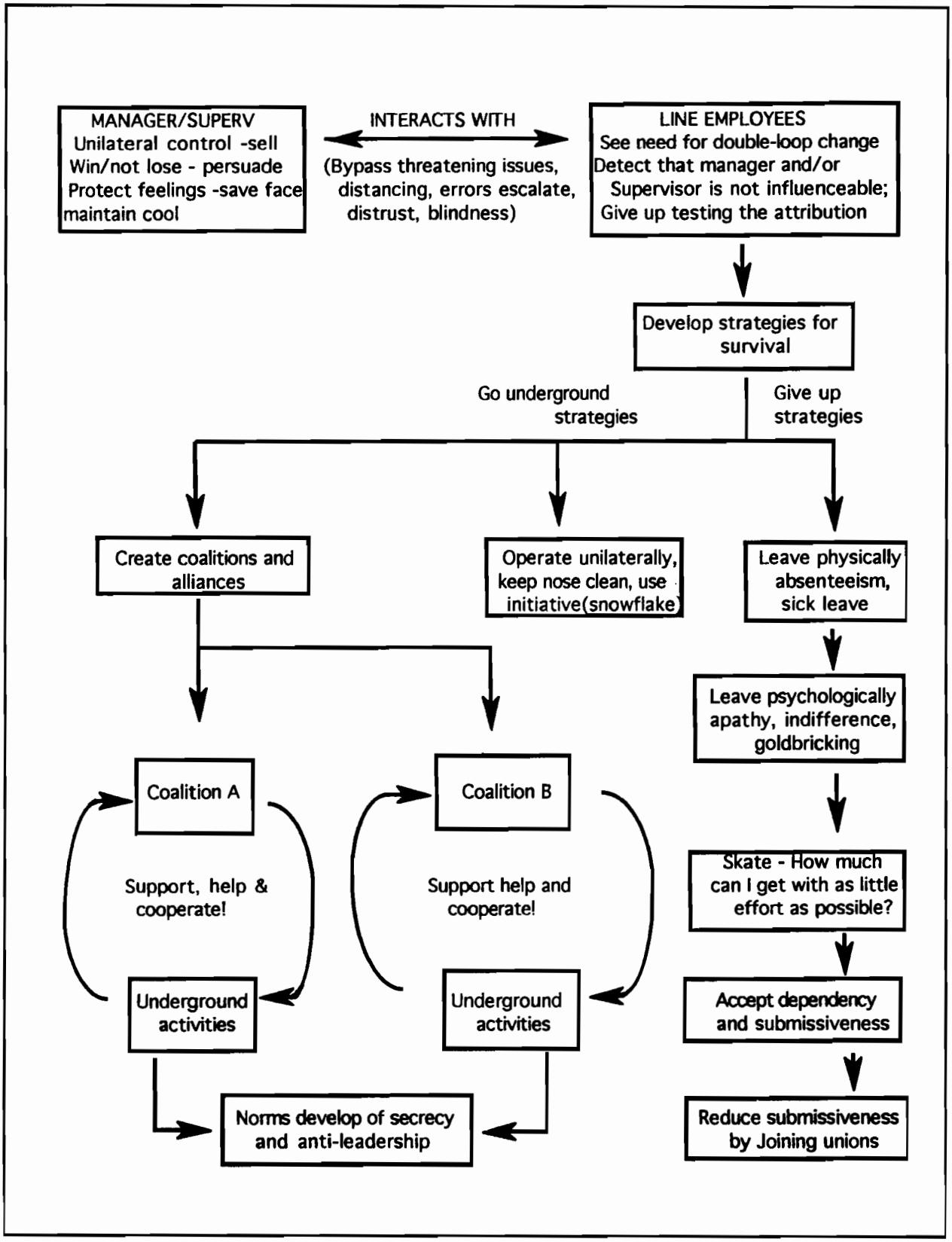

Figure 2. Organizational Defensive Pattern (ODP) as inferred from Argyris (1990).

2) Creating a Shared Vision - to create alignment and orientation for the employees,
3) Team Learning - to create knowledge and learn together,
4) Interpersonal Communication Skills - enabling employees to surface, test and improve their assumptions about problems,
5) Systems Thinking - to recognize how everything is connected to everything else.

The District implemented the re-organization in three phases: First, an "internally initiated intervention phase" when all employees took the responsibility to teach and learn the new skills. A consultant, Dr. Miro Valach, was brought in to teach systems thinking to the employees.

Second, an "external intervention phase" was initiated to expedite the process. Dr. William Reckmeyer was brought in to facilitate program planning sessions aimed at restructuring the District's biorational control of winter marsh mosquitoes and treehole mosquitoes. The sessions fostered a broader environmental perspective by way of stakeholder analysis; empowered employees to make the necessary structural changes in the District; and emphasized and institutionalized team approaches (Fig. 3). Perhaps most importantly, collaborative leadership was modelled (facilitation techniques). Dr. Reckmeyer's efforts resulted in the development of a successful biorational program for wetlands mosquito control.

And lastly, Mr. Jeffrey Dooley worked with the employees to develop interpersonal communication techniques emphasizing collaborative techniques described as Model II behavior (Table 1). He met with employees in monthly workshops designed to teach employees to surface, test, and improve their views of District issues. Problems that were only discussed at the water cooler began to be addressed in constructive ways.

RESULTS

In the District's monthly report of June 9, 1993, The District manager reported that The Alameda County Mosquito Abatement District had transformed itself to a learning organization and described the process of the transformation. Although, by definition, the transformation would never be complete, in his view, the District operated more like a learning organization than like a traditional organization. He reinforced his conclusions by citing data that revealed a highly successful biorational control effort that had just been completed.

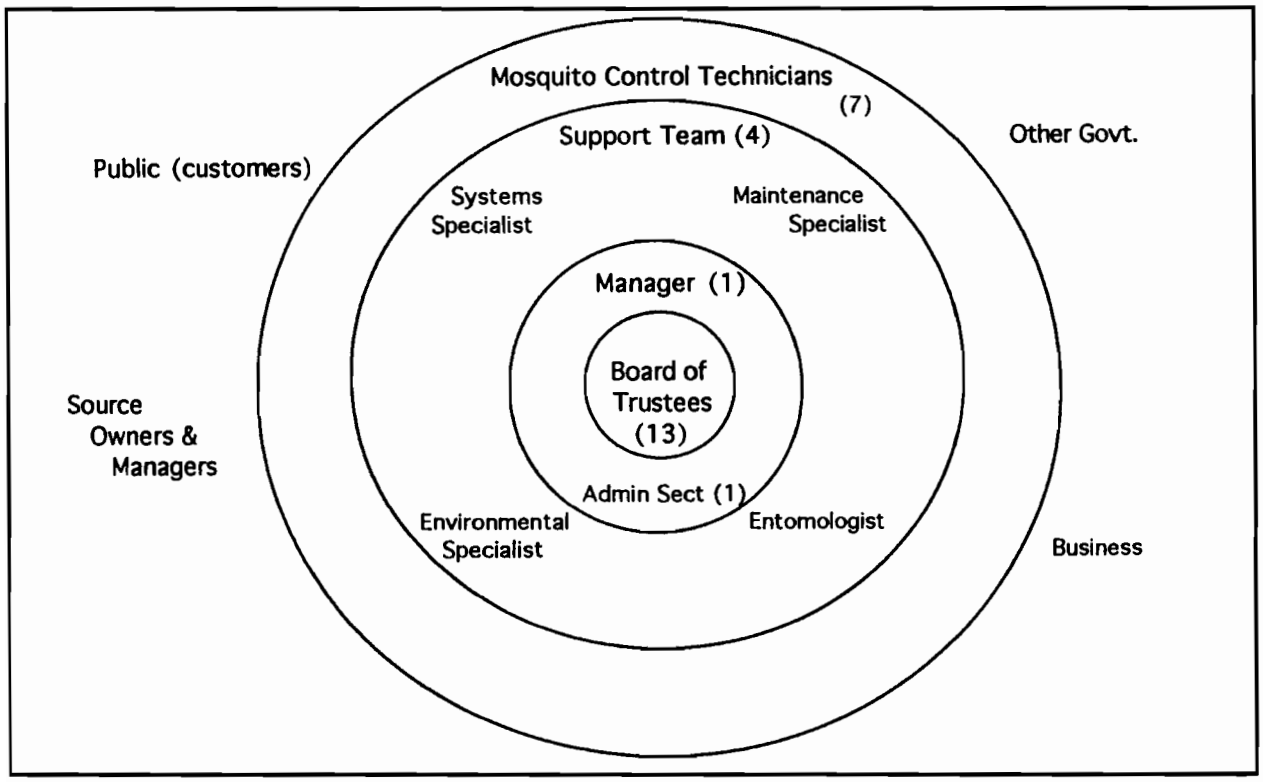

Figure 3. Organizational structure of the Alameda County Mosquito Abatement District after re-organization in 1993.

Today, in the Alameda County Mosquito Abatement District, major decision-making responsibility resides with the employees, including hiring, evaluating, and firing personnel, as well as equipping the District, and maintaining quality. Employees expect individual commitment to learning and to an employee-produced shared vision. Hard-won skills of interpersonal communication operate in weekly facilitated sessions to insure that tough issues are discussed openly. Management, in the meantime, has directed its efforts to negotiating the resource bargain between employees and the Board of Trustees, and auditing the results of District operations. Beyond audit and resource bargain, the manager's role is that of supporting the learning organization.

A learning organization is a new species of organization that has evolved in our chaotic world. It is designed to maximize flexibility to adapt to a rapidly changing environment. We do not know all of the problems or solutions that will be encountered on the way to more effective mosquito control in Alameda County. It is apparent, however, that the viability and longevity of the learning organization depends upon the mutual support of Trustees, management, and employees.

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MOSQUITO AND VECTOR CONTROL IN CALIFORNIA - A MODEL OF SELF ORGANIZING GOVERNMENT[1]

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Dear Chairwoman Brown, Host-Assemblyman Rainey, and Committee Members;

I am Fred Roberts, regional representative of eight special districts providing mosquito and vector control to the citizens of the San Francisco Bay region. I greatly appreciate this opportunity to be of service by presenting testimony to your committee.

