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of the

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California Mosquito and Vector Control Association, Inc.

January 27 thru January 30, 1991

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California Mosquito and Vector Control Association, Inc.

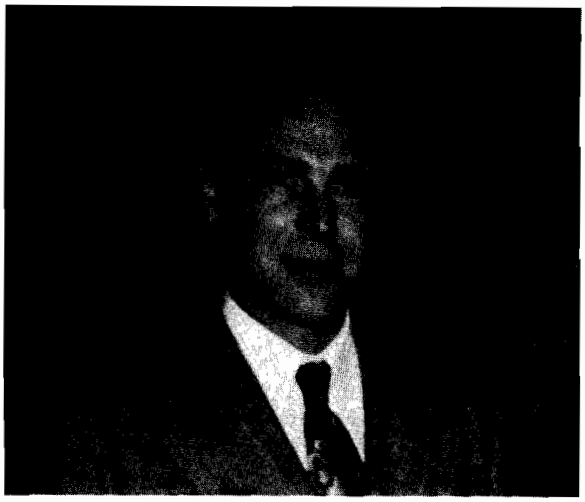
Volume 59 **January 28 thru January 31, 1990**

IN MEMORIUM

CHARLES DONALD GRANT

1919 - 1991

Past President and Honorary Member
of the California Mosquito and Vector Control Association



C. Donald Grant died on September 23, 1991, in Salinas, California, after a long and distinguished career as a biologist-entomologist. Don was born on October 9, 1919, in Oregon, and moved to San Francisco at an early age when his father accepted a teaching position there. Never one to put off a decision, he announced to his parents at age six that he wanted to be a biologist.

California was truly a golden state in the 1920s. Its streams were clean, its forests were healthy, its mountains were spectacular, and it offered a pristine natural environment. Don's father introduced him to the wonderful world of the Sierras. He learned how to camp and fish, and, most importantly, how to observe. Don became a woodsman and a naturalist. In addition to his knowledge of the insects, Don developed a vast store of knowledge of the birds, mammals, and natural flora of California. He was a talented artist and his line drawings of insects and watercolor paintings of shells, birds and native plants were superb. He was interested in all the biological phyla, but was an entomologist foremost.

In high school Don was introduced to the world of sports. He excelled in wrestling and football, and following graduation from high school he entered Stanford University on an athletic scholarship. He was a blocking halfback on the freshman football team and a member of the university boxing team. He was an outstanding boxer and was university heavyweight champion during his junior and senior years. He also received the annual Gene Tunney award for fine character and boxing ability in his sophomore, junior and senior years.

Don received his B.A. degree in the School of Biological Sciences in June of 1942, and promptly volunteered for military service. He was inducted into the Army Medical Service where he performed duties as a medical laboratory technician. He served in the European Theater of Operations and participated in the Normandy invasion. He was assigned to a medical battalion and remained in France until his separation from the U.S. Army in 1945.

Don returned to Stanford and received his

M.A. degree in 1947. He continued his graduate studies in entomology until the summer of 1950 when he was hired to organize a new mosquito abatement district. A decision had been made to consolidate the Pulgas MAD and the Three Cities MAD into a single district to be known as the San Mateo County Mosquito Abatement District. After the mosquito abatement district was established in Burlingame, the Trustees of the new organization prevailed upon Don to be the manager. This was his introduction to the old California Mosquito Control Association (CMCA) and a new world of challenges.

Don managed the San Mateo County MAD for 25 years. He spent considerable time at the beginning working with Harold F. Gray, Manager of the Alameda County MAD, Richard F. Peters, Chief, Bureau of Vector Control, California State Department of Public Health, and John R. Walker, Vector Control Specialist, Bureau of Vector Control, California State Department of Public Health. From Gray he learned the elements of managing a district; Walker trained him in editing and his close relationship with Peters resulted in the molding of a progressive and highly successful post-war California mosquito control program.

Other special friendships he formed within the Association included those with Theodore G. Raley, Manager of Consolidated MAD; W. Don Murray, Manager of Delta MAD; and Howard R. Greenfield, Manager of Northern Salinas Valley MAD. Each of these managers became integral members and were instrumental in shaping and directing the CMCA during their careers.

Don's association with the CMCA was mutually beneficial. The CMCA had emerged into a new pest insect control era in 1950. New insecticides and dispersing equipment, in addition to area treatment methods developed by the Army and Navy, were available. New MADs were being established and men with academic backgrounds in entomology and professional experience were being hired as managers and entomologists. Don knew that mosquito breeding could be controlled, and believed that all MADs, under the direction of a central (CMCA) guidance and with the support of the Bureau of Vector Control in the State Department of Public Health, could be successful. Having a part in accomplishing this vitally important statewide operation appealed to his professional interest and competitive nature.

For many years Don Grant's name and the CMCA were synonymous. In fact, Don devoted his life to the California mosquito control program. Beginning in 1950 and continuing through 1976, he knew every manager and all of the entomologists in the CMCA. Don held a variety of staff positions and was instrumental in developing numerous facets of the CMCA program. During his career he was Chairman and/or Member of the Budget; Education and Publicity; Forms, Records and Statistics; Legislative; Local Arrangements; Membership; Nominating; Program; Publications; Resolutions; Research; Survey Methods and Ecology; and the William B. Herms Award committees. He was also Moderator or Panelist for several CMCA Symposia. In 1953 he served as Vice-President of the CMCA, and he was elected President in 1954. In addition, he was Editor of sixteen volumes (Vol. 23-29, 44-52) of the *Proceedings and Papers of the California Mosquito Control Association*.

Don's military training as a medical technician added a new dimension to his biology background. He became interested in insect physiology and the importance of environmental factors in insect nutrition. Following his departure from San Mateo in 1975, he moved to Salinas and was employed by the Northern Salinas Valley Mosquito Abatement District. While there Don set up a water evaluation program to compare water quality of the permanent mosquito breeding sources in the District with fluctuations in the composition and numbers of the local mosquito populations annually. He also accepted the responsibility for editing the *Proceedings of the California Mosquito and Vector Control Association* until his retirement in 1984.

Don never really retired. He delighted in challenges because they made him think. One of the projects he took on was developing a synthetic media for mites for a company that wanted to develop an anti-allergenic serum for patients who were allergic to house-dust mites. For this a colony of American house-dust mites that had been fed only on a synthetic diet was required. Don was able to synthesize the essential foods and develop a dietary formula that would sustain a "pure" colony.

Don was a member of Sigma Xi, the American Mosquito Control Association, the Entomological Society of America, the Pan-Pacific Entomological Society, and the Society of Vector Ecologists. He was a green thumb gardener who specialized in the growing of orchids and nurturing fruit trees. His other recreational pleasures consisted of supporting

nearly two dozen cats and following his favorite sports teams.

Survivors include his mother, Madge Grant of San Francisco; a brother, Douglas of Mill Valley; a daughter, Donna Ruby of Randle, Washington; and two granddaughters. His wife of 49 years, Mary, predeceased him by four months.

Among the survivors one would have to list the legion of CMCA/CMVCA personnel whom he advised, trained, or otherwise influenced. He set many of the goals and developed numerous procedures for the association. We are all beholden to him in good part for whatever successes have resulted from our association with the CMVCA.

Don was not a perfectionist himself but he would not accept anything less than perfection and could not conceive any reason why things should not be done correctly from the onset. He was opinionated, but always favored progress. He was stubborn, but he understood his own limitations. He revelled in challenges and always tried to improve ongoing programs. No problem was too great; if he hadn't solved it, it was because time had run out. He was never too busy to help others, never turned down a request and expected only an expression of appreciation in return.

It is not possible to measure Don Grant's influence on the development and innate strength of the CMVCA because he produced over a long period of time through example and advice rather than by legislation. The scientific community has lost a wonderful naturalist, an outstanding entomologist, and an extremely valuable leader.

Wesley R. Nowell
Marina, California

Howard R. Greenfield
Salinas, California

1991 Proc. Calif. Mosq. Vector Control Assoc. 59: 4-9

SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1990

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This brief report summarizes arbovirus surveillance activities during 1990 and is the 21st report to the California Mosquito and Vector Control Association (CMVCA) 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 CMVCA and the CMVCA Research Foundation; county and local public health departments; the California Department of Food and Agriculture; physicians and veterinarians throughout California; and three branches of the California Department of Health Services - the Infectious Disease Branch, the Environmental Management Branch, and the Viral and Rickettsial Disease Laboratory of the Division of Laboratories.

Announcements about the program were disseminated from February to May, and 29 weekly bulletins (April 9 - December 21) were widely distributed during the season to provide detailed data. Many recipients now receive these via facsimile. We hope that eventually all recipients of the bulletin will have FAX equipment to receive reports as quickly as possible. In addition to the weekly bulletins, positive findings are telephoned immediately to the agency which submitted the mosquito pools or sentinel chicken sera.

Clinical and laboratory surveillance for human and equine cases of encephalitis, meningoencephalitis and meningitis detected only two cases of St. Louis encephalitis (SLE). This is in contrast to the 1989 season when 29 confirmed or presumptive-positive human cases of SLE were found. The first case was in a 19-year old man from Porterville, Tulare County, who probably acquired his infection from mosquitoes at a canal (Porter Slough) just east of his home. Onset of illness was 9/14/90.

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[5]California Mosquito and Vector Control Association, 197 Otto Circle, Sacramento, California 95822.

Unfortunately, serum samples taken 9/18/90 and 10/8/90 were not received for testing in the public health laboratory until October, so notification of the case was greatly delayed. The area was not part of an organized mosquito abatement program.

The second case was a 28-year old man with onset approximately 9/1/90. This individual had recently traveled in Texas, left there 8/31/90, stopped briefly in the Colorado River area of California/Arizona, and then returned home 9/2-3/90 where he became acutely ill and was hospitalized in Los Angeles. The place of contraction most likely was at the Colorado River site but this could not be determined exactly. Only a single blood sample obtained on 9/19/90 was available for testing. However, high SLE-IgM antibody, and both SLE-IgM and IgG antibodies in a cerebrospinal fluid sample substantiated the diagnosis.

There were several other suspect cases of SLE, in which SLE antibodies were detected. However, current infection was ruled out by careful, repeated testing which showed no changes in antibody titers and an absence of IgM antibodies. The occurrence of group B arbovirus antibodies which cross-react with SLE (Japanese B encephalitis, dengue, yellow fever, etc.) in immigrants or travelers who may have been naturally infected or received Japanese B or yellow fever vaccines in the past, complicates the interpretation of test results for SLE. If these factors are not considered, it may lead to false-positive test interpretations and case reports.

There were no confirmed cases of western equine encephalomyelitis (WEE) in humans or horses during 1990. This was the first year since the 1930's with no evidence of WEE activity.

Tests were done by the VRDL on 5,262 mosquito pools containing 222,636 mosquitoes (Table 1). The majority of the pools (73.7%) were submitted from Los Angeles, Riverside, Kern, and Orange Counties. *Culex tarsalis* Coquillett made up 54.8% of the pools, *Culex pipiens* L. complex 38.4%, and *Culex stigmatosoma* Dyar, *Aedes melanimon* Dyar and *Aedes vexans* (Meigen) the remainder. A total of 47 viral isolates were made by the VRDL (Tables 2 and 3). These included 18 SLE and 29 California (CEV) serogroup viruses. Additional pools from southern California (935 pools) and Kern County (70 pools) were tested by the U.C. Berkeley Arbovirus Laboratory, yielding Hart Park, Turlock and other viral isolates, but no additional SLE or WEE isolates. The VRDL program is now limited to tests for SLE, WEE and CEV group viruses, and has discontinued detection of other viruses. All SLE isolates were from *Culex tarsalis* pools, except two from the *Culex pipiens* complex. As usual, all CEV group isolates were from pools of *Aedes melanimon*.

Sentinel chicken flocks were located at 71 sites covering most endemic areas of the state. Serum samples were collected and tested for SLE and WEE antibodies monthly from May through November by the VRDL, and during the winter period for selected flocks by the U.C. Berkeley Arbovirus Laboratory (Tables 4 and 5). Only low-level SLE activity was detected in the San Joaquin Valley (5% seroconversion) and southern California (13% seroconversion). Most seroconversions occurred in late summer and fall, and no evidence of WEE activity was found (over 7,600 chicken sera were tested).

The surveillance effort during 1990 was the largest in the past 20 years. The surveillance program must be continued during the 1991 season, despite the low level of activity during 1990. The cyclic occurrence of virus activity continues to be unpredictable and there is an annual possibility of an epidemic.

Acknowledgements.

We thank Robert Kang, Dave Sasse, Kim Felder and Bernardo Cuevas for special assistance in the testing of mosquito pools and chicken sera. We are also grateful for the help of many other staff members of the Viral and Rickettsial Disease Laboratory, Environmental Management Branch and Infectious Disease Branch of the California State Department of Health Services; the Arbovirus Field Station and the Arbovirus Research Unit, School of Public Health, University of California, Berkeley; participating local mosquito control agencies; local health departments; the California Department of Food and Agriculture; and physicians and veterinarians who submitted specimens from suspect clinical cases. We especially thank the California Mosquito and Vector Control Association for providing some of the supplies needed for laboratory tests.

Table 1. Number of mosquitoes and pools tested from throughout California during 1990.

County	Cx. tarsalis		Cx. pipiens		Cx. stigmatosoma		Ae. melanimon		Ae. vexans		Totals	
	mosq.	pools	mosq.	pools	mosq.	pools	mosq.	pools	mosq.	pools	mosq.	pools
Butte	2154	44					2950	59			5104	103
Contra Costa	100	3					68	2			168	5
Glenn	1062	23									1062	23
Imperial	6193	143	1400	33					31	1	7624	177
Kern	16298	367	10558	230			1386	54			28242	651
Lake	723	15									723	15
Los Angeles	23920	608	31036	770	2918	90					57874	1468
Marin	144	5	39	2	38	2					221	9
Merced	662	15	61	2			569	12			1292	29
Orange	5602	143	15342	372	22	1					20966	516
Riverside	36872	811	16287	380	1336	50					54495	1241
Sacramento	5830	122	125	3	12	1					5967	126
San Bernardino	3998	105	1934	45	468	18					6400	168
San Diego	825	28	2076	47	793	20					3694	95
Sonoma	72	2	60	2	68	2					200	6
Stanislaus	1047	24	3261	70			1094	24			5402	118
Sutter	8406	184					662	15			9068	199
Tulare	1443	38	2422	58							3865	96
Ventura	1091	22	324	8	31	1					1446	31
Yolo	5958	123					50	1			6008	124
Yuba	2715	60					100	2			2815	62
Totals	125115	2885	84925	2022	5686	185	6879	169	31	1	222636	5262

Table 2. Viral isolates from mosquitoes collected throughout California during 1990.

Mosquito species	County	Virus isolates			
		WEE	SLE	CEV	Totals
Culex tarsalis	Imperial		6		6
	Los Angeles		1		1
	Riverside		7		7
	San Bernardino		1		1
	Tulare		1		1
Culex pipiens	Tulare		2		2
Aedes melanimon	Butte			17	17
	Kern			7	7
	Stanislaus			2	2
	Sutter			3	3
	Totals	0	18	29	47

Table 3. Complete listing of 1990 positive mosquito pools.

POOL NO.	SPECIES	DATE	PLACE	CO	LONG	LAT	COLLECTOR	MOSQ	POOL	WEE	SLE	CEV
** BUTTE COUNTY **												
BUCO 3	AE MEL	06/04/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 4	AE MEL	06/04/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 5	AE MEL	06/04/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 6	AE MEL	06/04/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 7	AE MEL	06/04/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 8	AE MEL	06/04/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 9	AE MEL	06/04/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 11	AE MEL	06/04/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 13	AE MEL	06/11/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 14	AE MEL	06/11/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 15	AE MEL	06/11/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 16	AE MEL	06/11/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 17	AE MEL	06/11/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 18	AE MEL	06/11/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 25	AE MEL	06/18/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 33	AE MEL	06/18/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
BUCO 81	AE MEL	07/17/90	GRIDLEY	BUCO	12140	3920	THOMAS	50	1	0	0	1
							** Subtotals **	850	17	0	0	17
** COACHELLA VALLEY **												
CHLV 812	CX TAR	08/21/90	MECCA	RIVR	11605	3335	DURSO	50	1	0	1	0
CHLV 814	CX TAR	08/21/90	MECCA	RIVR	11605	3335	DURSO	50	1	0	1	0
CHLV 816	CX TAR	08/21/90	MECCA	RIVR	11605	3335	DURSO	50	1	0	1	0
CHLV 817	CX TAR	08/21/90	MECCA	RIVR	11605	3335	DURSO	31	1	0	1	0
CHLV 821	CX TAR	08/21/90	MECCA	RIVR	11605	3335	DURSO	50	1	0	1	0
CHLV 853	CX TAR	08/28/90	MECCA	RIVR	11605	3335	DURSO	50	1	0	1	0
CHLV 861	CX TAR	09/04/90	MECCA	RIVR	11605	3335	DURSO	50	1	0	1	0
							** Subtotals **	331	3	0	3	0
** DELTA **												
DLTA 19	CX TAR	09/18/90	VISALIA	TULE	11920	3620	DELTA VCD	50	1	0	1	0
DLTA 28	CX PIP	09/18/90	VISALIA	TULE	11920	3620	DELTA VCD	40	1	0	1	0
DLTA 30	CX PIP	09/18/90	VISALIA	TULE	11920	3620	DELTA VCD	47	1	0	1	0
							** Subtotals **	137	3	0	3	0
** IMPERIAL **												
IMPR 257	CX TAR	09/12/90	HEBER	IMPR	11535	3345	IMPR CO H D	44	1	0	1	0
IMPR 258	CX TAR	09/12/90	SEELY	IMPR	11540	3250	IMPR CO H D	50	1	0	1	0
IMPR 261	CX TAR	09/12/90	SEELY	IMPR	11540	3250	IMPR CO H D	50	1	0	1	0
IMPR 262	CX TAR	09/12/90	SEELY	IMPR	11540	3250	IMPR CO H D	50	1	0	1	0
IMPR 263	CX TAR	09/12/90	SEELY	IMPR	11540	3250	IMPR CO H D	50	1	0	1	0
IMPR 264	CX TAR	09/12/90	SEELY	IMPR	11540	3250	IMPR CO H D	50	1	0	1	0
							** Subtotals **	294	6	0	6	0
** KERN **												
KERN 11	AE MEL	05/07/90	BUTTONWILLOW	KERN	11925	3530	ARBOVIRUS F S	50	1	0	0	1
KERN 12	AE MEL	05/07/90	BUTTONWILLOW	KERN	11925	3530	ARBOVIRUS F S	50	1	0	0	1
KERN 108	AE MEL	07/16/90	LOST HILLS	KERN	11935	3545	ARBOVIRUS F S	50	1	0	0	1
KERN 111	AE MEL	07/16/90	LOST HILLS	KERN	11935	3545	ARBOVIRUS F S	50	1	0	0	1
KERN 112	AE MEL	07/16/90	LOST HILLS	KERN	11935	3545	ARBOVIRUS F S	50	1	0	0	1
KERN 152	AE MEL	07/18/90	BAKERSFIELD	KERN	11910	3520	ARBOVIRUS F S	36	1	0	0	1
KERN 304	AE MEL	08/27/90	BUTTONWILLOW	KERN	11925	3530	ARBOVIRUS F S	50	1	0	0	1
							** Subtotals **	336	7	0	0	7
** SAN BERNARDINO **												
SANB 46	CX TAR	09/14/90	NEEDLES	SBND	11435	3450	MIAN	34	1	0	1	0
							** Subtotals **	34	1	0	1	0
** SOUTHEAST **												
SOUE 27	CX TAR	05/07/90	SEPULVEDA BASIN	L.A.	11830	3410	SPOEHEL	50	1	0	1	0
							** Subtotals **	50	1	0	1	0
** SUTTER-YUBA **												
SUYA 253	AE MEL	09/19/90	TROWBRIDGE	SUTE	12130	3855	LEMENAGER	50	1	0	0	1
SUYA 265	AE MEL	09/26/90	WINSHIP SCHOOL	SUTE	12150	3905	LEMENAGER	34	1	0	0	1
SUYA 268	AE MEL	09/26/90	SUTTER NATL W L	SUTE	12145	3905	LEMENAGER	50	1	0	0	1
							** Subtotals **	134	3	0	0	3
** TURLOCK **												
TRLK 22	AE MEL	08/06/90	GRAYSON	STAN	12110	3735	SELVIOGE	40	1	0	0	1
TRLK 51	AE MEL	09/10/90	CROWS LANDING	STAN	12100	3745	WALKER	50	1	0	0	1
							** Subtotals **	90	2	0	0	2
							TOTALS	2256	47	0	18	29

Table 4. SLE results of chicken sera testing in northern California during 1990.