I want to reveal to you today a form of government that is uniquely structured to meet the challenges of the 21st Century. It has emerged from our world of bewildering complexity, accelerating change, and seemingly intractable problems. It is flexible, dynamic, focused intensely at the local level, yet highly coordinated at a state-wide level. I believe that if you thoroughly examine this newly emerged form of government you will want to sustain it and help it continue to adapt to meet the needs of the citizens of California in the 21st Century.

This almost indiscernible organization is a highly coordinated, richly connected, state-wide network of agencies comprised of 50 local mosquito and vector control districts, the California Mosquito and Vector Control Association (CMVCA), the State Department of Health Services (DHS), and the University of California (UC). The organizational structure is an effective adaptation to increasing environmental and fiscal problems (Figs. 1&2). It has enabled the mosquito and vector control community to provide effective public health service.

This decentralized governmental structure meets state, regional, and local needs because:

1) The districts are small, manageable, operationally-oriented units (Fig. 2).
2) The local agencies are self-organized to coordinate regionally (Addendum 1).
3) The local agencies are coordinated state-wide by the CMVCA and DHS (Addendum 2).

Your committee asks that the "tangled web of government" be simplified to be more efficient and effective. I concur with your conclusions that new structures of government are required. Traditionally structured government is often too slow and too inflexible to solve many of today's problems (Fig. 3). We should recognize the difference, however, between government that is drowning in its own complexity and government that has self-organized to a necessary level of complexity to solve today's complex problems.

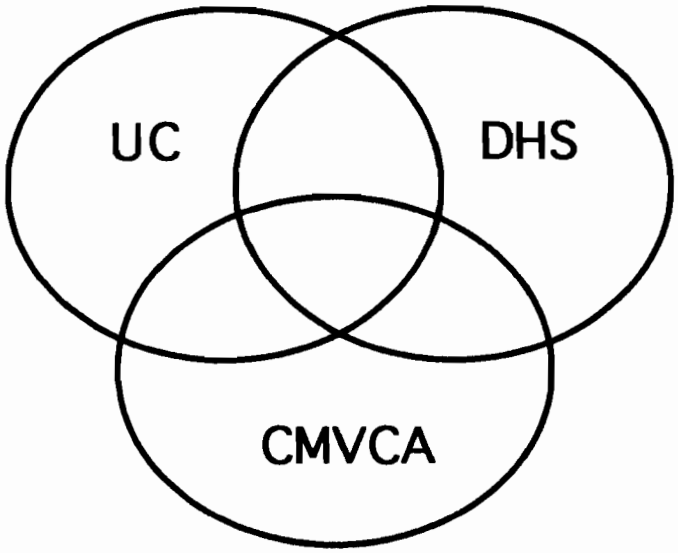
I appreciate the opportunity to offer the following recommendations to your committee in the three subject areas you have specified:

1) **Innovations.** Today, I have presented to you a new, innovative form of government that has emerged from the chaos (Fig. 2). It is a creative, self-organizing government that continues to restructure as we speak. It needs your support to sustain and help it evolve into the future. Enclosed is a list of additional innovations we have developed (Addendum 3).
2) **Barriers to Innovation.** A serious barrier to innovation is the seemingly impenetrable prevailing myths surrounding independent special districts (Addendum 4).
3) **Restructuring Government.** We have enclosed a list of recommendations that we hope will be of further assistance to you in consideration of future restructuring (Addendum 5).

[1] This article is a written summary of testimony presented on November 18, 1993 by the author before the California State Assembly Select Committee on Restructuring Government, Valery Brown, Chairwoman.

[2] Current Address: P.O. Box 8594, South Lake Tahoe, California 96158.

A Generalized Illustration of the Organization of Governmental Agencies in the State of California Collaborating to Accomplish Mosquito and Vector Control

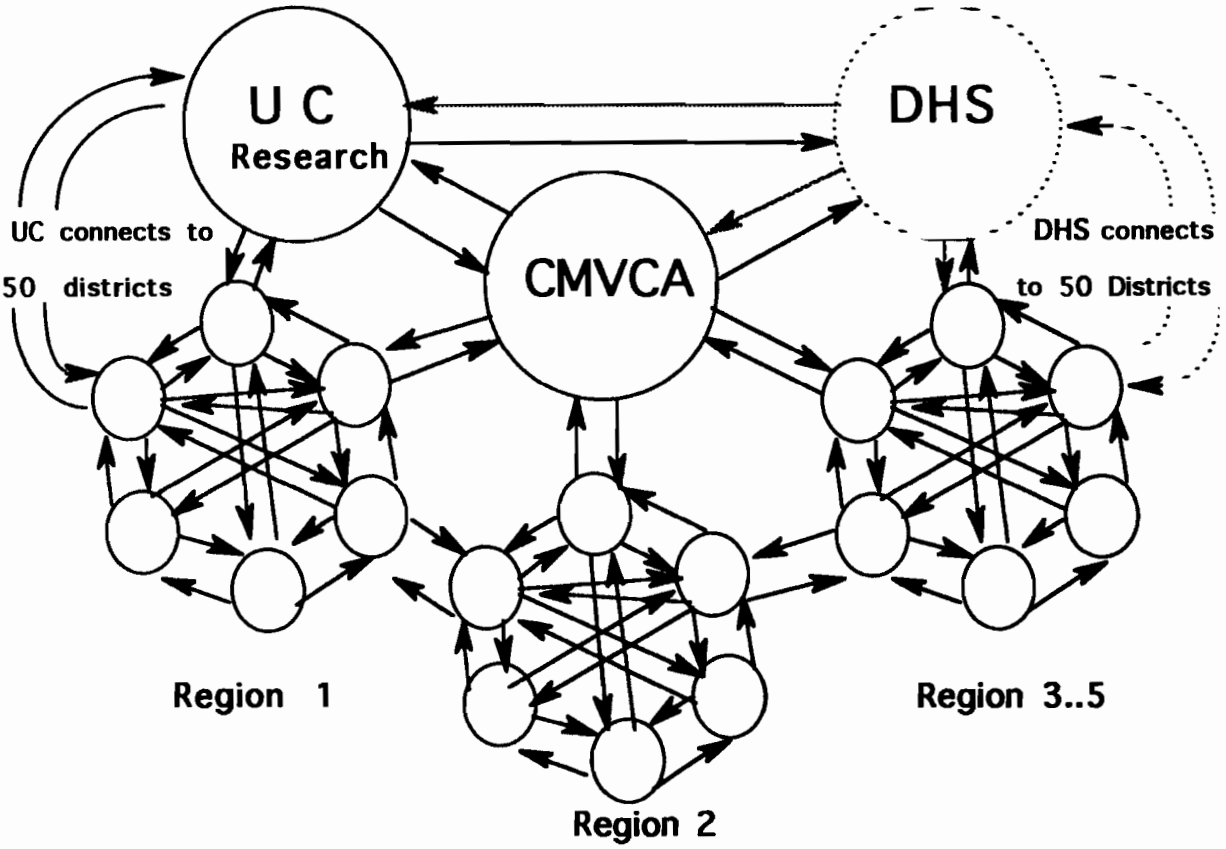


Three governmental organizations cooperate effectively together to provide mosquito and vector control programs to the citizens of the state. The overlapping circles underline the necessity that each agency function effectively and that the efforts be coordinated with those of the other agencies. The University of California Research Program (UC) provides the scientific and technical support that is so vital to effective mosquito and vector control. The California State Department of Health Services (DHS) has provided the coordinative function, quality assurance (e.g., certification and training of employees, audits of operational agencies), and laboratory support for disease surveillance. The role of the DHS has diminished in the face of budget cuts. The California Mosquito and Vector Control Association (CMVCA) is comprised of 50 local agencies conducting local public health programs.

Figure 1 is created to clearly illustrate that the ability to accomplish effective mosquito and vector control at the local level, while effectively coordinating vector-borne disease control at the state level, is dependent upon the viability and effective coordination of three state-wide agencies. Figure 2 shows in more detail the complexity of the organization that has evolved to meet the needs of mosquito and vector control in the state.

FIGURE 1

A Decentralized, Richly-Connected, Organization that Meets Local Needs and is Coordinated State-Wide



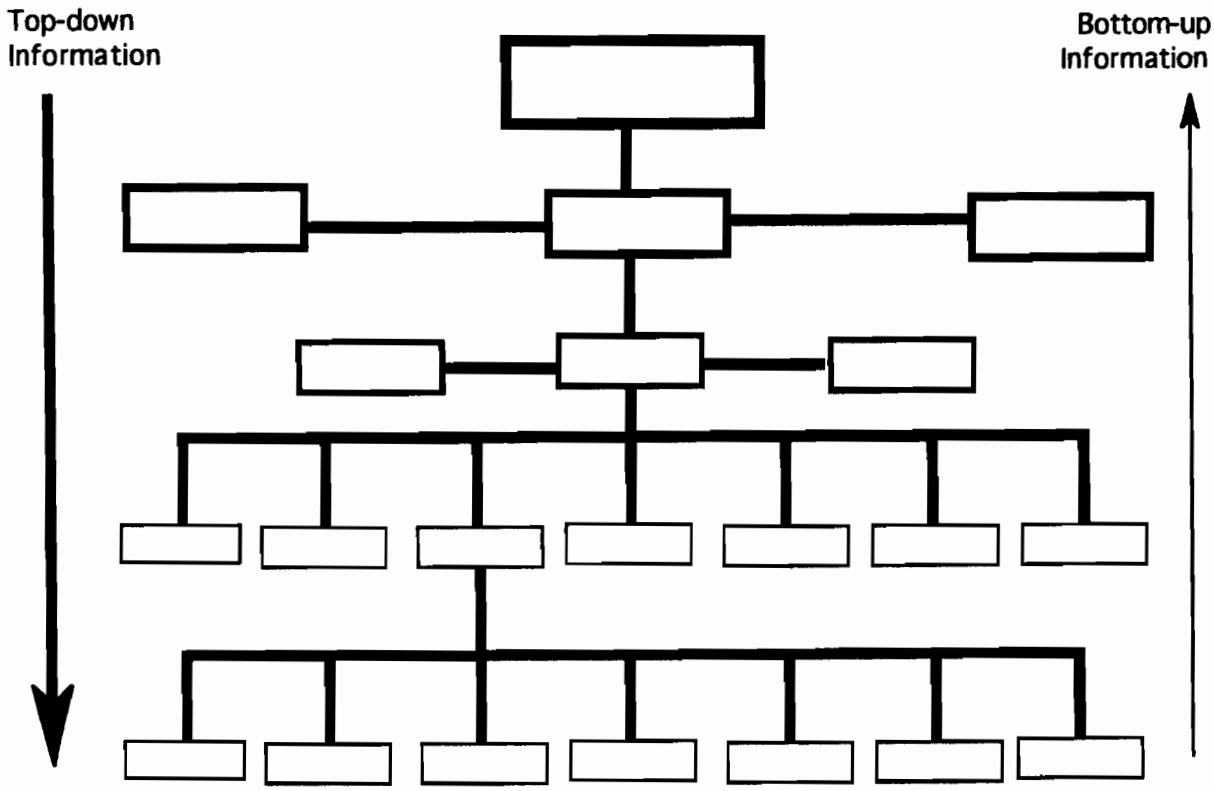
(Each of the five regions is composed of several local Mosquito and Vector Control Districts)

This flexible, decentralized organizational structure fosters multi-directional information flow supporting local program control and state-wide coordination of vector-borne disease control. Local districts are structured to move decision-making to line-employees. The California Mosquito and Vector Control Association (CMVCA) is comprised of 50 local mosquito and vector control districts and is taking on greater coordinative function as the California State Department of Health Services (DHS) is diminished due to budget cuts. DHS has provided the coordinative function, quality assurance, and laboratory support, but is now less able to perform. The University of California Mosquito Research Program (UC) provides scientific innovation to insure local agencies are efficient, effective, and environmentally sound. The 50 districts organize at a regional level (5 regions) to coordinate and solve regional problems.

This decentralized, state-wide, organization produces the positive characteristics of restructured government as described in "Making Government Make Sense." The special districts are, by legislative mandate, structured to be service oriented and close to the customer. The clarity of the objective and the consequences of failure (i.e., disease outbreaks, outbreaks of pest mosquitoes or vectors, complaining citizenry) compel the local districts to be customer focused, to focus on results and to take risks. Innovative solutions are provided both from the top of the structure (UC) all the way to line-employees.

FIGURE 2

Rigid Top-Down Traditional Organization



These rigid "command and control" organizations, common in government and in private corporations, are structured to send information (rules of action) from the top to the employees at the bottom. The flow or information that should be going from the line- worker, who is close to the problems, to those at the top who make the decisions is closed off. These organizations distort reality which results in bad decision-making. Increasingly bad decisions, in turn, cause increasing error, which in turn, causes those at the top to push harder to correct them - only compounding the error. Ultimately, those at the top of the organization are forced to create a defensive structure to stave off criticism. Because "line" employees see many of the problems most clearly and yet cannot make appropriate corrections (innovation), a counter-productive organizational defensive pattern evolves that is designed to protect workers from the irrationality of the organization. Unions are that structure undisquised.

These centralized, highly bureaucratic agencies show all of the negative characteristics of bad government featured in "Making Government Make Sense." Individuals in control of these organizations will often focus on process because results are embarrassing. The needs of the customers (service orientation) are not met because those at the top making the decisions are too far removed to know how to meet their needs. Innovative solutions and risk taking are unlikely because if it starts at the bottom it threatens the authority structure and if it starts at the top it has little likelyhood of breaking through the organizational defensive pattern that has emerged to protect employees. Centralized authority in these top-down organizations give you ineffective government, high costs, poor services, self-deceptive administrators, paralyzed workers, and frustrated customers (tax-payers).

FIGURE 3

ADDENDUM 1

REGIONAL COORDINATION OF MOSQUITO AND VECTOR CONTROL DISTRICTS

Local mosquito and vector control districts have formed strong regional organizations in the last few decades, driven both by economic and environmental concerns. Faced with diminishing financial resources, districts have come together in five regions of the state to share equipment, human resources, and knowledge. They have met the need for regional coordination of environmental programs by coordinating their activities with each other and with those of other environmental agencies. A partial list of specific regional activities follows. I have listed those of the California Mosquito and Vector Control Association (CMVCA) Coastal Region, which I represent:

1) The CMVCA Coastal Region, along with the California State Department of Health Services (DHS) acting as lead agency, has negotiated a general permit with the San Francisco Region of the U.S. Army Corps of Engineers. It allows the mosquito control districts in the San Francisco Bay Area to efficiently implement long-term, ecologically-sound mosquito control practices in wetlands within the jurisdiction of the Corps and the San Francisco Bay Conservation and Development Commission. A yearly plan is developed by the Region and distributed by the Corps for review by all environmental agencies in the region (local, state, and federal) and the interested public. The effort provides an example that local mosquito and vector control districts can be effective locally and coordinate regionally.

2) The Coastal Region has collaborated effectively with University of California researchers to improve the effectiveness of our mosquito and vector control efforts and to integrate them effectively with ecological systems. One particularly beneficial collaboration resulted in modifying long-term control efforts in wetlands to improve mosquito control, enhance the wetlands, and protect wildlife.

3) The Coastal Region negotiates a yearly contract with a helicopter firm to accomplish applications of biorational pesticides to control mosquitoes in wetlands. The number of applications accomplished by all of the districts in the region provides leverage to reduce the cost of the contract (economies of scale). The applications are coordinated through District staff assigned to regional duties as needed.

4) Staff of the Coastal Region districts meet periodically to coordinate mosquito and vector control programs in the region. This activity is especially helpful in reducing cross-border mosquito and vector problems.

5) The Coastal Region is currently in the process of developing a memorandum-of-understanding (MOU) with the U.S. Fish and Wildlife Service refuge managers of the San Francisco Bay Wildlife Refuge. The MOU is aimed a establishing wetland management practices that control mosquitoes and are compatible or beneficial to wildlife.

6) The Coastal Region actively participates in two state-wide agencies: the California Mosquito and Vector Control Association and the Vector Control Joint Powers Association (self-insuring agency). Both of these agencies are action oriented agencies that provide a vital state-wide coordinative function. Regional appointees serve on the Boards of Directors of both agencies and employees of Coastal Region districts serve actively on numerous standing and ad hoc committees of the agencies.

ADDENDUM 2

STATE-WIDE COORDINATION OF LOCAL MOSQUITO AND VECTOR CONTROL AGENCIES BY THE CALIFORNIA MOSQUITO AND VECTOR CONTROL ASSOCIATION AND THE CALIFORNIA STATE DEPARTMENT OF HEALTH SERVICES

The diverse and abundant ecosystems throughout California provide fertile ground for the production of some 47 species of mosquitoes and innumerable vertebrate and invertebrate vectors. Residents of the state are potentially exposed to numerous vector-borne diseases including: western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE), Lyme disease, plague, and relapsing fever. Changing conditions, such as the recent aggressive implementation of wetland creation and enhancement policies by environmental agencies, the unprecedented high level of activity of WEE this summer throughout Central California and in the Northern San Francisco Bay Area, and the impending invasion of the Africanized Honeybee underline a critical need to coordinate the activities of local mosquito and vector control agencies to continue to provide state-wide public health protection.

Until recent years, the leadership role of the state-wide coordinative function was performed by the State Department of Health Services (DHS). Since passage of Proposition 13, the DHS has been in steady decline, losing manpower and valuable expertise. In light of the growing public health risk that has resulted, the California Mosquito and Vector Control Association (CMVCA) has taken the responsibility to coordinate local mosquito and vector control agencies at a state-wide level. This should be recognized as a creative, "bottom-up" or "inside-out" approach by local government to solve problems beyond the boundaries of any one of the local agencies. some of the specific actions taken were:

1) The CMVCA Research Foundation was formed by action of the CMVCA to serve as a conduit to funnel revenues from local districts to fund vital mosquito and vector control related state laboratory functions. This approach has slowed the loss of technologies from the state laboratories that are essential to conducting an effective vector-borne disease surveillance and control program.

2) The CMVCA has hired a full-time executive director to head the association. He was chosen from the ranks of the U.S. Public Health Service and has an extensive background in the dynamics of vector-borne disease. His expertise has been critical as he has been compelled to take the lead role in the state-wide coordination of mosquito and vector control activities. This was never more evident than in the role he played in quelling the recent public health threat posed by WEE.

3) Beginning in the 1970s, the member districts of the CMVCA recognized the growing need for certification of the competency of their employees. The issue was heightened by the growing concern about pesticide use and associated environmental problems. The CMVCA and the DHS worked together to create a certification program that anticipated the needs of the U.S. EPA. An extensive program of continuing education was instituted in the 1980s to insure that employees in the field of mosquito and vector control kept their knowledge current. The certification training and continuing education of mosquito and vector control personnel, now mandated by law, illustrates the benefits of cooperation between state and local agencies.

ADDENDUM 3

INNOVATIONS IN MOSQUITO AND VECTOR CONTROL (A PARTIAL LIST)

An enormous number of valuable innovations have been generated in the name of mosquito and vector control in California. The University of California (UC) Research Program has been a veritable engine of scientific creativity in the field of mosquito and vector control. The local districts, the California Mosquito and Vector Control Association (CMVCA) Regions, and the CMVCA itself have also created many innovations in technical and governmental areas. Again. it must be said that the State Department of Health Services (DHS), once a vital partner in providing the bridge between research findings and application of the technology, can no longer perform that vital function. Following are some of the innovations:

1) The California Mosquito and Vector Control Association holds an annual conference at which time personnel of local mosquito and vector control districts, University of California researchers, employees of the DHS, vendors of vector control products, and others from throughout the nation come together to share the latest scientific, technical and practical innovations. A copy of the Proceedings or our 1992 meeting is enclosed.