Flock Location	Number SLE positive/number tested (percent positive)							
	Apr 30-	May 28- Jun 2	Jun 25- Jun 29	Jul 23- Jul 27	Aug 20- Aug 24	Sep 17- Sep 21	Oct 15- Oct 19	Nov 1- Nov 14
Northern California								
Shasta, Cottonwood	NB	0/19	0/20	0/20	0/20	0/20	0/19	.
Shasta, Pine Grove MAD	NB	0/17	0/17	0/17	0/16	0/16	0/16	0/16
Tehama, MAD Office	NB	0/20	0/19	0/20	0/20	0/19	0/19	.
Butte, Chico	NB	0/18	0/18	0/20	0/18	0/18[b]	.	.
Butte, Honcut	NB	0/20	0/20	0/18	0/18	0/18	.	.
Butte, Gray Lodge	NB	0/20	0/20	0/20	0/20	0/20	.	.
Glenn, Willows	NB	0/20	0/20	0/20	0/20	0/19	0/20	.
Colusa, Reading Oil	NB	0/18	0/17	0/17	0/17	0/17	0/17	.
Sutter-Yuba, P.V. Ranch	NB	0/18	0/18	0/18	0/18	0/18	0/18	.
Sutter-Yuba, Dean'a	NB	0/20	0/20	0/20	0/20	0/20	0/20	.
Sutter-Yuba, Barker	NB	0/18	0/17	0/17	0/17	0/17	0/17	.
Sacramento-Yolo, Merritt	NB	0/15	0/15	0/12	0/10	0/10	0/10	.
Sacramento-Yolo, Natomas	NB	0/19	0/20	0/18	0/17	0/18	0/18	.
Sacramento-Yolo, Elk Grove	NB	0/19	0/20	0/20	0/20	0/20	0/20	.
Lake, MAD Office	NB	0/19	0/19	0/19	0/19	0/19	0/19	.
Marin-Sonoma, W. Santa Rosa	NB	0/17	0/14	0/16	0/14	0/14	.	.
Solano, Grizzley Island	NB	.	0/20	0/18	0/18	0/18	0/18	.
Santa Clara, Gilroy	NB	0/20	0/18	0/19	0/18	0/19	0/19	.
No. California Totals		0/317	0/332	0/329	0/320	0/320	0/250	
San Joaquin Valley								
San Joaquin, Thornton	NB	0/19	0/17	0/14	0/12	0/12[c]	0/12[d]	.
San Joaquin, Escalon	NB	0/18	0/17	0/13	0/18	0/17	0/17[d]	.
Eastside, Oakdale	NB	0/19	0/20	0/19	0/20	0/20	0/18	.
Turlock, Vitoria	NB	0/23	0/10	0/9	0/11	0/10	0/11	.
Turlock, Modesto	NB	.	0/12	0/12	0/12	0/12	0/12	.
Merced, Gustine	NB	0/19	0/18	0/18	0/18	0/18	0/18	0/18
Merced, Veldhaus	NB	0/18	0/18	0/18	0/18	0/10	0/10	0/10
Madera, Madera	NB	0/20	0/20	0/18	0/19	0/17	0/17	0/18
Fresno Westside, Mendota Ref.	NB	0/19	0/20	0/20	0/17	0/19	0/20	0/18
Consolidated, Friant Rd.	NB	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Kings, MAD Office, Hanford	NB	0/19	0/19	0/19	0/19	0/19	0/19	0/19
Delta, Kingsburg GC*	0/20	0/20	0/20	0/14	4/13(31)	4/13(31)	7/13(54)	8/12(67)
Delta, Woodlake	NB	0/20	0/20	0/20	0/20	0/20	0/19	0/20
Dslta, Rocky Pt., Porterville*	0/20	0/20	0/20	0/19	0/19	0/19	0/18	0/18
Tulare, MAD Office	NB	0/18	0/15	0/17	0/17	2/17(12)	3/17(18)	3/17(18)
West Side, Belridge	NB	0/19	0/19	0/19	0/19	0/19	0/19	.
West Side, Maricopa	NB	0/17	0/17	0/17	0/17	0/17	0/17	.
Delano, Teviston*	0/20	0/19	0/20	0/20	0/19	1/20(5)	7/19(37)	9/19(47)
Kern, Wasco*	0/20	0/19	0/19	0/18	0/16	0/16	0/16	0/18
Kern, F.C. Tracy*	0/18	0/17	0/17	0/18	0/16	0/16	0/16	0/18
Kern, Buttonwillow*	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Kern, Wildlife Refuge*	2/21(10)[a]	2/21(10)	2/21(10)	0/8	0/7	0/7	0/7	0/7
Kern, Oildale*	0/20	0/20	0/19	0/15	0/14	0/14	0/14	0/14
Kern, John Dale*	0/20	0/19	0/18	0/16	0/18	0/15	0/15	0/15
Kern, River Bottom*	0/20	0/20	0/20	0/17	0/16	0/18	0/18	0/18
San Joaquin Totals	2/199	2/463(<1)	2/456(<1)	0/418	4/413(1)	7/403(2)	17/400(4)	

* New chickens put out in late January.
a. 1 seroconversion on March 5 and 1 on April 2.
b. Bled 9/14.
c. Bled 9/24.
d. Bled 10/23.
NB = not bled.

Table 5. SLE results of chicken sera tested in southern California during 1990.

Flock Location	Number SLE positive/number tested (percent positive)									
	May 14-May 17	Jun 11-Jun 15	Jul 9-Jul 13	Aug 6-Aug 10	Sep 3-Sep 7	Oct 1-Oct 5	Oct 29-Nov 2	Nov 26-Nov 30	Dec 27	Jan 22-Jan 24
Goleta, Gray's Ranch	0/20	0/18	0/18	0/19	0/18	0/19	0/19			
Ventura, Fillmore Hatchery,Oxnard	0/20	0/19	0/20	0/20	0/20	0/20	0/20			
Ventura,Hill Canyon,Thousand Oaks	0/10	0/10	0/10	0/10	0/10	0/10	0/10			
Ventura, Simi Valley	0/10	0/10	0/10	0/10	0/10	0/10	0/10			
Los Angeles (ANTV), Lancaster	0/18	0/18	0/18	0/18	0/18	0/18	0/18			
Los Angeles (LOSA), La Brea	0/19	0/19	0/19	0/20	0/19	0/17	0/17			
Los Angeles, Santa Fe, Irwindale	0/20	0/20	0/20	0/20	0/19	0/19	0/19			
Los Angeles (SOUE), Harbor Lake	0/19	0/19	0/18	0/18	0/18	0/18	0/18			
Los Angeles (SOUE), Sepulveda	0/20	0/20	0/20	0/19	0/19	0/19	1/19(5)			
Long Beach, El Dorado	0/20	0/20	0/19	0/19	0/19[a]	0/19	0/18	1/18(6)	1/18(6)	1/18(6)
Orange, 3 mini-flocks	.	0/15	0/14	0/12	0/12	0/11	0/11			
Orange, Duck Club	0/20	0/20	0/15	0/15	0/15	0/15	1/14(7)			
San Bernardino, Flood Control	0/11	0/10	0/11	0/13	0/14	0/14	0/14			
San Bernardino, Needles	0/14	0/7	0/11	0/13	0/11	killed	.			
West Valley, Chino, Tuenissen	0/18	0/19	0/20	0/20	0/19	0/20	0/20			
Northwest, Corona	0/23	0/22	0/24	0/20	0/21	3/21(14)[b]	4/21(19)	3/19(16)		
Coachella Valley, Palm Desert	0/20	0/20	0/12	0/9	0/9	0/9	.	.		
Coachella Valley, Indio	0/19	0/19	0/16	0/16	0/16	0/15	0/14	0/14	0/14	0/14
Coachella Valley, Mecca	0/20	0/20	0/15	0/15	1/15(7)	8/15(53)	8/15(53)	8/15(53)	8/15(53)	8/15(53)
Coachella Valley, Thermal	0/20	0/20	0/19	0/18	1/18(6)	1/18(6)	1/18(6)	1/18(6)	1/18(6)	1/18(6)
Coachella, Mecca, 5 mini-flocks	0/50	0/50	0/49	0/50	12/50(24)	18/50(36)	1/50(2)[c]	3/47(6)	2/46(4)	2/45(4)
Imperial, Drew Rd., Seeley	0/20	0/20	0/20	0/19	12/19(63)	19/19(100)	19/19(100)			
Imperial, Keffer Rd., Holtville	0/23	0/21	0/17	0/14	8/15(53)	13/14(93)	14/15(93)			
Imperial, Bard*	.	0/10	0/10	0/10	0/8	1/8(13)	1/8(13)			
Imperial, Palo Verde**	.	0/9	0/8	0/4	0/4	.	0/4			
San Diego, Tijuana River	0/11	0/11	0/11	0/11	0/11	0/11	0/11			
San Diego, Lakeside	stolen	0/15	0/14	0/14	0/15	0/15	0/14			
San Diego, Metate	0/8	.								
San Diego, Guajome Park	0/12	0/12	0/10	0/10	0/10	0/10	0/9			
So. California Totals	0/465	0/493	0/468	0/456	34/452(8)	63/434(15)	50/425(12)			

* Bled 5/24, 6/27, 7/25, 8/29, 9/26, 10/24.
** Bled 5/31, 6/29, 7/31, 8/31, 10/29.
a. Bled 9/10.
b. Bled 10/8.
c. All positive chickens but 1 replaced in October.

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POPULATION GROWTHS IN CALIFORNIA AND THEIR IMPACTS ON DISEASE TRANSMISSION

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When Chuck Beesley asked me to talk about "The Future Impact of Population Growths in the Central Valley on Disease Transmission" he indicated he had just read the recent book by Mike Service, *Demography and Vector-Borne Diseases*. His idea for this presentation had arisen from the chapter "Changes in Transmission Patterns of Mosquito-Borne Viruses in the U.S." that Marilyn Milby and I had written. We presented a primarily retrospective view of the impact of population and environmental changes on the occurrence of vector-borne diseases.

Today, I will use past experiences and current knowledge of vector-borne diseases to predict what may occur in California as our population grows and is redistributed geographically. I changed the title of this talk so it now covers all of California rather than being limited to the Central Valley. This change reflects my belief that we must consider the entire state as one unit of concern and share the responsibilities for its future.

Population Database.

My population data are from a report by the California Department of Finance entitled "Population Projections for California Counties, 1980-2020 with Age/Sex Data to 2020" and a supplemental issue entitled "Household Projections for California Counties, 1985-2020". Extension of my concerns to the year 2020 should interlock this presentation with that of Mary Heim who preceded me on "Population Changes in California" and those that follow by Dr. Reisen on the effects of possible climatological changes on vector populations and Drs. Hardy and Barr on possible emerging arboviral and other vector-borne diseases.

Population Projections.

I will begin with a quick summary of the population changes that are predicted to occur in California. I will then discuss potential relationships of these changes to specific diseases and problems that may be encountered in prevention of these diseases.

An almost unbelievable population growth is predicted for California. Our population was seven million in 1940 and increased to 29 million by 1990. It is predicted to soar to 40 million by the year 2020 and to 50 million by the year 2050. Between now and 2020, five counties in southern California will increase by seven million people and reach a total of 22 million. For comparison, this increase equals the total population of California in 1940 and is twice the population currently in the Central Valley of California.

At the same time the Central Valley will almost double its population from 4.7 million today to 8.5 million in 2020.

I do not have figures on the urban or rural distribution of these populations, but I must assume it will follow past trends towards increased urbanization. Estimates have been made of the percent increase of households in different regions between 1985 and 2020. A large part of these increases will be in areas that currently are agricultural or undeveloped. These are areas where vector-borne diseases are endemic today or will become so once surface water is introduced to meet the domestic and recreational needs of the population. I do not know where the water will come from, but I expect the populations will not go to where the limited water is available. They will undoubtedly expect it to be brought to them. I doubt that the current water development projects in California are sufficient or were designed to meet these needs.

I will go one step further to bring to your attention the resources currently being utilized for vector control in California. These are derived from the Year Books of the CMVCA. Today you are providing services for approximately 80% of the state's population of 29 million people. If there is any relationship of your activities to the state's population growth, you or some other agency will be providing a similar service to almost 40 million people by 2020 and the budget could be in the vicinity of one billion dollars. Furthermore, I predict that some level of vector and disease surveillance and control will be operating statewide, including recreational areas that now are sparsely inhabited. This will represent a major extension from the current coverage.

Relationships of Selected Endemic Diseases to Population Changes.

The projected population growth in California will provide many opportunities for endemic vector-borne diseases to become important and opportunities for new diseases to emerge. The diseases that most concern me already occur in California or are introduced frequently. They are western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE), Colorado tick fever, Lyme disease, tick-borne relapsing fever, plague, tularemia, dog heartworm and malaria. They all undoubtedly still will be here in the 21st century as they are firmly established in a cycle between wildlife hosts and mosquito, tick or flea vectors over extensive regions of California. Malaria is the exception, but is introduced so frequently in travelers or immigrants that it has to be considered to be here constantly.

I will use WEE as a model for the maintenance cycle for most of the preceding diseases and remind you that man is only an accidental victim of contact with this virus and is of no importance as a source of infection for vectors. With a few modifications, this type of infection chain occurs for all of the above diseases except malaria. In the case of WEE, young children are the most susceptible age groups. By the year 2020, there will be over five million children in this age group in California, and all of them will be susceptible and there will be no vaccine.

Next, I will use SLE to illustrate the interrelationships of population changes to increased risk of disease. We know that persons over 60 years of age are the most likely to develop encephalitis when infected with SLE virus. Evidence of this came from an epidemic in Houston, Texas in 1964. This population had very little prior exposure to SLE so few were immune. Old people were at unusually high risk of the disease and death. The age distribution of SLE in the non-immune population in Houston, Texas, can be compared with that in Kern County, California from 1943 to 1952. This was a period when a high proportion of the California population in the Central Valley was being infected every year and 40% or more of the older population had developed immunity. Again, it was clear that older people in Houston, who were not immune, were at very high risk of disease and in contrast, in Kern County, most of the cases were in children and older people with immunity had escaped.

The question is, what do we expect will occur if SLE virus remains endemic in California and the greatly enlarged population is exposed to infection? The age distribution of the 1985 population is quite different from that projected for 2020. By 2020, the baby boom population of early years will have moved up and entered the age group that is most susceptible to SLE. With the present low levels of SLE transmission in California, few individuals in this population will be immune. To give you a projection of what could happen in the future, we applied the attack rates from the epidemic in Dallas, Texas, in 1966 to the population projected for Los Angeles County in 2020. It resulted in over 2,000 clinical cases, which would be a catastrophe.

It also should be of concern that an increasing proportion of elderly persons as well as younger persons are moving into newly developed senior citizen communities or new population centers, many of which are located in former agricultural, foothill, desert and other underdeveloped areas. Frequently, these new communities are separated from older urban-suburban areas where vector control programs are established. Populations in these new environments are at increased risk of exposure to mosquitoes as vectors of encephalitis and to ticks and fleas that transmit Lyme disease and plague. You may have noted earlier that I included dog heartworm as a disease of concern. I believe that people will take their dogs with them and many will be taken into areas where infection is common. I believe there will be a need to develop effective vector control programs around such communities and to develop educational programs for individual protection.

I would be miss if I did not mention the 20 or more new viruses that have been found in Cali-

fornia, and that Dr. Hardy will discuss later. These infections were detected in wild animals or vectors. It remains to be seen if any of these will emerge as so-called "new diseases".

The Risk of Importation of Exotic Diseases.