2) Member Districts of the CMVCA have formed the Vector Control Joint Powers Agency (VCJPA) to self-insure, when appropriate, and to purchase insurance as necessary at reasonable cost. The VCJPA is governed by representatives of the local districts. This creative approach allowed small, locally-focused districts to form efficiently into a governmental unit large enough to obtain economies-of-scale.

3) The CMVCA created the CMVCA Research Foundation to serve as a conduit to funnel revenues from local districts to fund vital State laboratory functions. This approach has preserved vital functions and technologies at State laboratories that are essential to conducting an effective vector-borne disease surveillance and control program in California.

4) Since January 1, 1992, local mosquito and vector control districts, the University of California, The Department of Health Services and other relevant groups have been linked together by a state-wide computerized system called MosquitoNet. The system is operated by the Mosquito Research Program, the CMVCA, and DHS. The network serves as a means to exchange vital information in a timely and accurate manner to aid in the coordination of all of the agencies in an effective state-wide effort.

5) The Coastal Region of the California Mosquito and Vector Control Association, with the DHS acting as lead agency, has obtained a general permit from the San Francisco Region of the U.S. Army Corps of Engineers. It functions to allow the Mosquito Control Districts in the San Francisco Bay Area to implement economical, ecologically-sound mosquito control practices in wetlands of the Bay Area. The successful efforts serve as an example of small local districts coordinating their efforts effectively at the regional level.

6) The Coastal Region has collaborated effectively with University of California researchers to develop a highly effective "recirculation ditching" system to enhance the ecological control of mosquitoes on wetlands. The new approach provided excellent mosquito control, virtually eliminated pesticide use where it was implemented, and was found to be beneficial to endangered species.

ADDENDUM 4

BARRIERS TO INNOVATION

Change is everywhere if you have the eyes to see it. We are at a time in history that our "world view" is being transformed at a depth not experienced since the Copernican or scientific revolution of the 17th century. The transformation augers toward building a holistic world where socioeconomic systems are integrated with the natural world. It is being driven by the frustration of dealing with the chronic problems created by the old view. Perhaps the most serious problem being the inability to handle complexity.

A "crisis of control" is evident in our bureaucratic, top-down institutions (Fig. 3). Over-centralized governments and corporations are toppling everywhere; witness the USSR, United Airlines (almost becoming "Disunited Airlines"), and our own governments in the state of California. We should not place impediments in the path of organizations that are adapting to meet these challenges. Following are barriers to innovation that create serious problems for the coordinated network of organizations that provide mosquito and vector control to the citizens of the state:

1) The most serious barrier to innovation in local government may be the prevailing myth that special district government is uncoordinated, unresponsive, unaccountable and ineffective. This chronic misconception is perpetuated in the halls of the state legislature and the Governor's office by anecdotal evidence and is impervious to facts, figures, or ordinary observations that clearly show independent special districts to be the most effective government to provide certain public services. The myth has reached its height of destructiveness when it lurks at the foundation of a recommendation in the State Analyst's report "Making Government Make Sense" (page 125);

   > "Property tax revenues now allocated to special districts would instead be entirely allocated to counties or cities in the case of city-dependent districts. These counties or cities would be responsible for funding them or taking them over."

   The report, in one breath, defines a need to restructure government in a manner in which special districts clearly already lead the way and, in the next breath, produces a sentence (above) which would cripple special districts. Such action would destroy the most flexible form of government in the state. It would impose the top-down structures shown in Fig. 3.

2) The desire of the State Legislature and the Governor to take action to "simplify" government may plant seeds of destruction. The more diversity of governmental structures that are functioning, the more opportunity for innovative structures to appear and be selected.

3) The final barrier to innovation may simply be lack of vision. We need a shared vision to emerge in state and local government that can align all of the diverse governmental agencies in working towards a common goal. We need a transformational vision that will give us eyes to see the problems in new ways and gives us courage to create new solutions. The inability of leadership in the state to articulate a clear and coherent vision may be our most serious impediment to innovation. A powerful shared vision creates an environment where innovation, experimentation, and risk taking are possible.

ADDENDUM 5

RECOMMENDATIONS ON RESTRUCTURING GOVERNMENT

We make the following recommendations on restructuring state and local government:

1) Recognize and sustain the richly connected, locally focused, highly coordinated state-wide network of agencies comprised of 50 local mosquito and vector districts, the State Department of Health Services (DHS), the California Mosquito and Vector Control Association (CMVCA) and the University of California (UC). The organizational structure is an effective adaptation to increasing environmental and fiscal problems (Fig. 2). It is organized effectively at the local, regional, and state-wide level. This organizational approach has enabled the mosquito and vector control community to continue to provide effective public service in difficult times. This model of government, comprised of small units of local-government that are self-organized at a regional and state level, should be looked at seriously as "government of the future."

2) The State should support and adequately fund the DHS to carry out its legal mandates with respect to mosquito and vector surveillance and control programs (i.e., certification and training, interagency liaison, cooperative agreements, and coordination of multi-jurisdictional emergencies).

3) The State should review and amend existing laws that currently create contentious relationships, fragmentation, and lack of effective coordination between state and local agencies. Such laws should be amended to foster joint and harmonious problem-solving between state and local agencies.

4) The Legislature itself needs to seriously consider the over-riding principles and philosophy of "making government make sense." Top-down control at the state or local level is doomed to fail. The Legislature should foster decentralization of authority; empower local agencies that are close to the customer; insist they focus results (recognizing and rewarding good results, correcting consistently bad results); and create a climate to allow agencies to take risks and be innovative.

5) The Legislature should recognize that a diversity of structures in local government is a strength. Special districts and cities are and can continue to be our laboratories of innovation. If all of local government becomes uniform mono-structures, we will create rigid, brittle government that will snap in the onslaught of a changing world. The legislature needs to increase the plasticity of government by appreciating the diversity of forms of local government.

1994 Proc. Calif. Mosq. Vector Control Assoc. 62:115-116

THE CALIFORNIA MOSQUITO AND VECTOR CONTROL JOINT POWERS AGENCY OPERATIONAL EXPERIENCE OR HOW TO BEAT THE HIGH COSTS OF DISTRICT INSURANCE PROGRAMS

Grant W. McCombs

President - Board of Trustees
Orange County Vector Control District
P.O. Box 87
Santa Ana, California 92702

The genesis of the California Mosquito and Vector Control Association Joint Powers Agency (CMVCA-JPA, or simply JPA) program was in 1979, when district managers and trustees realized that spending a million dollars for insurance premiums to satisfy $40,000 in claims was not in the best interest of our taxpayers -- "our financial lifeline".

Many meetings and study groups were conducted statewide by the 29 interested districts and it was concluded that with a surcharge on our current insurance costs, a $500,000 pool could be developed to satisfy potential claim costs in three insurance programs. The first is the Pooled Liability Program in which our coverage includes personal injury and property damage, errors and omissions, public employee forgery, deposit and performance bonds, boiler and machinery, business travel, underground storage tanks, and aviation (when needed). Second is the Pooled Worker's Compensation Program and thirdly, the Pooled Physical Auto Damage Program.

It is obvious that a one-half million dollar pool could in no way protect the districts in the event of a catastrophic loss claim in the early growth period of the program, so it was necessary to go to the insurance market for supplemental coverage.

In order to generate a pool sufficient in financial magnitude in the Workers' Compensation Program pool, district premiums are based on payroll size of the district and a program of retained limits were imposed that range from $2,500 to $50,000 for larger districts.

Last year, the JPA joined several city and county special districts to form the Local Agency Workers' Compensation Excess Joint Powers Authority. In this program, losses from $250,000 to $500,000 are shared by the pool members on a contribution percentage basis. This formula gives a fair balance of financial responsibility for the smaller and less vulnerable participating districts. For claims in excess of one-half million dollars, the agency has purchased an umbrella policy at a relatively low rate for this excess coverage.

As in any pooled program, workers' compensation is no different in that districts with low claim losses, higher refunds, or retrospective payments result. For example, in the past three years $769,583 has been returned to low claim districts.

As an aside, it should be noted that last year our state legislature gave all business enterprises a bonus in workers' compensation costs when legislation was passed that reduced the amount of fraudulent claims for stress and other dubious injury claims. Needless to say, this legislation brought heartburn to tort lawyers and questionable medical clinics.

The Pooled Liability Program is generally recognized as the area of insurance coverage in which we find the source of greatest monetary losses. Accordingly, the JPA is working diligently to reach its full potential as self-insured with a pool of $10 million. Presently, the first $250,000 of claim costs are covered with pool monies, the next $750,000 through self-funding with excess contributions (currently at $1.3 million). For losses over one million dollars, we have gone to the insurance market for coverage.

On the other hand, the Pooled Auto Physical Damage Program has been one of the most successful programs. For the past two years, our member districts have paid no premiums in this area of insurance coverage, except for new vehicles added to their fleets. In this instance, a one-time flat fee of $575 is required.

Compare that figure to your personal auto damage costs, remembering that this coverage is for commercial use.

Now, a word about the administration of this program. As mentioned earlier, it was managers and trustees who built the framework of goals, responsibilities, regulations (such as pro-rating costs), development of programs, the governing Board of Directors (consisting of five regional district managers and two statewide trustees) and an Executive Board (to provide day-to-day oversight of administration). As the organization grew, it became very apparent that the respective managers had the experience to administer the program, but did not have the time from their responsibilities to provide public health services in vector control. Likewise, the trustees could not take the time from their respective business responsibilities. It was, therefore, determined to hire a professional management firm. The Executive Board established the criteria that a management firm should have on-board lawyers skilled in workers' compensation, general liability, risk management, and risk transfer, so that these legal skills could compliment the legal representation retained by the districts. There was also a requirement for skills in accounting and investment advice, underwriting, claim litigation and settlement, program formation, and safety loss control.

Since 1986, professional management has been contracted as self insurance program operational functions are not germane to the day-to-day operation of vector control. With an Administrator, we have a central repository for claim analysis and resolution, a library of films and manuals for safe operations, the development of risk management programs which are essential to the reduction of claim losses, and the monitoring of workers' compensation claims through the maze of redundant rules and regulations.

The Executive Board members and the Administrator work closely with the Board of Directors and the California Mosquito and Vector Control Association to insure that our legislative interests are expressed and followed in the state legislature; liaison through this relationship is possible with the California Department of Health Services, and researchers in vector control research, not only in the district labs, but also in the labs of the University of California at Berkeley, Davis, and Riverside.

In any pool, consistency of all operations is an essential for success. In our pool, we attain consistency by periodic inspections of the districts to insure that managers are aware of changes in regulations and they are in compliance with federal, state, and agency regulations, and to facilitate the exchange of ideas between the agency and other districts. Every effort is made to avoid the concept perpetuated by the myth "we are just here to help you syndrome" as in the beginning some managers, understandably, did not want someone looking over their shoulders. However, when they discovered that our non-punitive suggestions averted punitive fines by federal and state inspections, the reluctance was alleviated.

Our method to have our members maintain continuous support is accomplished through quarterly meetings with the Board of Directors and other members. In addition, once a year we hold a workshop in which all members participate in give and take discussions. Problems, new developments, and the goals of the JPA are reviewed to ascertain if our growth is going in the right direction, and to expand goals if required.

Now, in turning our attention to our fiduciary responsibility to the taxpayers as trustees and commissioners. For some reason or other, California politicians do not seem to be satisfied with the plethora of federal regulations concerning vector control, so they continuously come up with additional burdens for our managers; a phenomenom which might be true in many states as well. In California, some of these additional burdens include CAL-OSHA, fair housing and employment acts, pesticide application regulations, sexual and racial harassment (real or imagined) acts, and on and on.

In emphasizing the point of over regulation, we see that the JPA does not only save taxpayers' money, but also provides a great deal of administrative assistance in responding to many managerial responsibilities generated by legislation that only has peripheral value to a manager's primary responsibility of providing the best public health service in vector control at the least cost to the taxpayers. As an example, in our county, the JPA has returned $100,000 to the District in the last three years.

It is in this area of managerial burden and responsibility that trustees and commissioners should try to thoroughly understand the problems confronting the manager and support their recommendations to the governing board as far as possible without getting involved in micro-management, but still fulfilling their fiduciary responsibility to the taxpayers.

In conclusion, the Vector Control Joint Powers Agency now has 31 member districts with Compton Creek Mosquito Abatement District and San Gabriel Valley Mosquito Abatement District recently joining. Our future is very optimistic as the Board of Directors and the Administrator are working in innovative, financially conservative programs to become totally self-insured in all three insurance programs, as well as providing more services for our managers, trustees, and member districts.

1994 Proc. Calif. Mosq. Vector Control Assoc. 62:117-118

EXECUTIVE DIRECTOR'S ANNUAL REPORT 1993

Donald A. Eliason

Executive Director
California Mosquito and Vector Control Association, Inc.
8633 Bond Road
Elk Grove, California 95624

The State budget battles again dominated much of the attention of the California Mosquito and Vector Control Association (CMVCA) during the spring and summer months of 1993. Managers as well as some staff members and trustees of member agencies were again called upon to meet with legislators and their staffs in an effort to inform them of the important preventive efforts being carried out by the districts and the potential impacts further property tax shifts would have on these districts. During the late spring and early summer, the CMVCA worked with a public relations firm on a statewide media event day which was put on in four different areas to varying degrees of success. The best response appeared to be in the Southern Region where the event was hosted by the Orange County Vector Control District. The media event put on in Sacramento received very little attention, possibly because of saturation of the news media with efforts by many competing groups attempting to influence the state legislature. Many districts made excellent individual efforts in keeping their legislators informed of issues of importance. Finally, the State budget was past in September with further shifting of property taxes from some of our member agencies. Of considerable concern was the legislative interest in reinventing government in California and their apparent lack of appreciation of the contributions being made by special districts of any kind.

In February, a joint conference on wetlands was held in Davis with participation by CMVCA, the University of California and the U.S. Fish and Wildlife Service. While not considered a huge success, useful discussions on a variety of areas did take place and may result in some improved communications between these participating agencies.

Western equine encephalomyelitis (WEE) virus activity was widespread in most of northern California during the summer and fall months, as evidenced by antibody conversion in sentinel chickens and by isolation of virus from mosquito pools. No human cases were discovered despite all the virus activity and as yet, no explanation is available for the lack of cases.

On May 24,1993, the Association was informed by the Sheraton Harbor Island Hotel in San Diego that they were planning a major renovation of their hotel, which was the site chosen for the 1994 joint annual meeting of the American Mosquito Control Association (AMCA) and the California Mosquito and Vector Control Association. With only ten months left before the meeting, we were faced with finding a new site for a meeting for approximately 1,000 people. We had the good fortune to find space available in the Town and Country Hotel in San Diego and their staff was extremely helpful to us during the transition.

We ended 1993 by holding our fall Board of Directors meeting in Palm Springs, in the district of our President, Mike Wargo. In order to allow new officers to be seated and new committees to begin functioning with the new year and to comply with our bylaws, we designated the Palm Springs meeting as our Annual Conference. The technical sessions of the conference were postponed until April, 1994 as part of the joint AMCA/CMVCA meeting.

CALIFORNIA MOSQUITO and VECTOR CONTROL ASSOCIATION, INC.

STATEMENT OF CASH RECEIPTS, DISBURSEMENTS AND CHANGES IN CASH BALANCE

For the Years Ending December 31, 1993 and 1992

	1993	1992
Cash Receipts:		
Dues	$ 255,674	$ 251,872
Conference receipts	35,055	39,268
Publications and reprints	15,798	16,910
Interest	13,190	10,878
Workshops and equipment shows	784	9,277
Other	30	982
Total Cash Receipts	320,531	329,187
Cash Disbursements:		
Wages and benefits	141,745	136,615
Office expenses	23,141	20,903
Conference and workshops	23,641	34,570
Professional services	25,502	16,973
Printing costs	16,047	27,039
Operating expenses	6,119	14,479
Awards	1,750	1,500
Miscellaneous	3,689	1,770
Total Cash Disbursements	241,634	253,849
Cash Receipts in Excess of Cash Disbursements	78,897	75,338
Cash Balance, Beginning of Year	296,474	221,136
Cash Balance, End of Year	$ 375,371	$ 296,474

1994 Proc. Calif. Mosq. Vector Control Assoc. 62:119-124

C.M.V.C.A. YEAR-END COMMITTEE REPORTS: 1993

John R. Stroh

CMVCA Vice President
San Joaquin County Mosquito and Vector Control District
7759 South Airport Way
Stockton, California 75206

The following reports reflect the activities of the standing and ad hoc committees of the CMVCA for the year 1993 as submitted by the chairpserson(s) of each committee.

TRUSTEE CORPORATE BOARD

Carl O. Nichols (President)
William L. Boynton (Vice President), Grant W. McCombs (Secretary),
Earl W. Mortenson (Past President), Patricia Gillies, Leo F. Kohl,
Ernest E. Lusk, Chester Miller and Milton Wallace

The year 1993 proved quite challenging for the Trustee Corporate Board as we had tasked ourselves to the revision of some of the Trustee by-laws that were causing ambiguities in interpretation between the Trustees and elected members of the Board; to establish a meritorious achievement award for deserving Trustees; and the election of Trustee representation to the Vector Control Joint Powers Agency.

A by-law was crafted to provide a Meritorious Achievement Award which would recognize outstanding and exceptional service performed by a Trustee in support of the California Mosquito and Vector Control Association's (CMVCA) aims and goals.

A by-law revision was presented to and approved by the CMVCA Board of Directors concerning the election of Trustee representation on the Vector Control Joint Powers Agency Board of Directors. Elected Trustees will serve two-year terms of office. One Trustee will be elected from the Northern California Region and one Trustee will be elected from the Southern California Region. An alternate Trustee will be elected on a state-wide basis.

Article III, section 2, and Article VI, Section 3 of the by-laws were revised to relieve the ambiguity in voting procedure at quarterly and annual meetings. Elected Regional Representatives and Board Officers will vote on all agenda items requiring a vote and the entire membership will vote on amendments when required. Article, VIII Section 3 was revised to insure compliance with CMVCA Board by-laws.

Trustees throughout the State, where possible, made personal contact with members of the State Legislature to further the aims and goal of the Association's agenda in the field of vector control research and operations of essential health services provided by the districts throughout the state. This action by individual Trustees to explain the essential role of vector control operations was helpful in reducing the amount of financial support the State was withdrawing from the districts for funding of other State functions.

And finally, William L. Boynton was elected as President; Patricia Gillies as Vice President; and Grant McCombs as Secretary for the 1994-1995 terms of office.

MANAGEMENT COMMITTEE

Elizabeth Cline (Chair)
Dennis Boronda, Dave Brown, Jim Camy, Lue Casey, Gilbert Challet,
Craig Downs, Laura Haynes, Ron McBride, Chuck Myers,
Theresa Stratton and Jim Wanderscheid

The California Mosquito and Vector Control Association's (CMVCA) Management Committee had a productive year. Thanks to the hard work and cooperation of its members, the following tasks were completed:

1. Annual Management Workshop: After careful consideration and study, it was determined that a Management Workshop would not be possible this year because of the financial uncertainty caused by the State budget process. Instead, we arranged for Assemblyman Paul Woodruff to address the CMVCA at our quarterly meetings in December. Assemblyman Woodruff proved to be an extremely good speaker who was very helpful to everyone in attendance.

2. Update the Resource Directory: We have made the necessary changes to the Resource Directory to keep it current. This will be distributed to each member agency and it is also available on Mosquito Net.

3. Standardized Job Descriptions: The Committee studied the task of standardizing job descriptions to comply with the Americans with Disabilities Act (ADA). We came to the conclusion that it will not be possible for us to produce "standard" job descriptions which will comply with the ADA because they are necessarily very detailed. The committee will continue this into next year and investigate the possibility of developing guidelines for each agency to use in writing their individual job descriptions. The committee produced a "generic" application form to be distributed to the member agencies.

4. Review the Salary Survey: The Committee reviewed the salary survey to see if there might be a need for changes in the survey itself and with the way the results are reported. The Committee's findings were submitted to the President and Executive Director.