I frequently am asked if there is a danger that exotic diseases may be introduced and the relationship of such events to increases in human population or the susceptibility of domestic animals. At least eight vector-borne exotic diseases in the world concern me if they are introduced into California: Japanese B encephalitis (JBE), Murray Valley encephalitis (MVE), Ross River polyarthritis (RRP), Rift Valley fever (RVF), Venezuelan equine encephalo-myelitis (VEE), yellow fever, dengue fever and malaria. Each could be introduced by an infected person, animal or vector. Projected population growths will increase the likelihood of such events. The unanswered questions are, will such intro-ductions be recognized and become established? I already have referred to malaria introductions and believe that introductions will occur inevitably into the Central Valley and foothill areas of the Sierras. A doubling of population is predicted to occur in the rice-growing area of the Sacrament Valley and foothill areas where *Anopheles freeborni* Aitken and *Anopheles punctipennis* (Say) prevail. The predicted human population increase will assure the avail-ability of a pool of malaria susceptibles. It is conceivable that malaria will become established as an endemic disease. This could be abetted by person-to-person transmission through direct blood exchange by needle and syringe, as is happening with AIDS.

JBE, MVE, RRP, VEE and RVF are other examples of infections that could be introduced. These infections currently prevail in Asia, Australia, Latin America and Africa, and I expect we have competent vectors and hosts in California. I am not so concerned about dengue fever and yellow fever, as a competent vector such as *Aedes aegypti* (L.) or *Aedes albopictus* (Skuse) would have to become established before these viruses could become established. This could occur in the event of global warming and major changes in our climate. An infected person, animal or vector could be the source for introduction on any of the above eight diseases. We already have had incidents where cases of JBE, VEE and dengue fever were detected in travelers entering California. Fortunately, none of these infections became established.

Social Changes That May Accompany Population Increases.

As a final area of concern, I foresee there will be an extension of demands for physical and social changes as population increases. Many of these will have a major effect on vector control programs.

Let me pinpoint five of these concerns:

1. Most communities do not recognize that the increased growth and density of populations is being paralleled by an aging and poorly designed capacity for disposal of waste water, sewage and solid wastes. Epidemics of SLE in urban areas have reflected that *Culex* vectors readily utilize as breeding and harborage sites the underground conduits, gutters and catch basins built for waste water disposal. It is unlikely that these thousands of miles of drainage and disposal facilities will be redesigned and replaced in the foreseeable future. In the future, these aspects of environmental impact statements must be reviewed by vector control and health agencies. Currently, the continuous and endless monitoring of existing sites of this type is a costly and labor intensive task in vector control programs in urban and suburban environments.
2. As populations increase in size and density, these urbanized societies will demand establishment of green belts, aquatic environments and other recreational sites within or adjacent to population centers. Such developments are generally accepted as a social necessity. Almost inevitably, such sites also serve as a haven for vector breeding and a habitat for hosts of vector-borne diseases. Again, environmental impact reviews must include input from vector control and health agencies.
3. The continuing expansion of urban-suburban developments into agricultural and previously underdeveloped habitats will increase exposure to encephalitis viruses, Lyme disease, plague and other diseases endemic in such environments. An effort must be made to assure there is a demand that a competent agency take the responsibility to educate these populations and protect them from the risk of vector-borne diseases.
4. Increased populations and urbanization will lead to vastly expanded recreational use of areas that are now wildlife habitats with low human population density and geographically separated from urban centers. We have

already seen this nationally and in California. Many vector-borne diseases prevail in such habitats (Lyme disease, tick-borne relapsing fever, Colorado tick fever, tularemia, the encephalitides, dog heartworm and potentially malaria and emerging diseases that are not yet identified). Most such areas do not have organized programs for vector control or disease surveillance. I predict there will be a demand for such programs by our future society. If this happens, it will result in some level of vector-borne surveillance and control statewide.

5. As a last concern, it is critical that there be an adequate surveillance system for all the diseases mentioned as well as for those that may emerge. You cannot protect a population form an undefined problem and develop an effective abatement program without an adequate knowledge of the epidemiology of the diseases of concern. The CMVCA has a committee charged to make reviews and recommendations regarding diseases such as encephalitis, malaria, Lyme disease and its area of concern can be expanded. Two years ago a budget of $340,000 to support five staff members was added to the Environmental Management Branch of the State Department of Health Services specifically to improve surveillance of Lyme disease. I am informed that several weeks ago the State Legislature and the Governor removed that $340,000 item from the 1991-1992 budget. I take this as a challenge to vector control agencies that are increasingly concerned with this disease and related problems. To my knowledge, you may be the only organized group in California that is free to be an advocate for reinstatement or initiation of such programs.

Conclusion.

In conclusion, I have brought to your attention a series of potential problems with vector-borne diseases and their control that I believe can be associated with predicted population growths in California. Time does not permit me to expand this frontier further. I hope that this and other presentations in the plenary session will convince you that there will be an increased need for vector control agencies to serve the population of California in the 21st century and to do it well.

EFFECTS OF CLIMATOLOGICAL CHANGE ON THE POPULATION DYNAMICS AND VECTOR COMPETENCE OF MOSQUITO VECTORS IN CALIFORNIA[1,2]

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ABSTRACT

The effects of global warming on the population ecology and vector competence of the mosquito vectors of human pathogens in California were reviewed, emphasizing studies on *Culex tarsalis* and western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses. Increasing global temperatures due to the greenhouse effect were projected to alter seasonality, preclude diapause induction, and increase population turnover rates by increasing the rate of immature development, shortening the gonotrophic cycle and decreasing adult survivorship. Increasing temperatures also were projected to reduce the length of the extrinsic incubation period of arboviruses, the susceptibility of *Cx. tarsalis* to virus infection, and the *Cx. tarsalis* population infection rate with WEE virus due to enhanced modulation.

The seasonality of *Cx. tarsalis* abundance and WEE and SLE virus infection rates in Kern County was compared to Coachella and Imperial Valleys which average 5° C warmer. *Culex tarsalis* abundance peaked at comparable temperatures (monthly mean = 25-30° C); however, temporal abundance patterns were markedly bimodal in southern California with vernal and autumnal peaks and unimodal in Kern County with a late summer peak. In contrast, both WEE and SLE virus infections in *Cx. tarsalis* and seroconversions in sentinel chickens occurred during similar mid- and late-summer months at both localities when ambient temperatures were warmest. Planned research will investigate how virus transmission can persist in southern California during the mid-summer decrease in *Cx. tarsalis* abundance.

[1]An expanded text of this presentation will be prepared by Dr. W.C. Reeves for publication as a Forum Article in the Journal of Medical Entomology.

[2]Research upon which this review was based was funded, in part, by Research Grant 5-R22-AI-03028 from the National Institute of Allergy and Infectious Diseases, Biomedical Research Support Grant 8-S07-RR-05441 from the National Institutes of Health, special funds for mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California, and supplemental funding from the Southern Region of the California Mosquito and Vector Control Association.

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EMERGING ARBOVIRAL DISEASES IN CALIFORNIA

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Members and guests of the California Mosquito and Vector Control Association. I am very pleased to have an opportunity to present a paper at this plenary session on "California Mosquito Control in a Changing Environment." This topic is both timely and important since the emergence and re-emergence of arboviral diseases, as well as infectious diseases in general, frequently are linked to environmental changes brought about by human activities (Morse 1990). Thus, the anticipated human-induced thermal warming of the earth may well be associated with the emergence of new arboviral diseases in California, and perhaps the resurgence of some old arboviral diseases that currently are under control.

What Are Emerging Viral Diseases?

Viruses are but one of several types of infectious disease agents that historically have been associated with so called "emerging diseases" in human populations and have resulted in untold millions of deaths. An emerging infectious disease, including viral diseases, can be defined as an apparently novel disease entity associated with major epidemics in human populations and/or epizootics in domestic animal populations. Characteristics of emerging viral diseases are: a) they appear to arise suddenly out of nowhere, b) they are frequently foreign to humans and/or to a geographical region, and c) they are often highly virulent, causing significant morbidity and mortality. The latter characteristic usually is associated with the lack of immunity in human and domestic animal populations to a genetic mutant of an indigenous virus or to a new virus introduced either from another geographic region or from local fauna (i.e., zoonosis). As emphasized by Morse (1990), the movement or "travel" of viruses from one geographical area to another and from animals to humans frequently can be attributed to human activities.

It is unknown what the first viral disease was to emerge in humans. However, the first arbovirus to be associated with an emerging disease apparently was yellow fever which appeared as a disease entity in the Yucatan in the 17th century, but probably originated in Africa or South America (Taylor 1951). Yellow fever virus and *Aedes aegypti* (L.) frequently were introduced by ships into port cities along the eastern seaboard and gulf coast of the United States, which resulted in major epidemics beginning in New York in 1668 and ending in New Orleans in 1905. The first arboviral disease to emerge in California appears to have been western equine encephalomyelitis (WEE) which has been a significant public health and veterinary problem since 1930 (Milby and Reeves 1990, Reeves 1990).

Some Viral Diseases That Have Emerged Worldwide in the Last 30 Years.

A review of the literature for the past 30 years clearly indicates that both non-arboviral and arboviral diseases are continuing to evolve worldwide. The "Hong Kong" influenza A (H3N2) virus arose in 1968, most likely via genetic reassortment between human and animal influenza viruses, and this resulted in a pandemic causing millions of deaths worldwide (Laver and Webster 1973). A new eye disease, acute hemorrhagic conjunctivitis with neurologic involvement, arose during 1969 in humans in Ghana, Africa, and subsequently spread throughout the world and is now a leading cause of eye disease in Near and Far Eastern countries (Kono 1975). The picornavirus (enterovirus 70) causing this disease may have come from ungulates (Kono et al. 1981).

A number of non-arboviral hemorrhagic fevers have arisen in humans during the last 30 years and have been associated with extremely high mortality rates. Examples are Lassa (Casals 1975) and Ebola (Murphy et al. 1990) hemorrhagic fevers which

occur in Africa. Under natural conditions, humans acquire Lassa virus from peridomestic rodents, but the source of Ebola virus has yet to be determined. Another important non-arboviral disease that emerged recently is, of course, acquired immune deficiency disease (AIDS), caused by the retrovirus, human immunodeficiency virus (HIV). Millions of people are now infected with this highly lethal virus which may have originated from monkeys.

Several arboviral diseases have either arisen anew or have moved into new geographical regions during the last 30 years. A disease similar to Japanese encephalitis (JE) was seen in humans and horses in Japan as early as 1871 and has been associated with major epidemics in Japan during this century, with birds and pigs serving as the major sources of virus for infection of *Culex tritaeniorhynchus* Giles (Monath 1990). JE became a significant public health problem in Southeast Asia in the 1960's and in India and Nepal in the 1970's, and is currently the most important arboviral encephalitide in the world. JE virus is a flavivirus, similar to St. Louis encephalitis (SLE) virus that occurs in California. Another flaviviral disease, Rocio encephalitis, arose in 1975 in Sao Paulo State, Brazil, as an explosive epidemic that continued until 1977 when the virus seemingly retreated back into the jungle, not to be seen again (Iversson 1988). The presumed mosquito vectors and vertebrate hosts of Rocio virus were not determined.

A mosquito-borne bunyavirus, Rift Valley fever (RVF), has caused epizootics in domestic mammals, primarily sheep and goats, in sub-saharan Africa since at least 1912. The virus was introduced into Egypt, possibly via an infected animal or hematophagous arthropod, and caused an unprecedented epizootic and epidemic in 1977-78, with *Culex pipiens* L. serving as the major mosquito vector (Hoogstraal et al. 1979, Laughlin et al. 1979, Meegan 1979). There were at least 200,000 human cases with 600 deaths. The virus apparently disappeared from Egypt in 1981.

Arboviral Diseases That Have Emerged in California.

Four arboviral diseases of humans and/or domestic mammals have arisen in California since 1930 (Table 1). A major epizootic of equine encephalomyelitis occurred in the San Joaquin Valley during the summer of 1930, involving about 6,000 horses with an estimated 50% case fatality rate. Meyer and associates (1931) isolated WEE virus from the brain of a horse found sick near Merced in 1930. The disease spread into the Sacramento Valley during the summer of 1931, and by 1932 it had spread to many areas of California and the western United States. Meyer suspected that concurrent cases of encephalitis in humans were caused by the same virus, but this concept was not confirmed until 1938 when Howitt (1938) isolated WEE virus from the brain of a fatal case in a child in Fresno. Epizootics and epidemics of WEE in California continued to occur into the 1950's (Milby and Reeves 1990, Reeves 1990). Then annual levels of WEE viral activity in the Central Valley began to decrease, largely through mosquito and environmental control measures, until only sporadic and focal activity was detected in the 1970's and 1980's. However, WEE virus has remained active during most years along the Colorado River and in the inland agricultural valleys of southeastern California.

St. Louis encephalitis was first recognized as a significant human disease in the Central Valley of California in 1939 when serological tests became

Table 1. Emergence of arboviral diseases in California since 1930.

Year of emergence*	Arboviral disease	Vector	Disease associations
1930	WEE	*Culex tarsalis*	Equines and Humans
1938	SLE	*Culex* species	Humans and Equines(?)
1952	Bluetongue	*Culicoides varipennis*	Sheep and Cattle
1953	Colorado tick fever	*Dermacentor andersoni*	Humans

*Evidenced by viral isolation or serodiagnosis.

available for the specific diagnosis of SLE (Howitt 1942). Although equines were infected frequently with SLE virus, as evidenced by high antibody seroprevalence rates, fatal disease was probably rare. Like WEE virus, SLE viral activity became sporadic and focal in the Central Valley during the 1970's and 1980's while the virus has remained active in one or more of the inland agricultural valleys of southeastern California (Milby and Reeves 1990, Reeves 1990). Unlike WEE virus, however, outbreaks of human SLE have continued to occur in recent years (Murray et al. 1985, Tueller 1991).

Bluetongue was recognized as a disease entity of sheep in the Central Valley of California during the late 1940's and early 1950's, and the virus was first isolated from a diseased sheep in 1952 (Gibbs and Greiner 1988). The virus is an orbivirus that is transmitted horizontally among sheep and cattle in California by the biting midge, *Culicoides varipennis* (Coquillett). There is no evidence that wild vertebrates become involved in the transmission cycle. The virus continues to be epizootic in California, but the disease is controlled in part through vaccination.

Colorado tick fever (CTF) is caused by another orbivirus that is transmitted primarily by the wood tick, *Dermacentor andersoni* Stiles, in northeastern California and other mountainous areas of western Canada and United States where this tick occurs (Bowen 1988). The virus is maintained through the winter in hibernating nymphal and adult ticks and is amplified during the spring and summer in rodents. The existence of CTF virus in California was first demonstrated by researchers from the Rocky Mountain Laboratory in Hamilton, Montana, when they isolated the virus from pools of *D. andersoni* ticks collected in Modoc County during 1953 (Kohls 1955). The disease is sporadic rather than epidemic, and cases occur each year in California, primarily in campers, hikers, and people with outdoor occupations who enter tick infested areas. It is important to point out to this audience that a CTF-like virus has been isolated from a blacktail jackrabbit in Mendocino County and a western gray squirrel in San Luis Obispo County, which is outside of the range of *D. andersoni* (Lane et al. 1982). Thus, this virus and disease may be more widespread in California than is generally thought.

Mechanisms Involved in Emergence of New Viral Diseases.

Having discussed some of the non-arboviral and arboviral diseases that have arisen, let us now examine some of the possible mechanisms that could be associated with their emergence. The explosive appearance of many emerging viral diseases, coupled with the knowledge that mutants arise frequently during viral multiplication in vitro, supports the concept that some new viral diseases may have arisen through genetic changes in pre-existing viruses (Morse 1990). This appears to be the best explanation for the origin of the H3N2 subtype of influenza A virus that arose in the human population in 1968 and caused a pandemic. Human and animal influenza A viruses are known to exchange genetic material, through a reshuffling of RNA gene segments, when they co-infect the same cell. The 1968 influenza A (H3N2) virus may have obtained its HA gene from an avian or equine influenza A virus and the other seven genes from the pre-existing human H2N2 influenza A virus (Laver and Webster 1973). In addition, gene segments of influenza A (H3N2) virus coding for surface glycoproteins have been undergoing nucleotide changes since 1968 which have resulted in periodic epidemics associated with antigenic drift.

Similar mechanisms result in genetic changes of some arboviruses in the laboratory, and may have been associated with the emergence of some new arboviral diseases (Beaty et al. 1988). Unfortunately, there is relatively little definitive evidence at present to support this concept. On the basis of nucleotide and amino acid sequences in RNA genomes and proteins of New World and Old World alphaviruses, Hahn and associates (1988) concluded that WEE virus arose at sometime in the past through a genetic recombinational event between an Old World alphavirus, similar to Sindbis virus, and a New World alphavirus, similar to eastern equine encephalomyelitis (EEE) virus. This event could have occurred in a vertebrate host or mosquito vector infected simultaneously with both viruses, but it can not be predicted when this event occurred.

Trent and co-workers (1980) were able to group geographical strains of SLE virus into topotypes on the basis of genetic differences observed by oligonucleotide fingerprinting of the RNA genome. They also found that strains of SLE virus isolated from humans, mosquitoes and birds during SLE epidemics in the Central and Atlantic states tended to be more virulent for mice and monkeys and produced higher viremic responses in house sparrows than did endemic strains. California strains of SLE virus studied by Trent and co-workers usually fell

into an intermediate group on the basis of mouse virulence and viremogenic capacity in birds. In more limited studies with SLE viral strains from California, we also found that most strains had intermediate virulence for mice, but one strain was highly virulent to mice and another was avirulent (Hardy et al. 1985). Thus, genetic variability probably does occur in arboviruses in California and could provide a mechanism, along with genetic selection, for the emergence of new arboviral diseases as well as the resurgence of old arboviral diseases in the future.

A second mechanism associated with the emergence of new arboviral diseases is movement of virus from one geographical region to another (Morse 1990). This mechanism probably was associated with the 1977-78 RVF epidemic and epizootic in Egypt and possibly with the spread of JE into Southeast Asia, India and Nepal. Also, it is the most likely explanation for the appearance of bluetongue in the United States before the Second World War, as well as the sudden appearance of SLE in the Los Angeles and Orange Counties in 1984. If global warming precipitates significant changes in the ecology and distribution of mosquito species currently in California, or facilitates the establishment of introduced mosquito species, then some areas of California may become quite receptive to the introduction of new arboviral diseases and/or the re-introduction of old arboviral diseases, such as WEE which was not detected in California during 1990 by the state-wide arboviral encephalitis surveillance system.