5. Explore Regionalization: The Committee investigated regionalization of tasks, resources, services, etc. We identified many areas where regionalization is currently being utilized by CMVCA member agencies and some areas of possible regional cooperation to be developed in the future. The Executive Director or the Management Committee might want to take these findings and compile them for distribution to all member agencies so that the regions can benefit from each other's experiences and expand on what is working regionally, elsewhere.

6. Next Year: We looked at the Committee's potential for next year and would like to suggest that this Committee might be involved in organizing and tracking legislative contacts and activities in the future. This could be accomplished through the cooperation of the regional representatives and would ensure that these types of activities are undertaken and organized.

VECTOR CONTROL RESEARCH COMMITTEE

William Hazeleur (Chair)
James Caton, Arthur Colwell, Jack Hazelrigg, Ron Keith and John Stroh

During 1993, each committee member evaluated written research proposals in early February, prior to attending a Mosquito Research Conference organized by Dr. Bruce Eldridge, Director of the University-wide Mosquito Research Program. The conference, a joint meeting between the California Mosquito and Vector Control Association (CMVCA) Vector Control Research Committee and the University of California Mosquito Research Technical Committee, was held March 24 through 26 in Sacramento. At this conference, individual researchers made oral presentations of their research proposals and answered questions.

Following the verbal presentations at the Mosquito Research Conference, the CMVCA Vector Control Research Committee met and evaluated each research proposal and each committee member applied a numerical rating to those proposals. Two criteria - relevance to the needs of California mosquito control and past performance record - were used to rate each research proposal on a scale of 1 to 5, with 1 having the highest value and 5 having the lowest value. The rating for relevance was based on the value of these proposals as viewed by California mosquito control. The rating for past performance encompassed whether the researcher prepared an annual report, whether the research reported on in the annual report related to the research proposal which was funded, and whether the comments made by the CMVCA Vector Control Research Committee the previous year were taken into consideration. The researcher's collaboration with mosquito control personnel, attendance at the CMVCA conferences and other meetings of mosquito control people in California, as well as publication of past results were also taken into consideration for rating the research proposal on performance. Committee members' ratings were combined to arrive at an average rating within the 1 to 5 range for each proposal. Comments were also prepared for each research proposal. The comments, individual committee member's ratings and the combined average numerical ratings were compiled into a written report.

Chairman Hazeleur represented the CMVCA at the University of California President's Advisory Committee on Mosquito Research on May 20 at the University of California at Davis. The CMVCA Vector Control Research Committee's written report was submitted and reviewed at this meeting to determine mosquito research funding for the 1993-1994 year.

There were twenty-six mosquito research proposals submitted for funding in the 1993-1994 fiscal year. Funds requested from the twenty-six proposals totaled $550,935. Approximately $343,989 was available for funding mosquito research projects in the 1993-1994 year, which was a significant reduction from funds available in previous years. This reduction in funding available for mosquito research projects resulted in several good research projects receiving very limited or no funding in the 1993-1994 fiscal year.

WAYS & MEANS COMMITTEE

Chuck Hansen (Chair)
Charles Beesley, Lue Casey, Elizabeth Cline, Jerry Davis, Major Dhillon,
William Hazeleur, Leo Kohl, Donald Layson, Herbert Marsh,
Grant McCombs and Allan Pfuntner

In 1993, the California Mosquito and Vector Control Association's (CMVCA) Ways & Means Committee addressed the following four specific charges: 1) Analyze the standing charge of each standing committee and recommend language for adoption by the Board of Directors for the CMVCA 1993 Organizational Chart; 2) Continuation of a review of the Association's written policies and recommend any changes to the Board of Directors; 3) Review the current unequal representation of Corporate Members on the Board of Directors and make recommendations; and, 4) On request of the President, make recommendations on procedures and costs associated with moving the CMVCA office.

The action and recommendations on the four specific charges were as follows:

1. Committee members were given specific standing committee assignments and asked to analyze the proposed standing charge and recommend language for adoption by the Board of Directors per the CMVCA 1993 Organizational Chart. The Committee submitted proposed changes to the Chemical, Training and Certification, and Disease Control Committees. The Board of Directors took no action on these proposed changes.

2. Further review of the Association's written policies has been temporarily put on hold because we were given other more pressing charges to address.

3. The Committee feels that the issue of unequal representation of corporate members on the Board of Directors, due to the present configuration of our regional makeup, has been addressed several times before and is not an issue of great concern by the majority of our membership. Therefore, we have elected to not pursue this specific charge during 1993.

4. It is the committee's opinion that the Executive Committee should work with the Executive Director on the proposed move and not the Ways & Means Committee. If the Executive Committee would like a representative from the Ways & Means Committee to assist them, we are prepared to make an appointment.

In addition to aforementioned charges, the Committee addressed the following items:

1. We worked with the Trustee Corporate Board on proposed by-law amendments to the Trustee Corporate Board By-Laws. The two main issues were the election of members to serve on the Vector Control Joint Powers Agency Board of Directors and Trustee voting requirements. We approved the recommended changes to the by-laws and asked the Board of Directors to submit them to the Corporate Membership for ratification.

2. At the request of President Wargo, the committee addressed two concerns proposed by the Southern San Joaquin Valley Region: a) Establish guidelines (criteria) for Honorary membership eligibility - a sub-committee has been appointed to draft a policy for committee review at our next committee meeting in February, 1994; b) Review the Associate Member role on the Board of Directors - status of the Associate Member to remain unchanged unless Regions, through further discussion, decide to resurface this issue.

Both of the concerns proposed by the Southern San Joaquin Valley Region, late in 1993, will be worked on by the committee in 1994.

PUBLICATIONS COMMITTEE

Stephen Durso (Chair)
Ernest Lusk, Minoo Madon, Richard Meyer, Lal Mian,
Linda Sandoval, Ken Townzen and Glen Yoshimura

The California Mosquito and Vector Control Association's (CMVCA) Publications Committee was able to meet each of the five specific charges assigned to it for 1993. Among those tasks were:

1. The publication and dispersal of the *1993 CMVCA Proceedings and Papers of the 61st Annual Conference* and the *1993 CMVCA Yearbook.*

2. The Committee reviewed the format and presentation of the Yearbook and, along with the officer staff, will be implementing some changes in future issues which should aid in the preparation and dispersal of that publication in a more timely manner.

3. The Committee met several times with the authors of the four certification training manuals to keep each abreast of the desired development of those publications. A current time the first two of the manuals are scheduled to be published during the winter of 1994-1995 or the spring of 1995 with the other two manuals following by a year or two later.

4. In conjunction with the Entomology Committee, the Publications Committee has drawn up drafts of the desired needs and formats for the guides to the common arthropods which the former committee is now working on. Presently, with the statewide mosquito identification manual completed and published, the four regional companion guides to this publication should be completed and published in the winter of 1994/1995 or the spring of 1995.

AD HOC
AFRICANIZED HONEY BEE COMMITTEE

B. Fred Beams (Chair)
Ruben Arias, Mitch Bernstein, Major Dhillon, Frank Ennik, Bill Hazeleur,
Ed Lucchesi, David Martinez, Eric Mussen, Bob Schoeppner and Larry Shaw

The specific charges and actions by the California Mosquito and Vector Control Association's (CMVCA) Ad Hoc Africanized Honey Bee (AHB) Committee for 1993 were as follows:

1. Seek liability exemption for agencies working with AHB.
   Action: The committee is of the opinion that the same liability protection that currently protects mosquito and vector control agencies also protects in AHB related matters.

2. Coordinate with Chemical Control Committee for registration of AHB control pesticides.
   Action: On May 4, 1994, the California Department of Pesticide Regulation approved a special local need registration (24c) for an insecticidal soap compound (M-Pede) that is proving to be very effective on honey bee swarms and exposed colonies.

3. Develop model procedures for answering calls, emergency response, and other related problems.
   Action: Draft of operational model currently being developed by committee. It will be available in final draft form by January 1995.

4. In conjunction with the Ad Hoc Public Information Committee, develop a brochure for statewide use.
   Action: AHB brochures and other related educational materials are now available through the CMVCA office.

AD HOC
PUBLIC INFORMATION COMMITTEE

Stan Husted (Chair)
Dan Ariaz, B. Fred Beams, Dennis Boronda, Kriss Costa,
Susan Maggy, LuAnn Munns and Charlie Smith

During 1993, the California Mosquito and Vector Control Association (CMVCA) Ad Hoc Public Information Committee had a busy year and made much progress in completing the five charges originally assigned it as well as a number of additional tasks taken on during the year. Among the accomplishments of the Committee for 1993 are:

1. Two brochures were prepared and offered for sale from the CMVCA office. The two brochures were entitled "The Western Treehole Mosquito and Dog Heartworm" and "Are You Raising Mosquitoes in Your Backyard?"

2. A Spanish translation of the previously distributed brochure entitled "Preparing for the AHB" was provided by West Valley Vector Control District with a draft sent to the Executive Director for CMVCA Board of Directors approval.

3. Committee members prepared and gave two presentations at the AMCA/CMVCA Joint Annual Conference in San Diego. The two presentations were entitled "Advantages of a Statewide Approach to Public Education" and "How Media Events Can Improve Mosquito Control".

4. Other issues addressed by the Committee included possible ideas for redesign of the CMVCA logo, the preparation of a CMVCA newsletter article informing districts how to write newstips, the future value and work for this committee, the possible hiring of a statewide or regional news clipping service, possible preparation of generic mosquito and vector control videotapes for use by individual districts in their public education efforts, and the possible presentation of a Media Workshop for member districts.

AD HOC
MEETING/CONFERENCE PLANNING COMMITTEE

Allan Pfuntner (Chair)
Donald Eliason and Michael Wargo

It is the opinion of this Committee that the general arrangements for the California Mosquito and Vector Ccontrol Association Quarterly Meetings and the Annual Conference should remain a cooperatlve effort between the Local Arrangements Committee and the Executive Director. Interaction well in advance of the scheduled meeting allows input from all parties regarding site location, facility requirements, and transportation considerations.