The third and probably most common mechanism associated with emerging viral diseases is viral traffic from animals to humans (i.e., zoonoses) (Morse 1990). Most arboviruses are maintained in nature by horizontal transmission between arthropod vectors and wild vertebrate hosts. Humans are not essential for the maintenance of the transmission cycle, but can become involved tangentially when the flow of virus from vectors to humans is enhanced by events that increase their exposure to vector attack. Events that usually increase vector attack rates include atypical climatic conditions, such as heavy snowpack and spring runoff or heavy spring rainfall in California, which result in flooding, increased mosquito breeding and potentially increased viral amplification and transmission (Reeves 1967). Increased exposure to vector bites also occurs when humans enter areas where enzootic transmission normally takes place, such as during recreational and hunting excursions into natural wildlife areas in northeastern California that are infested with the tick which transmits CTF virus. Finally, changes in the environment brought about by human activities can enhance arboviral traffic from wildlife hosts and vectors to humans. Examples are irrigated agriculture and the emergence of arboviral encephalitides in the San Joaquin Valley of California in 1930, and the outbreak of SLE in Kern County in 1989 and the development of residential housing along the old Kern River bed southwest of Bakersfield.

Arboviruses and Wild Vertebrate Viruses in California Not Yet Associated with Significant Disease in Humans and/or Domestic Mammals.

I would now like to turn my attention to the arboviruses and some wild vertebrate viruses that are known to occur in California, but have not been associated with significant disease. Currently, there are 17 arboviruses, belonging to five families of animal viruses, that are transmitted enzootically among wild vertebrates by mosquitoes, ticks or biting midges in California (Table 2). As stated previously, only four of these 17 viruses have been associated with significant disease: WEE, SLE, bluetongue and CTF. In addition, four other viruses have been isolated from wild vertebrates that apparently are not vectored by any arthropod. Many of these arboviruses and wild vertebrate viruses were isolated while studying the ecology of WEE and SLE viruses. The potential of most of these viruses to produce disease in humans and horses has been evaluated by doing serological tests on the acute and convalescent sera from humans and horses with undiagnosed febrile and central nervous system disease (Milby and Reeves 1990, Reeves 1990). These sera were negative for antibody titer rises to WEE and SLE viruses as well as several non-arboviruses that are known to produce CNS disease in humans. The seroprevalence of antibodies to these viruses also was determined in normal humans and domestic mammals.

Table 3 summarizes what we know about the eight mosquito-borne arboviruses in California that have yet to be associated with significant disease. Three viruses, Turlock (TUR), Hart Park (HP) and Llano Seco (LLS), have been isolated frequently from *Culex tarsalis* and are most likely amplified in wild birds. Neutralizing antibodies to TUR, HP and LLS viruses were detected in only 1-6% of the undiagnosed human CNS cases tested. However,

Table 2. Arboviruses and some wild vertebrate viruses known to occur in California.

Viral family Genus Serogroup	Known arboviruses	Vertebrate viruses
Togaviridae		
Alphavirus	WEE	
Flaviviridae		
Flavivirus	SLE	Modoc
	Powassan	Rio Bravo
Bunyaviridae		
Bunyavirus		
California serogroup	California encephalitis	
	Jamestown Canyon	
Bunyamwera serogroup	Lokern	
	Main Drain	
	Northway-(like)	
Simbu serogroup	Buttonwillow	
Turlock serogroup	Turlock	
Reoviridae		
Orbivirus	Bluetongue	
	Colorado tick fever	
	Epizootic hemorrhagic disease	
	Llano Seco	
	Mono Lake	
Rhabdoviridae		
Rhabdovirus	Gray Lodge	Kern Canyon
	Hart Park	Klamath

diagnostic antibody rises were detected to HP virus in five cases. This is the first evidence which suggests that HP virus may be a human pathogen. Serosurveys of domestic mammals indicated that horses and dogs were infected frequently with TUR and HP viruses whereas relatively high antibody seroprevalences to LLS virus were found in horses, cattle, sheep, pigs and dogs. Diagnostic antibody rises to TUR virus were observed in six cases of undiagnosed equine encephalomyelitis, suggesting that TUR virus may be a equine pathogen. Gray Lodge virus has been isolated only from one pool of *Cx. tarsalis* and relatively little work has been done with this rhabdovirus.

California encephalitis virus is vectored primarily by *Aedes melanimon* Dyar in the Central and Owen Valleys (Reeves and Milby 1990). The virus is maintained through the winter in diapausing *Ae. melanimon* eggs and is amplified during the summer in leporids, at least in the Central Valley. Three clinical cases of acute encephalitis that occurred in humans in Kern County during 1945 were diagnosed as CE (Hammon and Reeves 1952), but no additional cases have been found subsequently by tests on paired sera from over 3,000 undiagnosed human CNS cases in California (Reeves 1990). Yet, neutralizing antibody prevalences to CE virus in Kern County residents during the 1940's and 1963 were 11% and 37%, respectively. The relative lack of human disease associated with CE viral infections in California is puzzling since two other California serogroup bunyaviruses, La Crosse and Jamestown Canyon (JC), are important human pathogens in upper mid-western and northeastern states (Grimstad 1988).

Eldridge and associates (1991) recently isolated

Table 3. Mosquito-borne arboviruses in California not yet associated with significant disease.

Virus	Year 1st isolated	Vector	Vertebrate host	Infection/disease* in Humans	Mammals
Turlock	1954	*Cx. tarsalis*	Birds	± / o	++ / ±
Hart Park	1955	*Cx. tarsalis*	Birds?	+ / ±	+ / o
Llano Seco	1971	*Cx. tarsalis*	Birds?	+ / o	++ / o
Gray Lodge	1971	*Cx. tarsalis*	?	?	?
California encephalitis	1943	*Ae. melanimon*	Leporids	++ / ±	± / o
CE-(like)	1989	*Ae. squamiger*	?	?	?
Northway-(like)	1970	*An. freeborni*	?	± / ?	++ / ?
	1971	*Ae. sierrensis*	?	± / ?	++ / ?
Jamestown Canyon	1963	*Cs. inornata*	Leporids?, Deer?	+ / o	++ / o
	1988	Snowpool *Aedes*	Leporids?, Deer?	+ / o	++ / o

*Infection/disease levels: o= nil, ± = 1-10%, + = 10-20%, ++ = >20% and ? = not known.

a CE-like virus from pools of adult *Aedes squamiger* (Coquillett) reared from larvae and pupae collected in a coastal tidal marsh near Morro Bay. This is the first arbovirus ever isolated from this species and represents a potential disease threat to the coastal human population. Studies are in progress to elucidate the ecology and epidemiology of this virus.

Jamestown Canyon virus is a California serogroup virus which has been associated with significant disease in upper mid-western states (Grimstad 1988). This virus has been isolated from *Culiseta inornata* (Williston) (Reeves and Milby 1990) and snowpool *Aedes* (Campbell et al. 1991) in California. Infections have been demonstrated by serological tests in deer, horses and humans in mountainous areas of California (Campbell 1990; Campbell et al. 1989, 1990), but no evidence of disease has been found thus far.

Campbell and associates (1989, 1990) also found relatively high antibody seroprevalences to Northway (NOR) virus in deer and some species of domestic mammals in California, but only one human serum was seropositive. In retrospect, four strains of NOR-like virus were identified that had been isolated from pools of *Anopheles freeborni* Aitken and *Aedes sierrensis* (Ludlow) collected in Butte County during 1970-71 (Campbell 1990). We recently obtained a virus from *Culiseta inornata* collected in Kern County that also appears to be a NOR-like virus. NOR virus was originally isolated from mosquitoes in British Columbia, Canada, and was found to infect humans, but without any evidence of disease (Calisher et al. 1986, Zarnke et al. 1983). We feel that it is important to learn more about the ecology and epidemiology of this virus in California, and such studies are in progress.

In addition to bluetongue, there are at least four other *Culicoides*-borne arboviruses in California (Table 4). Blacktail jackrabbits and desert cottontails serve as amplifying hosts of Buttonwillow (BUT), Lokern (LOK) and Main Drain (MD) viruses (Hardy and Reeves 1990b, Milby and Reeves 1990). Human infections have been detected only with MD virus, but no evidence of human disease has been found (Reeves 1990). However, all three of these viruses infect one or more domestic mammal species, especially LOK and MD. Diagnostic antibody rises were demonstrated to MD virus in five equine encephalitis cases and to LOK virus in one case (Milby and Reeves 1990). In addition, Emmons and associates (1983) isolated MD virus from the brain of a horse that died of encephalomyelitis in Sacramento County in 1981. Thus, MD virus and possibly LOK virus must be considered as potential equine pathogens. The other *Culicoides*-transmitted virus, epizootic hemorrhagic disease, is an orbivirus that is closely related to bluetongue virus and infects deer but not sheep and cattle in North America (Gibbs and

Table 4. *Culicoides*-borne arboviruses in California not yet associated with significant disease.

Virus	Year 1st isolated	Vertebrate host	Infection/disease* in Humans	Mammals
Buttonwillow	1962	Leporids	o / o	± / o
Lokern	1962	Leporids	o / o	+ / ±
Main Drain	1965	Leporids	± / o	++ / ±
Epizootic hemorrhagic disease	1981**	Deer	?	?

*Infection/disease levels: o = nil, ± = 1-10%, + = 10-20%, ++ = >20% and ? = not known.
**Refers to California only.

Greiner 1988). This virus has been isolated from diseased deer and bighorn sheep in California.

Four non-arboviruses have been isolated from the tissues of apparently normal wild mammals collected in California (Table 5). Rio Bravo (RB) and Modoc (MOD) viruses are flaviviruses, like SLE virus, but do not infect mosquitoes, *Culicoides* or ticks experimentally (Hardy and Reeves 1990a). Both viruses are probably maintained by persistent infections in their natural hosts: the Mexican free-tail bat for RB virus and the deer mouse for MOD virus. Low levels of antibody prevalences have been detected to both viruses in humans and some domestic animal species in California by hemagglutination-inhibition tests (Milby and Reeves 1990), but these could represent cross-reacting antibodies to SLE virus. Five laboratory workers became infected while handling RB virus in the laboratory and three of them developed moderately severe aseptic meningitis, orchitis or oophoritis (Sulkin et al. 1962). A febrile illness in a human also was diagnosed as a case of RB following natural infection (Karabatsos 1985). One case of aseptic meningitis in a child was diagnosed as a MOD viral infection (Reeves 1990). This child had been playing with a wild mouse near Porterville about a week prior to admission to the hospital. Thus, RB and MOD viruses must be considered potential human pathogens. Kern Canyon and Klamath viruses are both rhabdoviruses, but no studies have been done to evaluate their disease potential in humans and domestic mammals.

To summarize, only four of the 17 arboviruses known to occur in California have been associated with significant disease in humans and/or domestic mammals since the 1930's. However, some of the other arboviruses, as well as wild vertebrate viruses are known to infect humans or domestic mammals. These viruses are lurking in the wings, so to speak, as potential epizootic or epidemic disease agents of the future. It is impossible to predict, however, if and when this might occur and whether global warming will enhance this possibility. On the positive side, we know that these viruses are present in

Table 5. Viruses of wild vertebrates in California not yet associated with significant disease.

Virus	Year 1st isolated	Vertebrate host	Infection/disease* in Humans	Mammals
Rio Bravo	1954	Mexican freetail bat	± / ±	± / o
Modoc	1958	Deer mouse	± / ±	± / o
Kern Canyon	1956	Yuma myotis bat	?	?
Klamath	1962	Mountain vole	?	?

*Infection/disease levels: o = nil, ± = 1-10%, + = 10-20%, ++ = >20% and ? = not known.

the environment and we have acquired a significant amount of knowledge about their basic transmission cycles. This knowledge will be of value if any of these viruses emerge as significant human or domestic mammal pathogens at sometime in the future.

Will Global Warming, in Itself, Result in Arboviral Mutants?

The two previous speakers have discussed the potential effects that global warming may have on human populations and mosquito ecology in relation to arboviral diseases in California during the 21st century. One also might ask whether increased ambient temperatures due to future global warming will result in arboviral mutants with increased vector competence, viremogenic and virulence capacities. While this remains a possibility, it seems speculative since arboviruses already have to be able to multiply at 37° C and 42° C in order to produce viremia in their mammalian and avian hosts, respectively. Global warming is expected to cause ambient temperatures to rise by 5° C, and as you have heard, such a temperature differential already exists between the San Joaquin Valley and the Coachella and Imperial Valleys. We have tested SLE viral strains from each of these areas for mouse virulence and ability to produce viremias in one-week old chickens and to be vectored by *Culex* mosquitoes, and have found no consistent and significant differences between these viral strains. Thus, I feel that the emergence of new viral diseases or the resurgence of old arboviral diseases in the future, if they occur, will most likely be associated with human activities and environmental changes that alter vector and perhaps vertebrate host ecology and increase the flow of viruses from animals to humans.

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MOSQUITO AND ARBOVIRUS SURVEILLANCE PROGRAM OF THE KERN MOSQUITO AND VECTOR CONTROL DISTRICT

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Introduction.

An unexpected outbreak of St. Louis encephalitis (SLE) occurred in Kern County during the late summer and early fall of 1989. There were a total of 17 confirmed human cases with one near fatality of an elderly resident in southwest Bakersfield (Emmons et al. 1991, Tueller 1991). The existing surveillance system deployed jointly by the Kern Mosquito and Vector Control District and the Arbovirus Field Station (University of California, Berkeley) failed to provide sufficient information on the spread of SLE virus from the sparsely populated west side of the San Joaquin Valley into the more heavily populated areas in the eastern and central portions of the district (Reisen et al. 1991). The apparent lack of sensitivity in the existing system indicated that more intensive surveillance was needed along the Kern River and in areas rapidly expanding to the south and west of Bakersfield. The westward expansion of greater Bakersfield is increasingly putting residents at risk to exposure to mosquito-borne arboviruses in historically known viral foci along the lower Kern River and western valley drainages.

This paper briefly describes our current surveillance system and proposed changes that will hopefully provide additional support for detecting sporadic pulses in western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) virus activity and monitoring the spread of these viruses into populated areas.

Surveillance Program Overview.

Mosquito abundance monitoring and control evaluation: Numerous trap comparison studies have demonstrated that carbon dioxide-baited traps are more efficient than New Jersey light traps (NJLT) for sampling *Culex* and *Aedes* mosquitoes (Magnarelli 1975, Milby et al. 1978, Meyer et al. 1983). New Jersey light trap effectiveness has been severely limited as a consequence of increased competition from the proliferation of "security" lighting in both urban and rural areas, and limited access to AC electrical sources. The portability of battery operated CO_2 traps allows for deployment independent of competitive light sources and AC electrical availability. More importantly, CO_2 traps can provide data on three critical surveillance parameters: 1) mosquito abundance, 2) arbovirus activity (i.e. mosquito pools and virus isolates) and 3) evaluation of mosquito control efforts. Therefore, our operational decisions are largely based upon CO_2 trap versus New Jersey light trap indices.

Both CO_2 traps and NJLT's have proven ineffective for sampling *Culex quinquefasciatus* Say in metropolitan areas in Kern County. Urban surveillance of *Cx. quinquefasciatus* is accomplished by monitoring the oviposition rate of gravid females into five gallon (19.0 liter) plastic utility buckets (ovibuckets) containing an infusion of processed steer manure. The processed manure produces a less volatile oviposition medium and minimal surface film in comparison to the fresh manure media previously used by Parman (unpubl. data). The total number of rafts sampled by ovibuckets in one week is converted into the number of rafts/bucket-night as the unit of ovipositional activity or indirect measure of female abundance.

Arbovirus surveillance: The spatial deployment of sentinel chicken flocks in virus-sensitive areas within the district is based upon the history of long term surveillance and research efforts by the University of California, Berkeley (Reeves and Hammon 1962, Reisen et al. 1990a). Geographically, WEE and SLE viruses are active primarily on the floor of the San Joaquin Valley in

relative close association with marsh and riparian habitats. Few virus isolates have been obtained from mosquitoes collected in Sierra Nevada foothill riparian and oilfield waste water habitats along the eastern border of the district. This geographical pattern of activity has allowed for the judicious placement of 7 sentinel flocks at 2 riparian, 3 mixed agricultural and 2 urban sites.

Strategy for Trap Deployment.

SLE virus activity in 1989 was first detected in western Kern County and was subsequently tracked eastward along the Kern River drainage. The nature of the spread of the virus within the county clearly demonstrated a need for increased surveillance along the western boundary of the district and eastward following the Kern River to the Sierra Nevada foothills. A total of 15 CO_2 traps were arrayed in three transects to provide sampling of host-seeking female *Culex tarsalis* Coquillett and *Cx. quinquefasciatus*. The three transects consisted of: 1) the northwest transect (2 km WNW of greater Bakersfield) with 4 traps oriented NE to SW, 2) the southwest transect (1-2 km WSW of greater Bakersfield) with 4 traps oriented NW to SE, and 3) Kern River transect with a total of 7 traps evenly spaced from the mouth of the Kern River to a point 2 km W of California State University at Bakersfield. Additional CO_2 traps were operated next to the sentinel chicken flocks (2 traps/flock) at two mixed agricultural and two urban sites.

Biweekly sampling showed that *Cx. tarsalis* was more abundant than *Cx. quinquefasciatus* along the Kern River, but much less abundant than *Cx. quinquefasciatus* along the northwest and southwest transects (Fig. 1-A,B,C). *Culex tarsalis* was most abundant along the southwest transect (Fig. 1-D) and at both riparian and mixed agricultural sentinel chicken flock sites (Fig. 2). Populations of *Cx. tarsalis* and *Cx. quinquefasciatus* peaked in August and September and *Cx. quinquefasciatus* continued to be abundant through October. The relative abundance of *Cx. tarsalis* in late summer (in a time frame coincidental with SLE transmission) along the southwest transect demonstrated the necessity for more intensive virus surveillance in that area. Carbon dioxide-baited traps were positioned at sites that were within 1-2 km of heavily populated neighborhoods where a significant number of residents could come in contact with potentially infective *Cx. tarsalis* and *Cx. quinquefasciatus*.