After the general parameters have been established, the actual negotiations and contract finalization should be the responsibility of the Executive Director. Such a procedure promotes efficiency and continuity as the Executive Director is knowledgeable regarding meeting/conference needs and can effectively negotiate with the hotel or facility to meet those requirements. An inexperienced negotiator would be at a gross disadvantage in this arena.

As experienced in San Diego in 1994, contact and coordination through a single location, with delegation of tasks to assigned Local Arrangement committee members, promotes effective communication and results in a minimum of confusion and frustration for all parties involved.

WILLIAM C. REEVES
NEW INVESTIGATOR AWARD

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

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

Merry L. Holliday-Hanson was the recipient of the 1994 award at the 62nd Annual Conference held in San Diego. The other finalist was J. Wakoli Wekesa. The two finalists' papers are printed on pages 125-130.

Previous William C. Reeves New Investigator Award Winners:

1994 - Merry L. Holliday-Hanson
1993 - Jeffrey W. Beehler
1992 - Darold P. Batzer
1991 - David R. Mercer
1990 - Gary N. Fritz
1989 - Truls Jensen
1988 - Vicki L. Kramer

1994 WINNER

WILLIAM C. REEVES NEW INVESTIGATOR AWARD

1994 Proc. Calif. Mosq. Vector Control Assoc. 62:125-127

SIZE-RELATED COST OF SWARMING IN *ANOPHELES FREEBORNI*

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ABSTRACT

Size-related energetic costs of swarming in male *Anopheles freeborni* were assessed using males from three behavioral groups: patrollers (swarm initiators), early swarmers, and late swarmers. Small and medium-sized males exhibited decreases in total fuel (trehalose and glycogen) levels during the swarming period. However, there was no difference in fuel consumption by large males during the swarming period, suggesting that these males are more energetically efficient than small or medium sized males. Patrolling males contained a higher proportion of glycogen than did early and late swarmers, indicating that glycogen is mobilized for flight before trehalose.

The mosquito, *Anopheles freeborni* Aitken produces mating swarms, which are highly predictable. Approximately 5-10 minutes after sunset, a small number of males appear at a swarm location. Gradually, the number of swarming males increases, reaching a peak within 15 minutes, at which time most mating occurs. Females fly into the mass and leave *in copula* with a male. Occasionally, when mosquito density is at or near its peak, several males vie for a single female.

It has been established that nectar sugars do not fuel swarming flight, but the storage sugar, trehalose, and glycogen do (Yuval et al. 1994). The energetic costs of swarming have been quantified, indicating that swarming consumes, on average, greater than 50% of available fuel calories. Investigations of how male size shapes energetic constraints must be conducted to answer fitness associated questions, since size is a central factor in sexual selection (Thornhill and Alcock 1983). Accordingly, the objectives of this study were to analyze the size-related cost of swarming and to assess the nutritional status of swarm initiators.

MATERIALS AND METHODS

Swarming male *An. freeborni* were collected with aerial nets adjacent to rice fields in Sutter County, California on three evenings during July and August, 1993. Based on the behaviors of males in swarms, we established three behavioral groups: 1) patrollers, 2) early swarmers, and 3) late swarmers. Patrollers (swarm initiators) were defined as those males first appearing and hovering at a swarm site (<15 males)and arriving less than five minutes after the first male appeared. Early and late swarmers have been previously described by Yuval et al. (1994). Early swarmers were sampled approximately five minutes after the patrollers arrived. Following this collection, males remaining in the swarm were marked by blowing fluorescent dust (Hercules Incorporated, Wilmington, Delaware) into the swarm.

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Males were collected 15 minutes later and transported to the lab. We retained marked males as our late swarmer group.

Based on earlier studies, wing length was used as an index of mosquito body size. The collected male *An. freeborni* were separated into three size classes based on wing length measurements: 1) <4.23 mm, 2) 4.23-4.46 mm, and 3) >4.46 mm, to assess the size-related cost of swarming.

We followed the procedures outlined by Van Handel and Day (1988) to measure the amounts of lipid, nectar, storage sugars, and glycogen in individual males. One-way analysis of variance was employed to compare mean concentrations of these chemicals among the behavioral categories.

RESULTS AND DISCUSSION

Reserves of storage sugars and glycogen are converted to flight energy in male *An. freeborni* for swarming (Yuval et al. 1994). Therefore, we focused our analyses on these fuels. In the smallest size class (<4.23 mm) fuel levels among the patrollers and early swarmers were significantly lower than males sampled from the late swarm category ($P<0.01$; $F=6.209$) (Fig. 1a). Late swarmers in the second size category (4.23-4.46 mm) contained significantly higher levels of fuel than did the patrollers from the same size class ($P<0.05$; $F=3.821$) (Fig. 1b). However, no size difference was detected between the late swarmers and early swarmers in this size class. Although differences were evident among individuals in both the first and second size classes, no differences in fuel levels were found in the largest size class (>4.46 mm) ($P=0.586$; $F=0.551$) (Fig. 1c). This suggests that large males are more energetically efficient than small males.

The proportion of glycogen found in individual males decreased during the swarming period (Fig. 2). The patrollers contained a higher proportion of glycogen than did the early and late swarmers; however, based on our small sample sizes, these proportions were not statistically significant. Thus, a general trend can be seen in which the rate of glycogen consumption was greater than that of storage sugar. It appears that males utilize energy from glycogen before using trehalose. If males exhaust their glycogen reserves, they are still able to mobilize trehalose for flight energy.

Lipid levels were not statistically different among the behavioral groups or among the size classes (Fig. 1), and only one male was nectar-positive. This illustrates that lipid and nectar sugars are not utilized to fuel swarming, a phenomenon observed by others (e.g., Yuval et al. 1994; Nayar and Van Handel 1971).

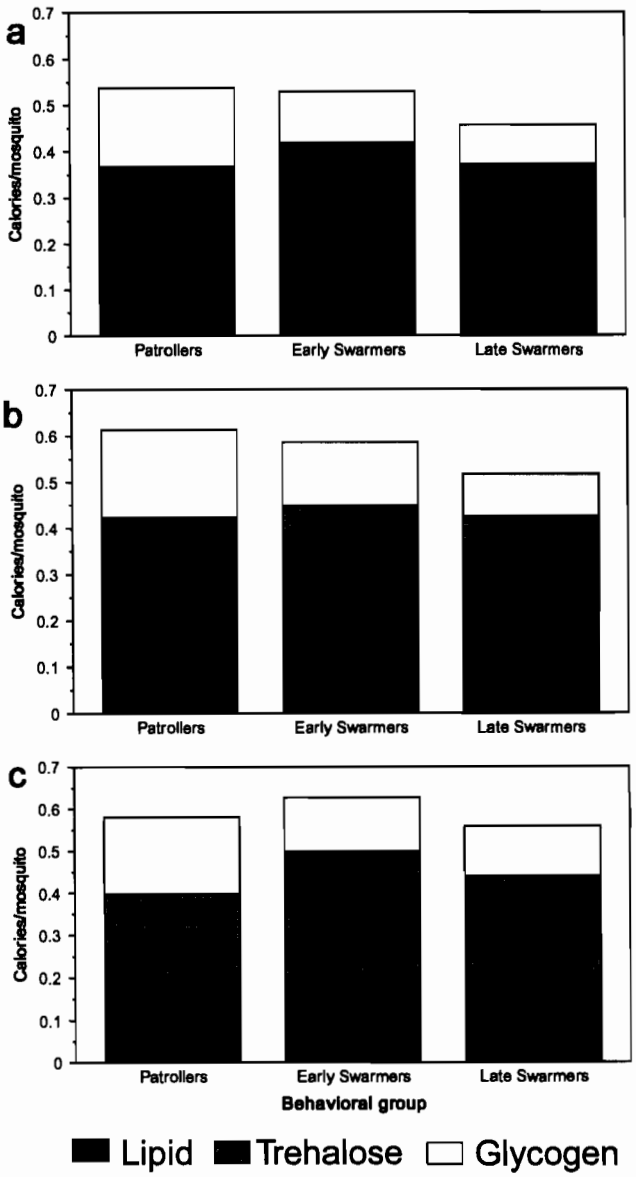

Figure 1. Average caloric reserves of lipid, trehalose, glycogen, and total fuel in swarming *An. freeborni* males of three size classes [a) wing length <4.23 mm; b) wing length =4.23-4.46 mm; and c) wing length >4.46 mm] and three behavioral groups (patrollers, early swarmers, and late swarmers).

Large *An. freeborni* males have a reproductive advantage over small males, as large males mate more frequently than small ones (Yuval et al. 1993). This advantage is enhanced by the reduced energetic costs incurred while swarming. Our study indicates that being large also has an energetic advantage-it is energy cheaper for large males to swarm.

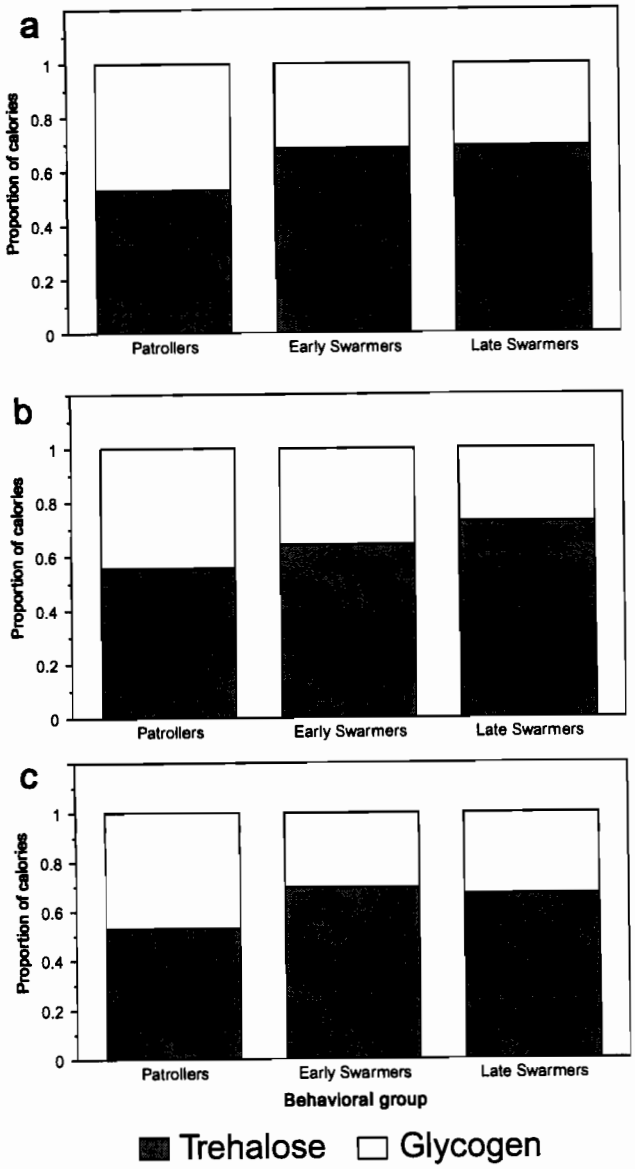

Figure 2. Caloric proportions of glycogen and trehalose in swarming *An. freeborni* males of three size classes [a) wing length <4.23 mm; b) wing length =4.23-4.46 mm; and c) wing length >4.46 mm] and three behavioral groups (patrollers, early swarmers, and late swarmers).