There was a distinct dissimilarity in the peak abundance of *Cx. quinquefasciatus* measured in urban areas by ovibucket and CO_2 traps operated along the two western and river transects. Oviposition rates measured by 40 ovibuckets randomly placed throughout greater Bakersfield were highest during June and July. By comparison, host-seeking female abundance determined by CO_2 traps peaked in August and September (Fig. 3). The temporal differences in abundance patterns of *Cx. quinquefasciatus* in urban and rural areas will require further investigation.

Summary and Augmentation.

Placement of CO_2 traps and sentinel chicken flocks in the Kern District was based upon historical arbovirus activity patterns and an assumption that virus is either activated within or disseminated from wetland foci (i.e. Kern River, Main Drain, etc.) into residential and commercial developments in the expanding western sections of greater Bakersfield. The geographical distribution of confirmed human cases of SLE in Kern county during the summer and fall of 1989 did not reveal any directional component in the spread of the epidemic. Similarly, the rates of sentinel chicken conversions among flocks exclusive of the Kern River flock progressed spontaneously during the same time period; precluding any definitive assessment of SLE dissemination.

The sporadic occurrences of SLE cases in 1989 and the results of CO_2 trapping along the transects in 1990 indicated that changes in trapping strategy and sentinel chicken deployment required further modification to increase surveillance sensitivity. Carbon dioxide trap counts among traps in the eastern section of the Kern River transect revealed that *Cx. tarsalis* production was relatively low in comparison to the other areas sampled. Therefore, those traps will be redeployed to 1) supplement the western transects and 2) increase surveillance efforts to the south and southeast of Bakersfield.

The sites of existing sentinel flocks are too widely dispersed to provide a uniform continuum of viral detection in areas where urban expansion (i.e. southwest Bakersfield) is placing residents in potential direct contact with viral dissemination. Current research with "mini-flocks" in southern California indicates that dispersing flocks and reducing flock sizes to 5-10 birds each can enhance detection sensitivity (Hazelrigg unpubl. data, Reisen unpubl. data). Therefore, the District will augment the existing sentinel chicken program (7 standard

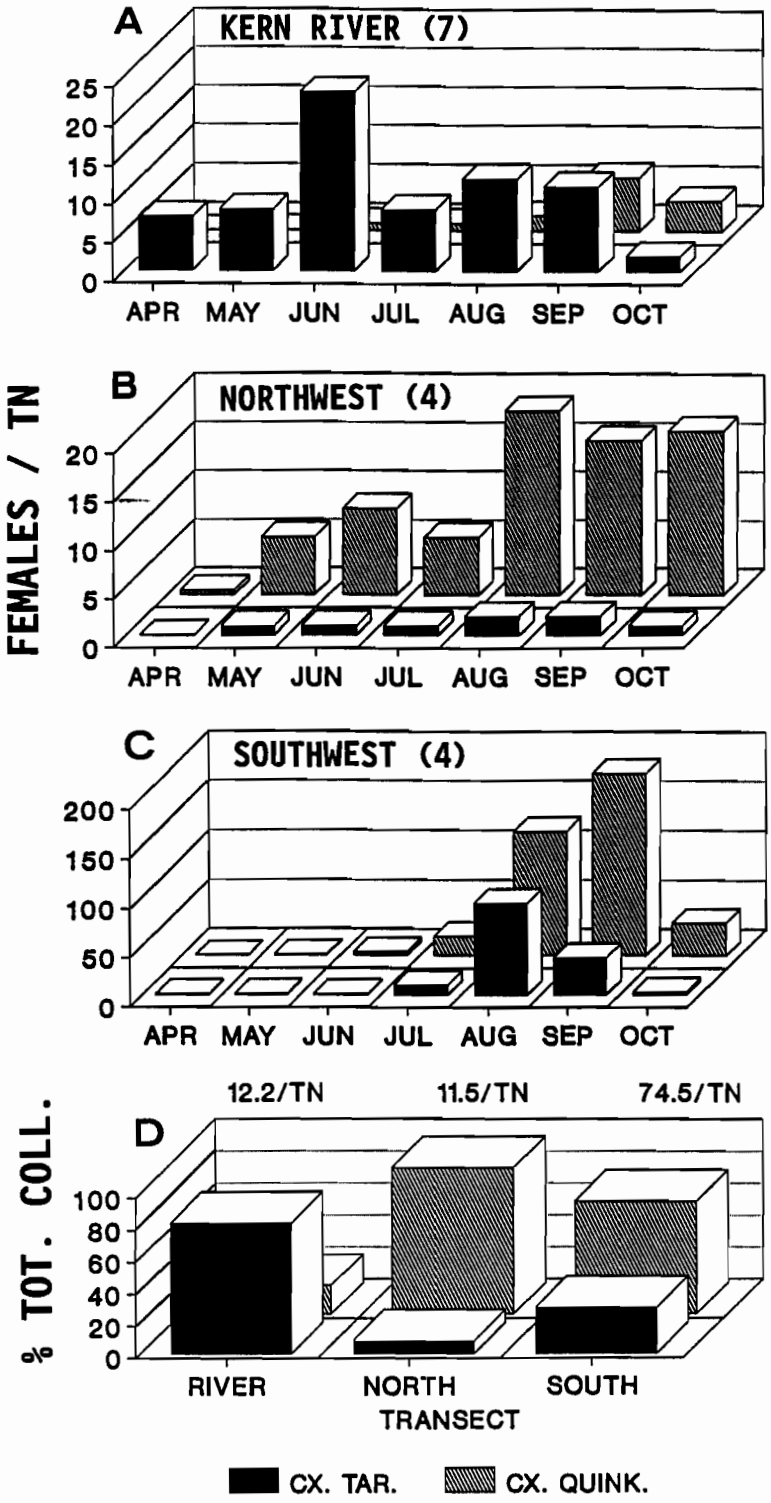

Figure 1. Monthly abundance (females/trap-night) of *Cx. tarsalis* and *Cx. quinquefasciatus* determined by CO_2 traps in each of the three transects (A,B,C) and the number (in parenthesis) of traps in each transect. Also indicated is the comparative abundance of the two *Culex* species within each transect (D) and the total number of mosquitoes collected per trap-night.

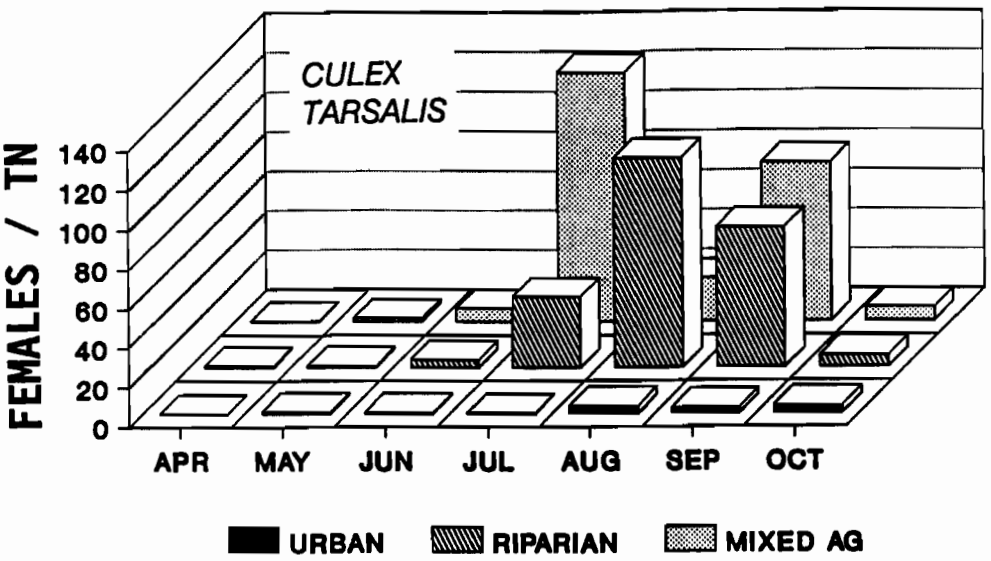

Figure 2. Monthly abundance of *Culex tarsalis* at sentinel chicken flock sites.

flocks) with 5 additional flocks to the northwest, southwest and south of greater Bakersfield. Birds will be allocated to support 3 standard flocks (20 birds each) and 9 "mini-flocks" (10-12 birds each).

The distribution of human SLE cases in the southern San Joaquin Valley and Los Angeles basin in 1989 and 1983 respectively, implicated the possible involvement of *Cx. quinquefasciatus* as a secondary vector of SLE virus (Webb et al. 1987, Reisen et al. 1990b). In urban areas where *Cx. quinquefasciatus* is significantly more abundant than *Cx. tarsalis*, the gravid trap (Reiter 1983) has proven to be more effective than the CO_2 trap for sampling large numbers of *Cx. quinquefasciatus* (Reisen et al. 1990b, Reisen and Meyer 1990). Although our existing ovibuckets provide information on the relative abundance of *Cx. quinquefasciatus* in urban areas, this surveillance tool fails to collect adult females for pooling and arbovirus isolations. The Kern District will augment the ovibucket surveillance program by deploying 10 gravid traps at key sites throughout the greater Bakersfield area. Thus, the urban and rural arbovirus surveillance program will include, in addition to female *Cx. quinquefasciatus* pooled from rural CO_2 traps, additional females pooled from the urban gravid traps.

The arbovirus surveillance program and augmentations described herein represent the Kern Districts' first attempt at developing a systematic method for monitoring mosquito-borne arbovirus activity in the southern San Joaquin Valley. The physiography and land utilization pattern of agriculture and urbanization allows for an organized effort to detect virus activity and spread, and concurrent evaluation of mosquito abatement practices. Although our current surveillance program is labor intensive, the efforts are justified considering the fact that recent epidemics of SLE in California have occurred without any early indication from environmental parameters (i.e temperature, rainfall, mosquito abundance, etc.) that have been associated with past outbreaks.

Acknowledgements.

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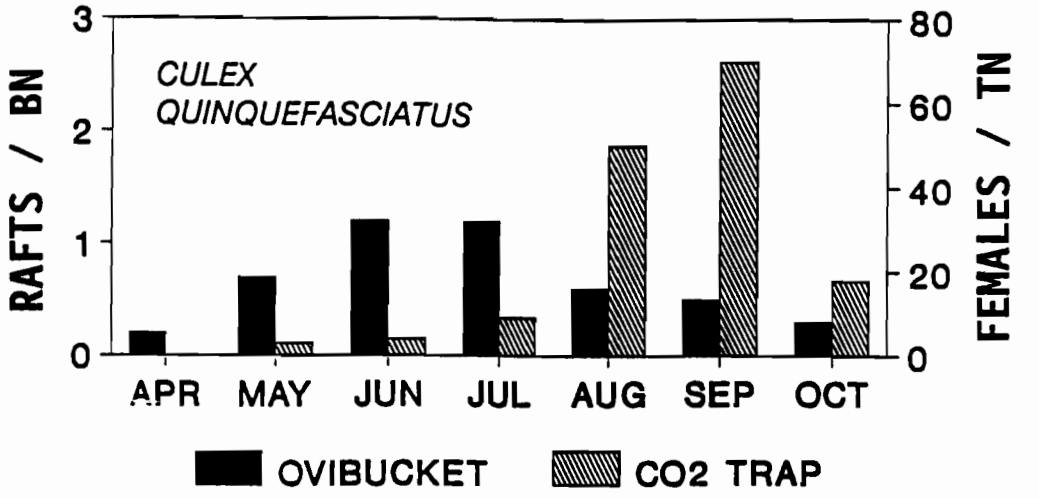

Figure 3. Monthly abundance of *Culex quinquefasciatus* determined by urban ovibuckets (n = 40) and rural/periurban CO_2 traps (n = 15).

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MOSQUITO ABUNDANCE AND ARBOVIRAL ACTIVITY IN SAN BERNARDINO COUNTY DURING 1990

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ABSTRACT

Of the total mosquitoes collected in New Jersey light traps and dry ice (CO_2)-baited traps in San Bernardino County during 1990, *Culex tarsalis* (55.4%), *Culiseta inornata* (21.2%), and *Aedes vexans* (20.3%) were the main species collected in the desert region while *Cx. tarsalis* (34.7%), *Culex stigmatosoma* (24.6%), *and Culex quinquefasciatus* (24.4%), were predominant in the valley region. Mosquito activity in the desert region was the lowest in the suburban habitats (7.0%) as compared to the rural (45.9%) and urban sites (47.1%). In the valley region, however, more mosquitoes were found at the suburban sites than the other two habitats. Seasonally, mosquito populations peaked in September to November in the desert region and May (urban), July (suburban), and August (rural) within the habitats of the valley region.

One mosquito pool collected in mid-September in the desert region from the City of Needles was positive for Saint Louis encephalitis virus. All other mosquito pools and sera samples from sentinel chicken flocks in both regions showed no viral activity.

Introduction.

As part of the California encephalitis virus surveillance (EVS), the San Bernardino County Vector Control Program has been carrying out EVS and other mosquito control activities in both the valley and desert areas of San Bernardino County. Geographically, the county consists of three distinct regions; the desert, mountain and valley regions. Demographically, the valley region houses over 80% of the nearly 1.4 million county population with the remainder 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 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 adjoining areas along the Colorado River. Due to the periodic incidence of encephalitis disease, mosquito control and EVS activities have been routinely carried out in the desert and valley regions of this county. The data generated in routine EVS activities are appraised here in relation to the population dynamics of adult mosquitoes and arboviral activity in San Bernardino County during 1990.

Materials and Methods.

The general procedure used for EVS studies consisted of the three main components.

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 region, the traps were stationed at six locations; Yucaipa Regional Park, Fifth Street and a Flood Control Basin in San Bernardino, Fontana Regional Park, Ontario and Upland sites. 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 these traps were counted and identified to species. The adult mosquito occurrence reports were submitted to the California Department of Health Services.

Arboviral activity in adult female mosquitoes: To monitor arboviral activity in local mosquito populations in both the desert and valley regions, dry ice (CO_2)-baited traps were used to collect host-seeking adult female mosquitoes. Eight or more such traps were operated on a biweekly (valley region) or monthly (desert region) basis.

Female mosquitoes collected overnight were anesthetized using triethylamine, counted, identified to species, and then pooled together by species with 10-50 adults per each labelled vial. All pools (vials) were stored in dry ice in the field or at -60° F in a deep freezer 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 chicken flocks: 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 15 white leghorn chickens was maintained in both the valley and desert regions. The valley flock was stationed near a horse ranch at the northeastern corner of Meridian Avenue and Olive Street in the City of San Bernardino. This site is within one mile of the last year's flock site (Randall Basin) which had a history of one human SLE case each in 1987 and 1988. The desert flock was initially stationed near a golf course and later maintained at the sewage treatment facility in the City of Needles. New Jersey light traps were regularly operated at both flock sites. Blood serum samples from all sentinel chickens taken on pre-determined dates during the mosquito season were sent to the VRDL for detection of arboviral activity.

Results and Discussion.

Of the total 17,316 mosquitoes collected in New Jersey light traps and CO_2-baited traps at various sites in the county during 1990, the most abundant culicine species in both the desert and valley regions was *Culex tarsalis* Coquillett (35-55%, Table 1). In the desert region, *Culiseta inornata* Williston was the second most abundant species with 21.2% of the total collection, followed by *Aedes vexans* Meigen (20.3%). Other species totaling <2.0% each of the total included *Anopheles franciscanus* McCracken, *Culex erythrothorax* Dyar, *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar, and *Psorophora columbiae* (Dyar and Knab). Earlier studies in this area, have shown *Cx. tarsalis* as the most abundant species comprising as much as 72%, 62% and 86% of the mosquitoes collected in 1986, 1987 (Reisen et al. 1988) and 1988 (Mian, unpublished data), respectively.

In the valley region, mosquito composition by species was *Cx. tarsalis* (35%), *Cx. stigmatosoma* (24.6%), *Cx. quinquefasciatus* (24.4%), *Cx. erythrothorax* (9.1%), *An. franciscanus* (5.0%), with *Culiseta incidens* (Thompson), *Cs. inornata*, and *Ae. vexans* each comprising <2.0% of the total. In the Chino area of this valley region, the three culicine species in order of their relative abundance have previously been were reported to be *Cx. quinquefasciatus, Cx. stigmatosoma* and *Cx. tarsalis* (Pfuntner 1988). The Chino area is composed of various agricultural biotopes included but not limited to dairy farming. These biotopes provide ideal habitats for the breeding of mosquito species in the aforementioned order.

Based on New Jersey light trap data, the distribution of mosquitoes by habitat in the desert region, was 47.1% urban, 45.9% rural and 7.0% suburban (Table 2). Both the urban and rural trap sites were closer to the Colorado River than the suburban site. In the valley region, mosquitoes were found in higher numbers in both suburban (52.6%) and rural (33.2%) habitats than at urban sites (14.2%). This distribution pattern could be attributed to the proximity of trap sites to mosquito breeding habitats ranging from domestic or residential swimming pools to flood control structures in the urban and suburban habitats, or to seepage water in ponds, ground depressions and irrigation ditches in cultivated crops by the Colorado River in rural areas.

Data on the seasonal abundance of mosquitoes from the desert region show a small population peak in June in the rural habitat followed by a larger peak in the suburban and rural habitats in November (Table 3). Mosquito activity peaked in the urban habitats during the months of September and October. High spring and fall mosquito population levels in the urban and suburban habitats

Table 1. Mosquito composition from all traps in San Bernardino County during 1990. Total collected mosquitoes are 13,543 and 3,773 in the desert and valley regions, respectively.

Species	% Composition Desert	Valley
Aedes vexans	20.3	<0.1
Anopheles franciscanus	1.6	5.0*
Culex erythrothorax	1.1	9.1
Culex quinquefasciatus	0.2	24.4
Culex stigmatosoma	0.1	24.6
Culex tarsalis	55.4	34.7
Culiseta incidens	0.0	1.2
Culiseta inornata	21.2	0.9
Psorophora columbiae	0.1	0.0

* Includes *An. freeborni* or *hermsi* (<0.1%).

Table 2. Distribution of mosquitoes collected in New Jersey light traps at various locations in San Bernardino County during 1990. Total collected mosquitoes are 8,156 and 928 in the desert and valley regions, respectively.

Trap location	% Mosquitoes/trap-night Desert	Valley
Rural	45.9	33.2
Suburban	7.0	52.6
Urban	47.1	14.2

necessitated adulticidal applications of Pyrenone® MAGC, (a mixture of pyrethrins and piperonyl butoxide) during the last week of May and the first and last weeks of November.

Seasonal mosquito faunal composition from New Jersey light trap collections in the desert region showed that *Cx. tarsalis* and *Ae. vexans* were predominant during the spring and summer months, whereas *Cs. inornata* prevailed during the fall and winter months (Table 4). Mosquito abundance as measured by CO_2 traps revealed a similar pattern with *Cx. tarsalis* as the most abundant species during June through October, and *Ae. vexans* as the main species during July and August (Table 4).

In the valley region, the suburban sites had the highest mosquitoes caught thus far in New Jersey light traps. Mosquito populations peaked in the urban sites in May, suburban sites in July, and rural sites in August (Table 3). Mosquito activity at these habitats during May through October was mainly due to *Cx. quinquefasciatus, Cx. stigmatosoma* and *Cx. tarsalis*, whereas during the later part of the year (October), *Cs. inornata* was the most prevalent species (Table 5). Similarly, data on mosquitoes caught in CO_2 traps presented the same pattern of mosquito abundance with *Cx. quinquefasciatus, Cx. stigmatosoma*, and *Cx. tarsalis* as the predominant species during June through October (Table 5).

Regarding the arboviral activity in San Bernardino County, one out of the 74 submitted mosquito pools which was collected during mid-September in the desert region was found positive for SLE virus. Based on the serology of blood samples from the sentinel chicken flock in Needles, no seroconversion was found during the earlier part of the 1990 mosquito season. Due to the loss of the chicken flock by wild dogs, no sampling was carried out during September and October. All mosquito pools and sera samples from the sentinel chicken flock maintained in the valley area did not show any arbovirus activity during the 1990 season.

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Table 3. Seasonal abundance by habitat of mosquitoes collected in New Jersey light traps from the desert and valley regions of San Bernardino County during 1990.

		% Mosquitoes/trap-night			
Region	Month	Urban	Suburban	Rural	Mean
DESERT	JAN	0.6	3.8	2.5	2.3
	FEB	0.2	3.4	3.8	2.5
	MAR	0.7	2.5	0.6	1.3
	APR	4.3	0.8	4.4	3.2
	MAY	2.9	2.9	5.7	3.8
	JUN	3.0	1.3	19.3	7.9
	JUL	7.0	0.4	4.3	3.9
	AUG	8.9	1.3	6.2	5.4
	SEP	42.2	6.7	3.1	17.3
	OCT	23.3	12.1	17.2	17.5
	NOV	4.9	48.1	32.2	28.4
	DEC	2.0	16.7	0.7	6.5
VALLEY	MAY	50.2	11.0	2.0	21.1
	JUN	30.9	15.0	6.1	17.3
	JUL	10.3	41.0	26.4	25.9
	AUG	7.0	15.6	42.9	21.8
	SEP	1.6	12.0	11.3	8.3
	OCT	0.0	5.4	11.3	5.6

Table 4. Seasonal abundance by species of mosquitoes collected in New Jersey light traps and CO_2-baited traps from the desert region of San Bernardino County during 1990. Total collected mosquitoes are 8,156 and 5,387 in the New Jersey and CO_2-baited traps, respectively.

Month	% Mosquitoes/trap-night								
	Ae. vexans	An. fran.	Cx. erythro.	Cx. quinque.	Cx. stigmat.	Cx. tarsalis	Cs. inorn.	Ps. columb.	Totals
N.J. TRAPS									
JAN	0.0	0.0	0.0	0.0	0.0	<0.1	1.7	0.0	1.7
FEB	0.0	0.0	0.0	0.0	0.0	0.1	2.0	0.0	2.1
MAR	<0.1	0.0	0.0	<0.1	0.0	0.5	1.0	0.0	1.5
APR	0.2	<0.1	<0.1	0.0	0.0	3.4	0.2	0.0	3.9
MAY	0.1	0.1	0.1	0.0	0.0	3.8	<0.1	0.0	4.1
JUN	0.6	1.2	0.2	0.0	0.0	6.0	0.0	0.0	8.1
JUL	1.7	0.4	0.1	0.0	0.0	2.8	0.0	0.0	5.0
AUG	2.0	0.2	<0.1	0.0	0.0	4.3	<0.1	0.1	6.6
SEP	0.3	0.2	0.0	0.1	0.0	25.0	0.4	<0.1	26.0
OCT	1.1	<0.1	0.1	<0.1	<0.1	7.6	7.3	0.0	16.0
NOV	1.0	<0.1	0.0	0.0	0.0	9.6	20.3	0.0	22.2
DEC	0.0	0.0	0.0	0.0	0.0	0.2	2.6	0.0	2.8
CO2 TRAPS									
MAR	0.0	0.0	0.0	0.0	0.0	0.8	0.1	-	0.9
JUN	3.4	0.6	1.1	0.0	0.4	10.9	0.0	-	16.4
JUL	24.5	0.0	<0.1	0.0	<0.1	3.6	0.0	-	28.2
AUG	10.0	<0.1	0.9	<0.1	0.0	9.6	0.0	-	20.6
SEP	0.4	0.8	0.3	<0.1	0.2	14.3	0.0	-	16.0
OCT	0.7	0.0	0.2	0.2	0.0	16.6	0.2	-	17.9

Table 5. Seasonal abundance by species of mosquitoes collected in New Jersey light traps and CO_2-baited traps from the valley region of San Bernardino County during 1990. Total collected mosquitoes are 928 and 2,845 in the New Jersey and CO_2-baited traps, respectively.

Month	% Mosquitoes/trap-night								
	Ae. vexans	An. fran.	Cx. erythro.	Cx. quinque.	Cx. stigmat.	Cx. tarsalis	Cs. incidens	Cs. inorn.	Totals
N.J. TRAPS									
MAY	-	0.0	0.1	2.4	8.2	0.8	1.3	1.1	13.9
JUN	-	0.3	0.0	1.5	7.0	3.6	2.0	0.1	14.5
JUL	-	5.2	0.3	2.9	15.0	7.8	0.5	0.1	31.8
AUG	-	8.3	0.0	1.3	8.9	4.2	0.4	0.0	23.1
SEP	-	0.0	0.0	2.1	4.1	3.9	0.1	0.0	10.2
OCT	-	0.2	0.1	1.4	0.8	1.1	0.3	2.6	6.5
CO2 TRAPS									
JUN	0.0	<0.1	1.3	3.1	7.8	15.3	0.0	0.6	28.1
JUL	0.2	0.5	5.2	0.5	0.3	3.4	<0.1	0.0	10.1
AUG	0.0	0.8	5.6	13.3	2.8	8.4	0.0	0.0	30.9
SEP	0.0	3.4	0.2	4.7	3.0	5.7	0.0	0.0	17.0
OCT	0.0	<0.1	2.2	6.9	3.4	1.3	0.0	<0.1	13.9

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EVALUATION OF MOSQUITO AND ARBOVIRUS ACTIVITY IN ORANGE COUNTY, CALIFORNIA, 1990

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In 1990 the Orange County Vector Control District (OCVCD) continued its mosquito and encephalitis virus surveillance. Mosquitoes were collected all year at ten permanent sites throughout the county (Bennett et al. 1991). Nineteen CDC/CO_2 traps were utilized as well as five Reiter ovipositional traps. Additionally, blood-fed female mosquitoes were collected inside three stable traps (Magoon 1935) containing five chickens each.

A total of 20,946 mosquitoes was collected from which 516 mosquito pools were submitted for virus testing (Table 1). The collections included 372 pools of *Culex quinquefasciatus* Say, 143 pools of *Culex tarsalis* Coquillett, and 1 pool of *Culex stigmatosoma* Dyar. None of these pools tested positive for St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) viruses. However, one pool of *Cx. tarsalis* from within the boundaries of the nearby Southeast Mosquito Abatement District (SEMAD) at the Sepulveda Basin in Los Angeles was found positive in early May.

In Orange County, sentinel chicken flocks included one large flock (25 chickens) at the 20 Ranch Duck Club in Irvine and three mini-flocks (5 chickens each) held in stable traps located in Fullerton, Buena Park and Irvine. Seroconversions for SLE antibodies occurred in the large flock (1 chicken) in Irvine on October 29. Shortly afterward, a single chicken seroconverted from the Sepulveda Basin in Los Angeles on November 2 and another from Long Beach on November 29. Though in Los Angeles County, both locations border Orange County.

Wild bird sera collected through this lab by Dr. John Gruwell and Becky Brown were tested for SLE and WEE antibodies at the Orange County Health Department. Nine modified Australian Crow traps (McClure 1984) were used in 1990 to trap a total of 18,356 birds from which 6,760 blood samples were taken. Rock doves (*Columba livia*) from Irvine showed the highest number of SLE positives at 4.80 percent (4.38% in 1989)(Table 2). However, rock doves (pigeons) were not collected after April because of the closure of Bonita Canyon Landfill. House finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*) were infected at rates of 1.4 and 3.0 percent, respectively (1.1 and 3.0%, respectively for 1989). Five of 206 (2.4%) white-crowned sparrows (*Zonotrichia leucophyrs*) were positive for SLE this year. All of these white-crowned sparrows were recaptures from Irvine sites, were previously negative for SLE, and more than likely acquired the infection locally.

When examining infection trends in the wild bird populations for 1990 (Fig. 1), positive birds were found every month of the year with the highest SLE activity occurring in April (4.9% positive) even though the sample size for April was much lower than previous months. During 1989, 1.5% of the birds were positive in April and the highest periods of viral activity appeared in mid-June and December. Comparison of SLE-positive birds collected from Huntington Beach and the collection of host-seeking mosquitoes from the same area (Fig. 2) shows a correlation between increased viral activity and numbers of mosquitoes collected from this backyard source. Again, wild birds appeared to be an effective sentinel for virus activity in the Los Angeles Basin. There consistently were seroconversions months before the positive mosquito pools or chicken seroconversions appeared (Fig. 2).

Culex quinquefasciatus was the predominant mosquito species collected in suburban sites and was present through the fall and winter months, while *Culex tarsalis* was predominant in rural areas and, in most cases, was gone by October (as in 1989). This is illustrated by comparing a residential backyard site in Irvine (Fig. 3) with the rural site at

Table 1. Number of mosquito pools submitted for SLE and WEE virus surveillance by species and trap type from Orange County, California during 1990.

Species	Stable Traps	Oviposition Traps	CDC Traps	Red Box
Culex quinquefasciatus	162	85	116	9
Culex tarsalis	19	-	124	-
Culex stigmatosoma	1	-	-	-

Table 2. Small bird seroconversions for SLE and WEE antibodies in Orange County, California during 1990.

Species	No. Positive		No. Bloods Sampled	% Positive	
	SLE	WEE		SLE	WEE
Rock Dove	49	0	1,027	4.80	0
House Sparrow	54	0	1,782	3.00	0
House Finch	51	0	3,747	1.40	0
White-crowned Sparrow	5	0	206	2.40	0

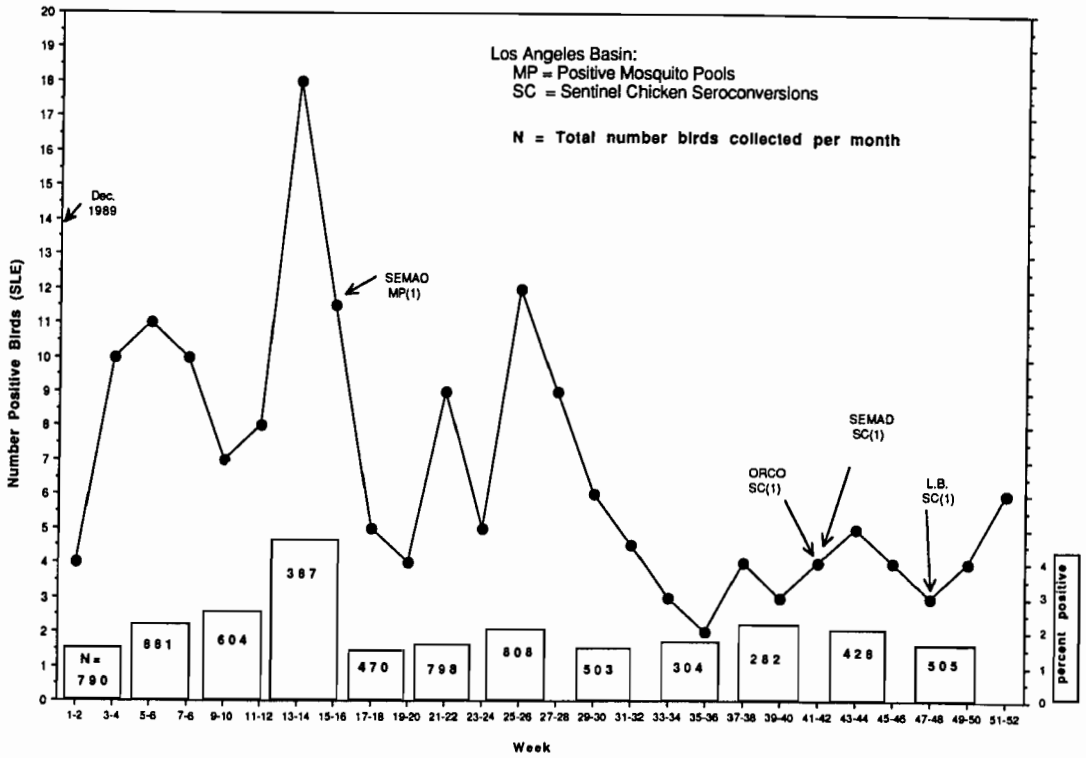

Figure 1. SLE virus activity in the Los Angeles basin and wild bird seroconversions in Orange County, California during 1990.

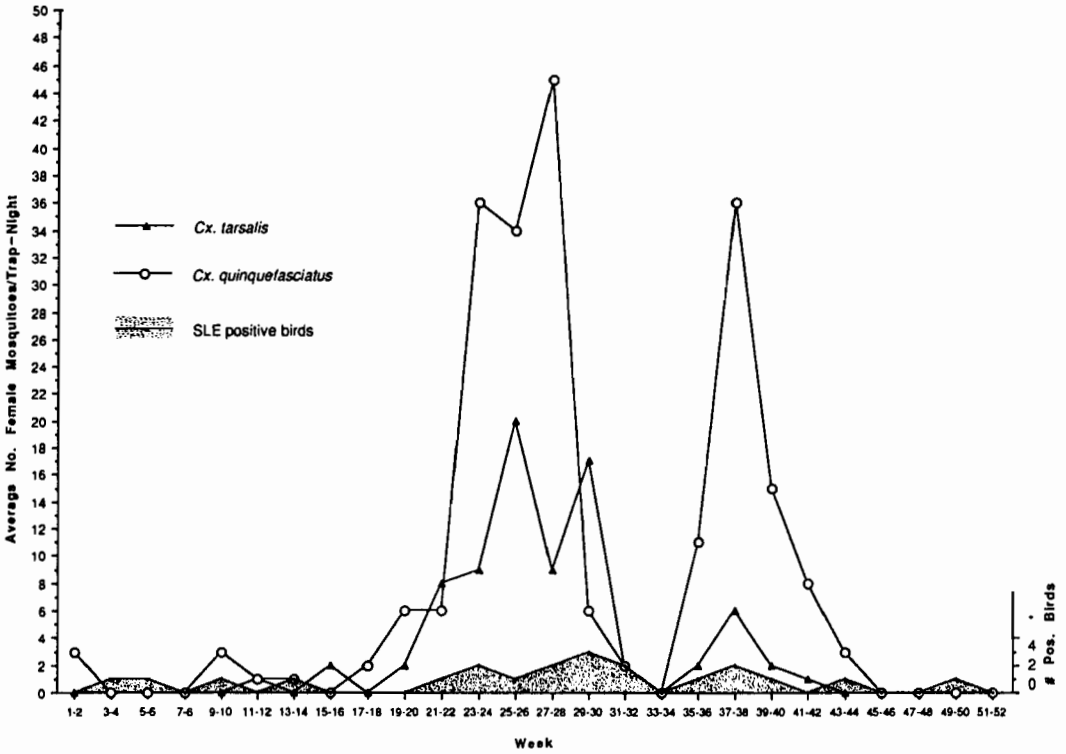

Figure 2. Host-seeking female mosquitoes and SLE activity in wild birds from a suburban residence in Huntington Beach, California during 1990.

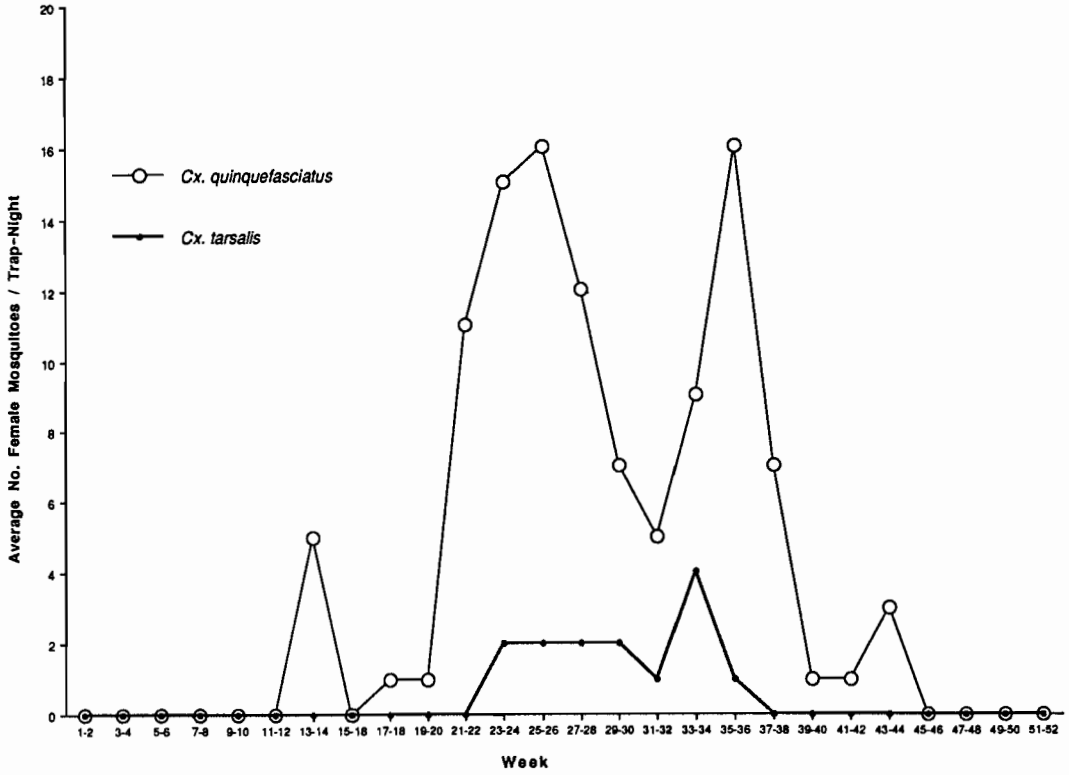

Figure 3. Host-seeking female mosquito activity at a suburban residence in Irvine, California during 1990 as determined by CDC/CO_2 traps.