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A HISTOLOGICAL TECHNIQUE FOR DETECTING MULTIPLE BLOOD FEEDING IN *ANOPHELES FREEBORNI*

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ABSTRACT

A histological technique was used to determine the frequency of multiple blood feeding in a single gonotrophic cycle of *Anopheles freeborni*. Fifty-six laboratory-reared mosquitoes, all known to have taken two blood meals, were examined. When the interval between meals was from 1 to 24 hours and the time from the second meal until fixation ranged from 0 to 24 hours, we detected 78% (39/50) of known double meals. At intervals outside this range, 50% (3/6) of multiple meals were detected. Seventy-nine percent (44/56) of the double meals detected were distinguished by a physical blood meal separation and the amount of heme that developed around each meal. Thus, histological techniques can provide a direct and more accurate measure of multiple blood feeding in anopheline mosquitoes.

Dissemination of vector-borne diseases is dependent on the frequency of host-vector contact (Garrett-Jones 1964, Klowden 1988). For female mosquitoes the purpose of this contact is to take a blood meal. Female mosquitoes usually rely on a blood meal to acquire nutrients necessary for developing their eggs. Assumptions that female mosquitoes feed only once during a gonotrophic cycle (Gillies and Wilkes 1965) conflict with data that suggest mosquitoes feed more often (Klowden 1988). The prevalence of multiple blood meals varies among mosquito species, and within species, spatial and temporal variation is not uncommon. Consequently, the frequency of multiple blood feeding in several field-collected anophelines (as determined by immunological methods) has been found to vary from 5 to 50 % (Burkot et al. 1988).

Multiple blood feeding within a single gonotrophic cycle appears to be a widespread behavior among anophelines. However, in *Anopheles freeborni* Aitken, a species that is a potential vector of malaria and the Northway virus (Campbell et al. 1991), immunological tests have estimated multiple blood feeding at less than 2% (Washino and Tempelis 1967, McHugh 1989, Wekesa et al. 1992). Here we present results from a study on *An. freeborni* using a histological technique (adopted from the work of Romoser et al. 1989 on *Culex nigripalpus* Theobold) that is a more direct method, and that improves detection of multiple blood feeding.

MATERIALS AND METHODS

To determine whether female mosquitoes had taken single or multiple blood meals we adapted the histological technique described by Romoser et al. (1989) for *Culex nigripalpus* and modified by Scott et al. (1993) for *Aedes aegypti* (L.). This technique increases our ability of detecting cryptic blood meals; which are meals completed on the same host species and cannot be detected by the standard immunological tests. Mosquitoes were sedated by placing them on wet ice, after which they were fixed by placing them in Smith's modified Alcoholic Bouin's fixative (45:45:5:5 solution of saturated aqueous picric acid, 95% ethanol, reagent-grade formalin, and glacial acetic acid) at 60°C. The specimens were then dehydrated and infiltrated with paraplast (Sigma Chemical Co., St. Louis, MO), sectioned and mounted on microscope slides. Mounted sections were stained using a modified Azan trichrome technique (Hubschman 1962), and examined under bright-field microscopy. The status of engorged females was determined by the following parameters (defined by Romoser et al. 1989 and Scott et al. 1993) the peritrophic membrane, peritrophic plug, heme or zone of digested

blood, partially digested blood meal, and physical or multiple blood meal separation.

We adapted this method on laboratory-reared *An. freeborni* females. Adult females were maintained on a diet of raisins and sucrose solution. At various intervals, individual females were allowed to take blood meals from the hand of one of the authors (J.W.W.). Eight hours before the first blood meal, the sucrose solution was removed, but the solution was not removed in subsequent meals. In most cases, the first meal was deliberately interrupted to facilitate re-feeding at 1 to 48 hours later. After the second blood meal, females were assigned a code number and processed histologically. The specimens were mounted and presented randomly, in a blind test, to another person who attempted to determine their blood feeding history. Female mosquitoes not taking the second blood meal were used as controls.

RESULTS AND DISCUSSION

Fifty-six laboratory-reared *An. freeborni* females, all known to have taken two blood meals, were examined histologically (Table 1). When blood meals were separated by 1 to 6 hours and fixed within 2 hours after the second meal, the most useful histological parameter for detection of multiple feeding was physical blood meal separation. When mosquitoes were fixed immediately after the second meal, multiple meals were detected in 94 % (15/16) of the specimens. Generally, 79% (44/56) of the double meals detected were distinguished by a physical blood meal separation and the amount of heme that had developed around each meal. The peritrophic membrane was seen in 55% (31/56) of blood meals; only 19% (8/43) of mosquitoes with multiple blood meals had detectable peritrophic plugs from the first blood meal.

When the interval between meals was from 1 to 24 hours and the time from the second meal until fixation ranged from 0 to 24 hours, we detected 78% (39/50) of known double meals. At intervals outside this range, the first blood meal was no longer detectable in 67% (4/6) of mosquitoes examined. However, in 50% (3/6) of these mosquitoes, multiple meals could also be detected by the presence of a peritrophic plug. This study confirms that histological parameters described by Romoser et al. (1989) and Scott et al. (1993) are useful for identifying multiple blood meals in *An. freeborni*. A comparison of our data with those of Romoser et al. (1989) and Scott et al. (1993) indicates that the detection rate of known multiple meals for *An. freeborni* (78%) was similar to that for *Aedes aegypti* (80%, Scott et al. 1993), however the detection level for these two species was lower than for *Culex nigripalpus* (93%, Romoser et al. 1989). The differences in the detection level of the two sets of mosquito species could be accounted for by our low sample size or by interspecific differences, e.g., a faster rate of blood meal digestion in *An. freeborni* and *Ae. aegypti* than in *Cx. nigripalpus*.

Table 1. Detection of multiple blood meals (numbers examined) by 56 *An. freeborni* during a single gonotrophic cycle.

Time Between Meals (hrs)	Time from Second Meal Until Fixation (hrs)				
	0	1	2	10	24
1	4 (4)				2 (3)
2		1 (1)		2 (2)	1 (1)
6	5 (5)		3 (4)	0 (2)	4 (5)
12	3 (3)	2 (2)	1 (1)	2 (2)	1 (3)
24	2 (3)	0 (1)	3 (3)	3 (4)	0 (1)
48	1 (1)			1 (3)	0 (2)

The same specimens were evaluated by two individuals in a blind test. Of the 64 laboratory-reared females examined, we made 61 correct determinations, an accuracy of over 95%. We examined field-collected females and found (as did Scott et al. 1993) that the histological parameters for detection of multiple blood meals were similar to those of the laboratory-reared females.

In a field study reported elsewhere (Wekesa et al. 1995), we examined 134 field-collected blood-engorged *An. freeborni* females for multiple blood meals, 9.7% (13/134) of the females were found to have taken at least 2 blood meals. This estimate is higher than any other reported multiple blood feeding event for resting populations of *An. freeborni*. In studies by Washino and Tempelis (1967), McHugh (1989) and Wekesa et al. (1992), conducted in the Sacramento Valley, < 2 % of the *An. freeborni* examined were estimated to have taken multiple blood meals. Yet our findings may still underestimate the number of host contacts per gonotrophic cycle of the mosquitoes for several reasons, e.g., when the interval between meals is so brief (< 1 hour) that meals mix (meal mixing was observed by Briegel and Horler 1993), if the entire blood meal is digested beyond detection, or if the first meal has been digested and excreted. Also, the number of host contact in *An. freeborni* may be higher than what we found due to the gonotrophic cycle for this species which is 4 days for parous and 6 days for nulliparous females (McHugh 1989). The gonotrophic cycle of 4-6 days for *An. freeborni* is long compared to tropical species e.g. *Anopheles gambiae* Giles (3-4 days, Gillies and Wilkes 1965) and *Anopheles albimanus* (2-4 days, Rodriguez et

al. 1992).

The current conventional wisdom of one blood meal in a single gonotrophic cycle (gonotrophic concordance) implies that *An. freeborni* with such a long gonotrophic cycle will have fewer host contacts in its lifespan. However, knowledge that this species takes multiple blood meals increases the frequency of vector-host contact in a given gonotrophic cycle and may enhance its ability to transmit diseases.

Host defensive behavior (Edman and Scott 1987) and nutritional status of the vector (Klowden 1988, Breigel and Horler 1993) are among factors that may influence multiple blood feeding. Female mosquitoes that are not often provided with a carbohydrate (sugar) meal tend to take multiple blood meals (Klowden 1988). Unlike Briegel and Horler (1993), we provided mosquitoes from our lab study with a sugar meal during the experiment, hence the observed results were not limited by inadequate nutrition. Overall, we found that the histological technique is accurate and reliable in detecting multiple blood meals in *An. freeborni*. The application of this technique to other anopheline vectors may provide a better understanding of their frequency of multiple blood feeding, and specifically, provide information for accurate determination of their vectorial capacity.

ACKNOWLEDGEMENTS

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