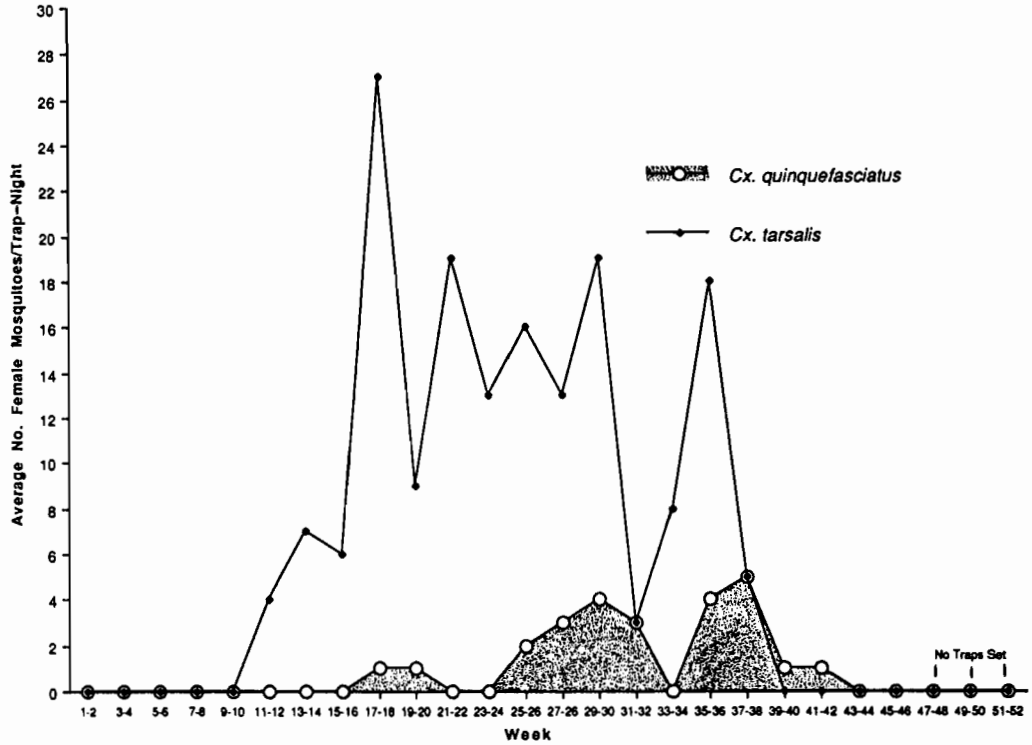

Figure 4. Host-seeking female mosquito activity in the San Joaquin Freshwater Marsh, Irvine, California during 1990 as determined by CDC/CO_2 traps.

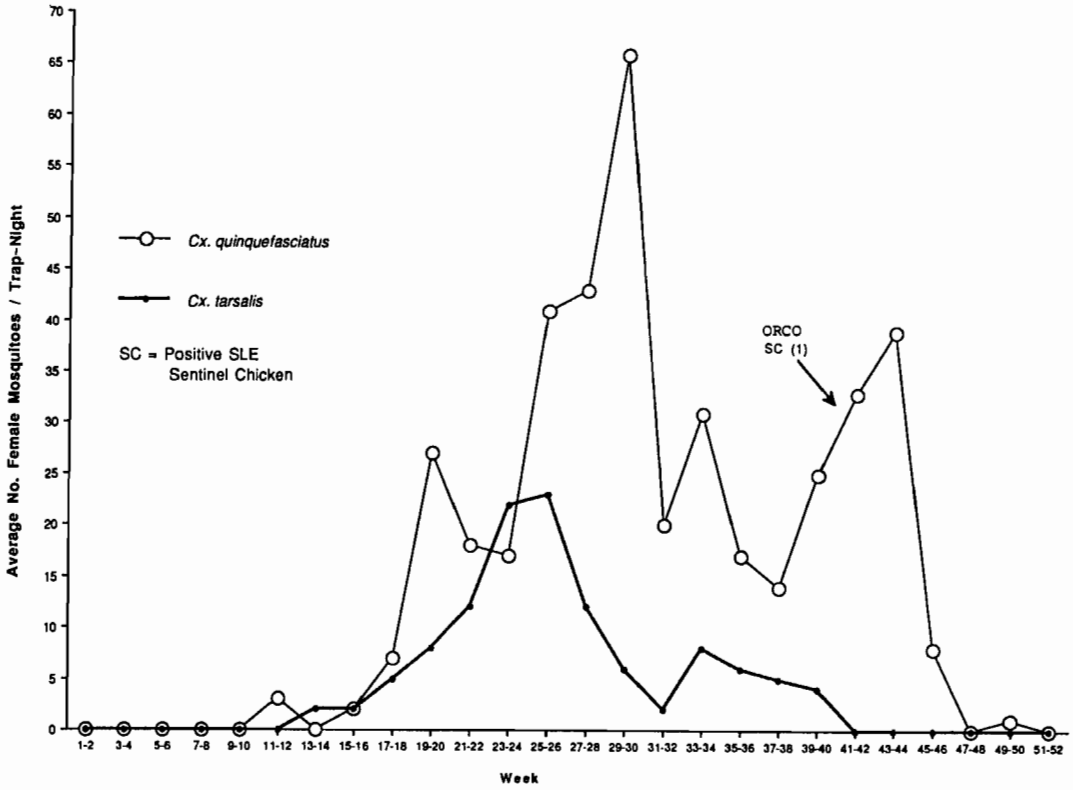

Figure 5. Host-seeking female mosquito activity at 20 Ranch Duck Club in Irvine, California during 1990 as determined by CDC/CO_2 traps.

the San Joaquin Marsh, also in Irvine (Fig. 4). Populations of *Cx. tarsalis* in the marsh were highest in May, but only averaged 27 females per trap-night; much lower than both 1989 (100 per trap-night in August) and 1988 (400 per trap-night in August).

The 20 Ranch Duck Club was the only site in the county that had a sentinel chicken seroconvert for SLE. Unlike past years, the *Culex quinquefasciatus* population at 20 Ranch was higher than that of *Cx. tarsalis* (Fig. 5). *Culex quinquefasciatus* averaged 45-65 per trap-night in July as compared to 10-25 per trap-night for *Cx. tarsalis*. Between October and November, *Cx. quinquefasciatus* averaged 25-40 females per trap-night during the time when the chicken seroconverted at this site. In contrast, *Cx. tarsalis* was averaging <5 per trap-night at the same time.

In addition to the host-seeking females collected in the CDC/CO_2 traps, females were collected from the stable trap at the 20 Ranch site four days a week (Monday through Thursday) throughout the year (Fig. 6). At the peak of mosquito activity between August and September, 300 female *Cx. quinquefasciatus* were collected during each four day period (75 per trap-night). Two weeks prior to the chicken seroconversion, 180-250 females were being taken each four day period (45-63 per trap-night). Although *Cx. tarsalis* was most abundant between June and July (23 females per trap-night), the collections were down to almost zero by the end of September.

Gravid female *Culex quinquefasciatus* were obtained from Reiter ovipositional traps at both suburban and rural sites. A standard comparison of gravid female mosquito activity from the 20 Ranch Duck Club in Irvine and a nearby residential site (Fig. 7) reveals that peak activity periods varied between both sites this year with 20 Ranch showing the most change from 1989. High numbers of gravid females (100-120 per trap-night) were present starting in August and continued through November. Prior to August, very few were collected. In 1989, gravid *Cx. quinquefasciatus* were present starting in March at 20 per trap-night, built up to 50 per trap-night in July and September and then dropped gradually until December. Possible causes for the changes that have occurred at 20 Ranch may be the extensive landscaping and

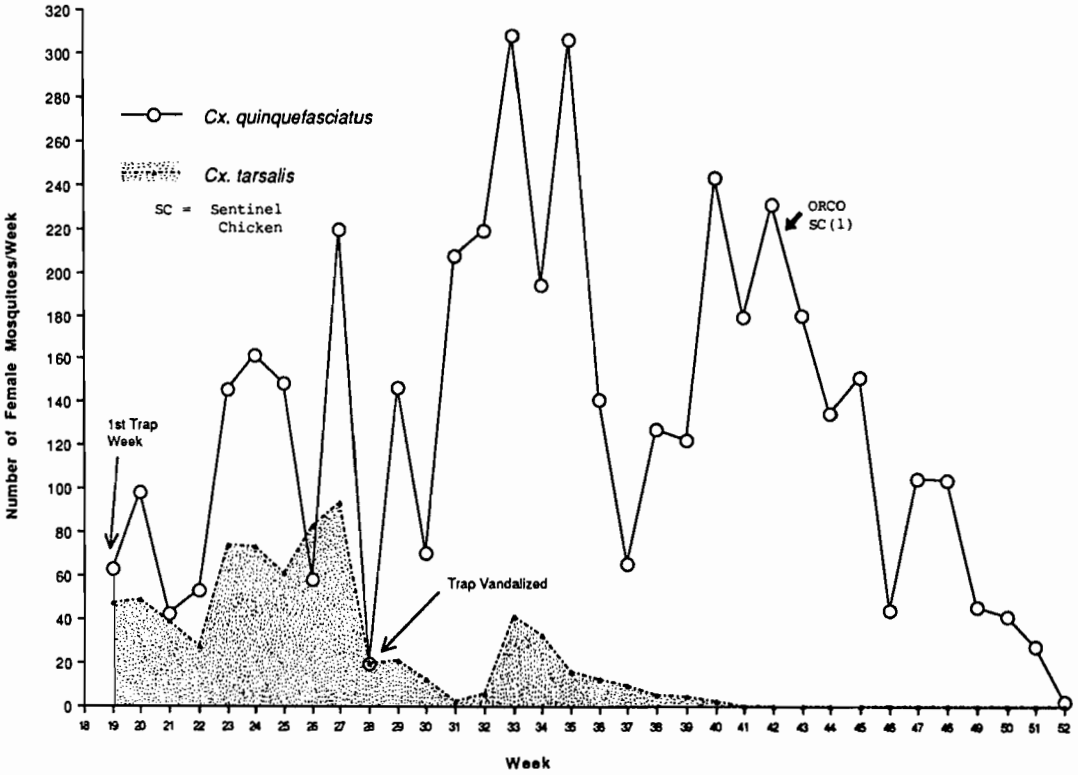

Figure 6. Host-seeking (blood-fed) female mosquito activity at 20 Ranch Duck Club, Irvine, California during 1990 as determined by collections from a stable trap.

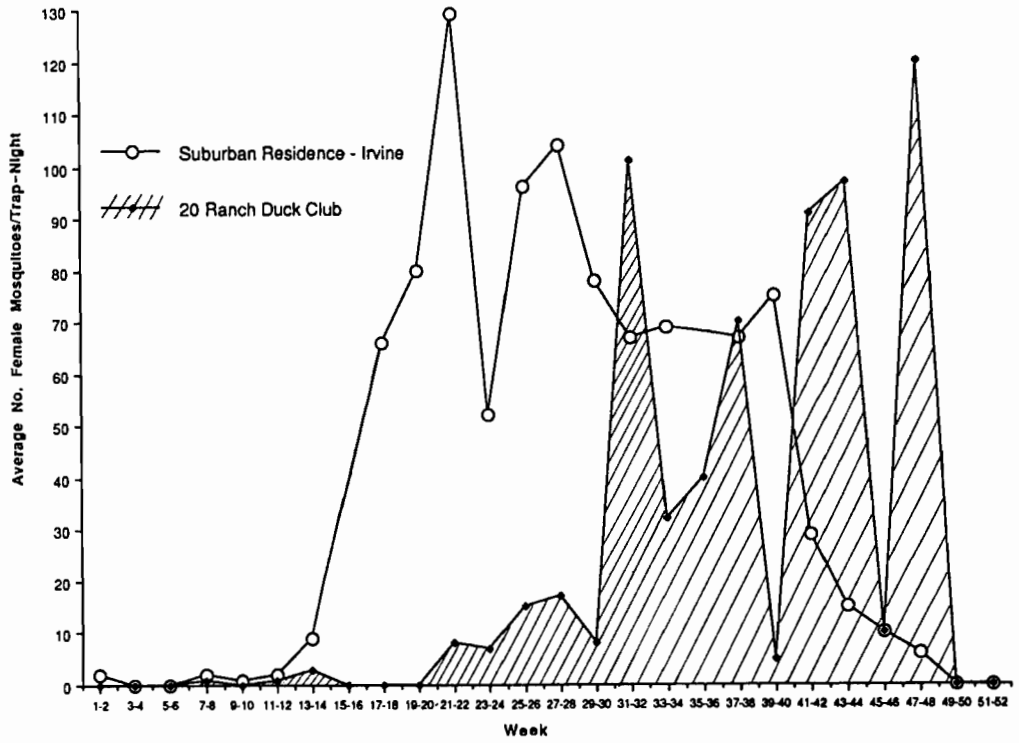

Figure 7. Gravid female *Culex quinquefasciatus* activity in Irvine, California during 1990 as determined by Reiter ovipositional traps.

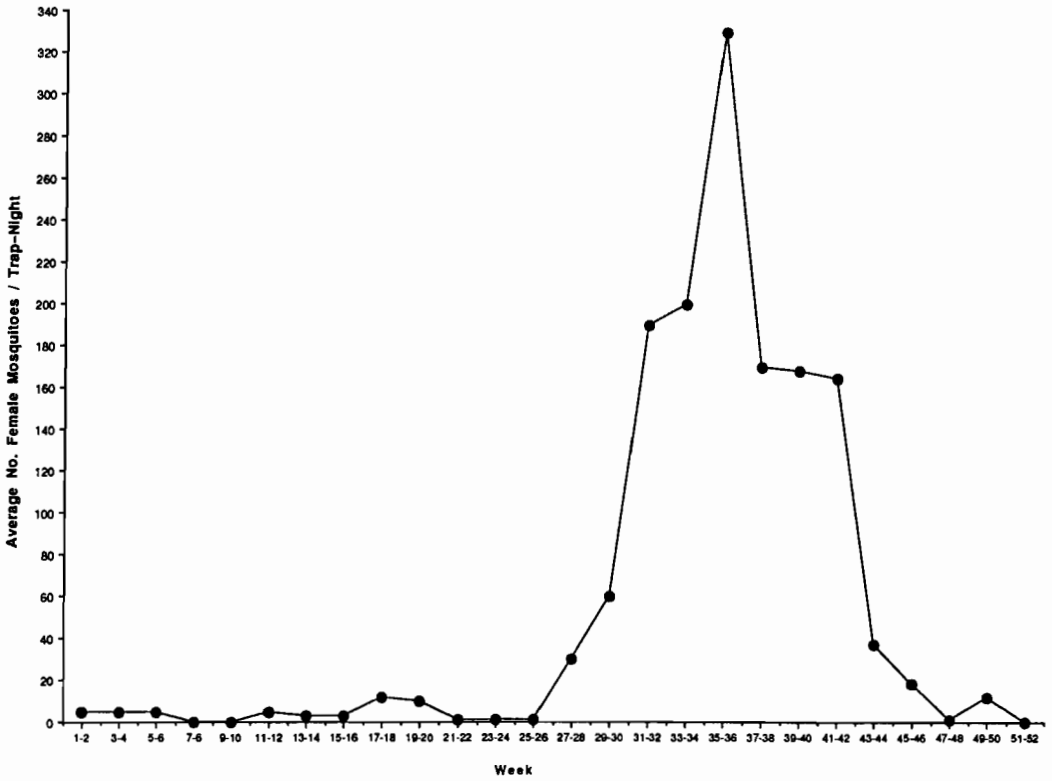

Figure 8. Gravid female *Culex quinquefasciatus* activity in Huntington Beach, California during 1990 as determined by Reiter ovipositional traps.

housing projects which have altered the area adjacent to the collecting sites with the removal of vegetation, ground leveling, and pond drying. Changes in the local environment may have modified the mosquito life cycles.

Temporal activity of gravid females at the Irvine residential site was almost identical to 1989 except the number per trap-night collected this year was much higher; ranging from 80-130 per trap-night in May (40-50 per trap-night for 1989), 90-100 per trap-night in June (50-60 per trap-night for 1989), and 65-75 in October (30-35 per trap-night for 1989).

In 1989 the highest numbers of gravid female *Cx. quinquefasciatus* were obtained from a suburban park in Huntington Beach and reached 240 females per trap-night in December. The counts for 1990 were even higher; reaching 320 per trap-night in September. However, the counts were down to 160-200 per trap-night in August and October (Fig. 8). Although the numbers were lower in 1989, high counts of gravid *Cx. quinquefasciatus* females were obtained starting in April, whereas very little activity was noted between January and July of 1990. The December increase in gravid female activity that occurred in 1989 did not occur in 1990.

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MOSQUITO AND VECTOR CONTROL: WHERE DO WE GO FROM HERE?

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Introduction.

Looking into the future seems to be becoming a habit of mine, but I'm not sure I'm getting any better at it. You have heard excellent presentations from Jack McGurk, Steve Nations, and Jim Lyons on the present and future status of public health, science and technology, politics, and higher education, on both a state and national level. It would be hard for anyone to conclude, after hearing these presentations, that we are not facing new and entirely different challenges in California mosquito and vector control than we were just a few years ago. I don't have enough time to give a comprehensive overview of all of the present and future issues that I see in mosquito and vector control, but I would like to touch on a few topics that seem to me to be particularly important. These topics stem from trends that have been mentioned by the previous speakers, and that I see attracting increasing attention almost daily.

Trends Affecting Mosquito and Vector Control.

Water: It is hard to pick up a daily newspaper or a weekly news magazine without seeing something about water. Most of the articles mention the lack of it, and it is ironic that it is the lack of water which may be responsible for the greatest challenges to mosquito and vector control. Problems with water abound in California. As a leading agricultural state, California is also a leader in the use of water for agriculture. But groundwater pollution is a serious problem, and about half of all such pollution is from nonpoint sources. The leading cause of nonpoint pollution is agriculture, stemming from sediments, salts, fertilizers, pesticides, and manures (National Research Council 1989). A classic example of a water problem produced by agricultural practices can be seen in the western San Joaquin Valley. There is little question that mosquito production is related to nutrient availability, and thus is directly related to pollution. This relationship is apparent in highly polluted waters in wetlands constructed for treatment of wastewater. Recently, a different type of water-related challenge has arisen. Wetlands constructed primarily to provide habitat for water fowl are planned for locations all over the state. It is difficult to forecast just what mosquito problems will result from these wetlands, but it seems certain that the wetlands will be built, and that there will be consequences of concern to vector control agencies.

Bill Reeves has repeatedly called attention to another potential water-related problem, but few people have paid much attention. I think it is time to consider some facts. In this century there has been an increase in the mean annual temperature in the Northern Hemisphere of 0.2-0.4° C, and global sea-level has risen 25-30 cm (Gornitz and Lebedeff 1987, Barnett 1988, Peltier and Tushingham 1989). But present forecasts call for increases in mean annual temperature of 1-4° C within the next 50 years; linked to a probable doubling of atmospheric CO_2 levels. The historical record shows that sea-levels are very responsive to climate change, and studies of Pleistocene climatic changes show abrupt changes in sea-level between glacial and inter-glacial periods (Shackleton and Opdyke 1973, Bloom 1983). This difference has been estimated to be more than 100 m and to have had a profound effect on coastal marshes and estuaries in California. If the forecasts for global warming are correct, then we should expect to see a further increase in sea-level with substantial salt water intrusion into coastal lowlands. This will undoubtedly affect mosquito breeding and has the potential to influence patterns of mosquito-borne disease transmission.

Pesticides: We seem to have left an era of great uncertainty about public health pesticides and entered into an era of relative stability. In the vernacular, the other shoe may have dropped. The general framework of the relationship between pesticides and rare and endangered species has been developed, and the relationship between this

Table 1. Selected insecticide usage reported by California mosquito abatement districts, 1987-1989 (Source: CMVCA Yearbooks).

Year	Malathion	Chlorpyrifos	Methoprene	*Bti*
1987	43,659	7,345	494	64,238
1988	23,025	3,780	516	103,380
1989	15,771	4,971	2,463	113,616

Units are pounds of actual toxicant for malathion, chlorpyrifos and methoprene; billions of biological units for *Bti*.

framework and preventing or abating an outbreak of vector-borne disease has been addressed. Against a backdrop of discussion about registration, physiological resistance, and economics, a downward trend continues in use of public health pesticides in California. I described this trend on a long-term basis three years ago in San Mateo. Since that time, the reduction in pesticide use has continued. Examination of data furnished by California MADs to the State Department of Health Services and published annually by this organization, leads one to an inescapable conclusion: The reliance on conventional broad-spectrum pesticides for vector control in California is essentially a thing of the past (Table 1). The State Department of Health Services no longer monitors for OP resistance, and Dr. George Georghiou has re-directed his OP resistance research program toward resistance problems with microbial pesticides.

People: The preliminary results of the 1990 census confirmed what we already knew: There are a lot of people living in California, and the numbers are increasing all the time. More important, from the standpoint of vector control, is the rapid urbanization of areas in the Central Valley and in some other areas, which is resulting in an ever-growing interface between high density housing and land which serves as habitat for a variety of biological organisms, including mosquitoes, water fowl, predators, rodents, and flies. As this interface increases, so does the risk of zoonotic disease transmission. And as the urbanization increases, so do the challenges in vector control.

Solutions.

These are some of the challenges. What should be the response of those involved in vector control? Where do we go from here?

Ecology of aquatic habitats: The present emphasis on water use in all forms, and on the preservation of existing wetlands and the construction of new wetlands will not go away soon, in my view. The challenge for us is to accelerate studies on natural and constructed wetlands, and to continue in our roles as responsible environmentalists. We have insisted, and rightly so, that public health concerns be factored into planning for wetlands management. Because the reasons for doing so are compelling, wetlands managers will come to us for scientific and technical advice on minimizing mosquito and other vector problems. At this point do we have all the answers for them? You know we don't. We have only begun to develop information on the relationship between aquatic vegetation and insects. There is undoubtedly a strong relationship between non-point sources of pollution and mosquito breeding, but we can't say what it is for all species in all situations. The most extreme example of this is the use of constructed wetlands for preliminary treatment of wastewater, but the inability to solve mosquito problems for this otherwise promising technology has stalled it.

There has been a recent trend in the University of California to re-emphasize its role in conservation and natural resource management. Dr. Kenneth Farrell, Vice President of the Division of Agricultural and Natural Resources, underscored this emphasis in his recent editorial in *California Agriculture* (Farrell 1991). We need to be sensitive to this re-emphasis, which is driven largely by increasing public concerns about deterioration of the global environment and deterioration of natural diversity within California. I believe there will be many opportunities for us to work with organizations such as the Wildland Resources Center, the Natural Reserve System, and the Water Resources

Center. The challenges we face in understanding and managing aquatic ecosystems cannot be met without broad interdisciplinary approaches.

Management of pesticides: The need for limited, specialized use of public health pesticides will continue far into the future. Even though we have largely shifted use to selective, insect-specific compounds, we must not ignore the potential problems with these materials. Mosquitoes can develop resistance to materials such as growth regulators and microbial insecticides. None of these materials are completely non-toxic, nor are any of them without some potential risk to non-target organisms. I see the need, therefore, for strong emphasis on research on resistance mechanisms and resistance management of new materials. This is going to require some new testing methods, because in most instances, the physiological resistance mechanisms in insects will be different than those involved in detoxification of conventional pesticides.

Because there are so many aspects of toxicity that differ with these new materials, regulators are having difficulty assessing safety, and the registration process has been slowed substantially. Of particular concern to mosquito abatement agencies is the very long registration process involved in the cases of *Lagenidium giganteum* and *Bacillus sphaericus*.

Along with other new technology associated with new materials, we must take a fresh look at application technology. Ken Giles and Bill Steinke of the Agricultural Engineering Department at UCD tell me that the presently-used ULV technology is years behind fluid atomization technology in general and as presently used is noisy, energy inefficient, and provides only crude control over droplet size. There may be off-the-shelf technology available now which may be adaptable for insecticide dispersal use, and which may represent a significant improvement over presently available equipment. Any new technology must accommodate modern materials now being used, as well as future new materials.

Finally, the biological basis for adulticiding and larviciding must be re-examined. We can no longer afford to be guided only by meteorological factors, while ignoring diel activity cycles of target insects. This research is vital if we are to have available workable emergency response plans. What an empty victory it will be if we develop an otherwise sound emergency exemption plan to EPA's Pesticide Restriction Plan under the Endangered Species Act, only to find that we have no usable emergency technology when we really need it.

Information storage, retrieval, and dissemination: We face many problems associated with an increased human population, varied and sometimes conflicting priorities, environmental regulations, and an exploding information base. In responding to these factors, we will gather the fruits of the seeds that have been planted by the CMVCA during the last decade. The efforts must continue and be accelerated. These areas are (1) training of vector control personnel, (2) public education, and (3) information storage, retrieval, and dissemination. It will not be easy to expand these activities in a declining economy, but unless we can further develop these activities in the state, I don't see how we will be able to cope with the many problems we will face during the next several decades.

Conclusions.

I have discussed three problems which we will face in the coming years: water, pesticides, and people. I could have added more to the list. The challenges we face today are similar to those faced by the previous generations of vector control professionals. They met the challenges of their day through the application of science and technology. This is also the key to our future. However, it remains to be seen whether or not we in California, and indeed, we in the United States, are willing to make a commitment to science and technology sufficient to meet the many challenges we face. Leon Lederman, President-elect of the Board of Directors of the American Association for the Advancement of Science, paints a dismal picture of the status of science in the United States (Lederman 1991). He makes a persuasive argument that as a country, we have become a second-rate scientific power. It is absolutely vital to all of us that we do what we can to reverse this trend. Otherwise, the answer to the question "Where do we go from here?" is "nowhere".

I would like to end on a personal note. The CMVCA has made a number of critical decisions over the past five years which have positioned the Association to face future challenges from a solid foundation of organization, communication, and training. These decisions derived from the efforts of many people, but there is one individual in this association who has distinguished himself by unusual dedication and vision. That individual is John Combs, our outgoing Executive Director, and I am pleased to publicly acknowledge his contribution to

CMVCA and to vector control in California.

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PUBLIC ATTITUDES AND PESTICIDE USAGE IN CALIFORNIA

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I am pleased to be here today to address you as a group of scientist and public-serving professionals whose efforts are aimed at reducing the impacts of our mosquito population. Although I work in the social sciences, I too have an interest in your friend the mosquito. Growing up in Ohio, I was sure we had the largest, meanest and greatest number of mosquitoes. I spent many summer evenings swatting mosquitoes and watching the town's truck sprayer spreading its mists - usually a welcome sight. I doubt such a truck would be as welcome today. I had further encounters with mosquitoes in the tropics of Ecuador where I popped chloroquin and fancidar pills weekly - suspecting that fancidar may be more hazardous than the mosquitoes. I also saw and participated in the spraying of DDT in the 60's and the 80's as a means for combating malarial mosquitoes.

My comments on the topic "Public Attitudes and Pesticide Usage in California" are not anecdotal. Rather, they will focus on the public, the publics' perceptions of pesticides and possible implications for you. My remarks are offered from the perspective of a concerned professional interested in human behavior, not that of mosquitoes.

As I began to construct this presentation, I reviewed documents from some of your previous meetings. Here I want to mention only two. In a 1984 article on the "Development of Mosquito Control in California", Richard Peters (1984) noted:

> "The status of mosquito control in 1946 must be appreciated. There were twenty-five local control agencies collectively covering only 5,000 square miles with the budgets of all barely totalling $500,000. People, their lives disrupted by the war, were swarming to California, mostly choosing to settle in the suburbs proximal to mosquito production. Irrigated agriculture was booming. DDT, a new miracle insecticide was becoming available which gave promise that it might well obliterate mosquitoes. Certainly the public was now in prospect of undisturbed outdoor living in full protection from mosquitoes and mosquito-borne diseases."

Today, in 1991, California's population is still booming. People are moving in large numbers to the Central Valley and all other parts of the state. Californians find their lives disrupted by a war. Irrigated agriculture is still booming, but DDT is no longer the miracle, and neither are the other chemical pesticides. However, the public still expects to live undisturbed by the pesky mosquito.

Has much changed in the intervening forty-five years from 1946 to 1991? It is appropriate to state "the more things change, the more they stay the same". For you, professionals who combat the mosquito, much has changed, especially in terms of mosquito abatement, the public's trust of you and the public's perception of risk.

In 1988, Bruce Eldridge, in a presentation entitled "Conventional Chemical Pesticides for Mosquito Control: Past and Future", using the metaphor of the "pesticide ship" encountering an iceberg, said "We have hit the iceberg, but the band plays on". He ended his presentation warning:

> "Have we hit the iceberg? Perhaps not yet. But I'm putting away my instrument and putting on my life preserver."

In 1990, the iceberg loomed as Big Green, Proposition 128. Although you may have dodged it, remember: The mass of most icebergs lies just below the surface. The submerged iceberg hit the ship (with the ship's help) and it is sinking. It may not sink in 1991, but I hope your mosquito abate-

ment life preserver is close at hand. Some publics are telling you they lack trust in you and you are advised to abandon your familiar ship.

Just as the publics are powerful shapers of events and actions, they too are influenced by events, especially those most prominently presented in the media and most closely related to their health and safety. In the area of pesticides and pests, let me list some shaping events of the last decade:

-Mediterranean Fruit Fly, San Jose, 1981
-Mediterranean Fruit Fly, Los Angeles, 1981
-Mexican Fruit Fly, Los Angeles, 1981
-Japanese Beetle, Sacramento, 1983
-Gypsy Moth, statewide, on-going
-Farmworker boycott, nationwide, ongoing
-Watermelon and Temik, nationwide, 1986
-Proposition 65, statewide, 1986
-Killer Bees, Bakersfield and Mexico, ongoing
-Apples and Alar, nationwide, 1990
-Chilean grapes, nationwide, 1990
-DBCP and groundwater, statewide, ongoing
-NutriClean, Sacramento and Los Angeles, ongoing
-Mediterranean Fruit Fly, Los Angeles, 1989-90
-Big Green Initiative, statewide, 1990
-Big Brown Initiative, statewide, 1990
-"Arachniphobia" (the movie), nationwide, 1990
-Gerber Organic Baby Food, nationwide, 1991
-Gas masks on CNN reporters, 1991
-National Geographic article on California's Central Valley, 1991

That is a quick overview of the decade. A consequence of these events has been an acceleration in the change of the social definition of risk. While years ago risk may have been based on a chance model (i.e., taking the risk meant winning or losing), today risk means danger. Today, in 1991, the California publics perceive danger.

Please note I said publics, not public. All too often the public is thought of as a single, homogenous group. In fact, what exists are "California publics", some of whom are concerned about pesticides and others unconcerned. Here I want to distinguish among three roles these publics play. The first role is as voters. The second role, as economic consumer, is the most commonly understood role. The third role is as citizen and consumer and processor of information. Let me first discuss the most hallowed role of the public - the public as voters.

Public as Voters.

Big Green! You all know what Big Green is - not was, it hasn't gone away! Proposition 128 was soundly defeated in the November election by roughly a two-thirds majority. It was fought by chemical, oil, business and agricultural interests. Reasons for its defeat likely include, among others, economics and voter distrust of government. One conclusion may be that the public as citizen-voter expressed its will, saying that pesticides aren't all that bad, that the risks are overstated, and that the economic costs that might result are more important than the environmental and public health ones.

However, look more closely at the voting results. The press reported that only about 36% of all eligible voters exercised their right to vote nationally and in California. That is, roughly one out of every three persons voted. Big Green was defeated by almost a two-third to one-third vote. If my fractions are correct, one-third of all potential voters voted, and two-thirds of those voting were against Big Green; then only about 20% of all potential voters (or the public) expressed formal opposition to the Proposition. Conversely, 10% of all potential voters expressed their favor. Approximately 70% of all voters did not express a voting preference. For those of you with statistical backgrounds, I can suggest that this 70% resembles the 68% of any normally distributed population one standard deviation unit from the mean or average. Is it possible that this 70% not voting is a population one standard from the mean and that it lacks concern about pesticides? Or about the environment? Or about human health?

With these three groups, I can construct a pesticide risk perception continuum. One pole on this continuum is occupied by the 20% anti-Big Green group and on the other pole sits the 10% pro-Big Green group. Both are "no risk" groups: The 20%'ers may say that there is no "real" risk from pesticides, that the regulations and risk management programs ensure the public's safety, whereas the 10% say no level of risk, real or potential, is acceptable. Does this mean that the remaining 70% of non-voters are passive, disinterested and non-vocal? Some are, but many more are parents, homeowners and taxpayers. Many don't trust the government. Most have what they consider an "informed" opinion. Many can and will be moved to vote and to take other action, especially if they feel they are coerced into a situation without benefit to them or one with the

potential of ill effects.

Public as Consumers.

The second role for the public is as economic consumer, the most commonly understood role of the public. The economic consumer role can be a powerful one and many of the issues of pesticides expressed at a societal level are focused on this role. Alar, for instance, certainly jolted the consumer (as it did the apple growing industry) and temporarily, at least, resulted in a surge of interest and purchases of pesticide-free and organic food. In a recent but pre-Alar study, University of California researchers interviewed 795 consumers in terms of consumers' concerns about food quality and safety (Pastore and Bruhn 1991). Half of those interviewed indicated concern about food safety. Of those, nearly two-thirds expressed concern about the use of pesticides and herbicides on produce. It may be noteworthy that these fractions (one-half and two-thirds) again result in a percentage of 36% of respondents who express concern. Meats, fish and poultry were also suspected for possible chemical contamination. The researchers state that their "study reveals that consumer concerns about food safety are not simply the result of the Alar controversy. Rather, long-standing unexpressed concerns appear to have been brought to the surface by recent coverage in the mass media." Responses to this concern are visible. In late-1990, Gerber Baby Foods announced the marketing of a line of organic baby food.

Consumers also consume pesticides in another important way. It is estimated the California homeowners annually spend over $2 billion on lawn and home products including pesticides, fertilizers and similar products. In terms of consumers' use of pesticides, inconsistencies between their attitudes and practices have been noticed. A study of homeowners in Sacramento (Grieshop and Stiles 1989) reported significant positive correlations between perceived safety or danger of home use of pesticides and the users' risk taking behavior. However, there was still considerable risk taking (e.g., using stronger than recommended doses, not using safety equip-ment, etc.) among those who perceive great risk.

All consumers are not alike. They express a variety of attitudes, they perceive danger information differently, they possess different information, they trust and believe different sources and they follow different practices.

Public as Citizen Actors.

The third role, the public as citizen actors, I believe has special meaning for you. Big Green also provides entry into analysis of this role. Although Big Green was defeated, it hasn't melted away to nothing. If history is a valid predictor, this outcome is highly unlikely. Rather, Big Green will be chipped into smaller chunks that ultimately become law and regulation. An example illustrates:

Thomas Elias, writing in his November 1, 1990 syndicated column "California Focus", suggested that if the past is a teacher then the provisions articulated in Big Green will not go away. He points out that in the 1972 state election Proposition 9 called for a ban on DDT, a ban on lead gasoline, a moratorium on nuclear power plants as well as other provisions. Those who campaigned against Proposition 9 (chemical and oil companies, agricultural interests among others) argued: "This proposition has some good aims, but it's poorly drafted and tries to do too much. Besides, it would cost too much". Is there a sound of familiarity to this argument? Proposition 9 was soundly defeated. Nevertheless, by 1990 most, if not all of the proposition's provisions are in fact law.

Why did this happen? First, the problems addressed by Proposition 9 did not go away, and second, neither did the publics. Publics concerned with Proposition 9 provisions acted as citizens with values and preferences to mobilize others, including legislators, to implement the regulations and laws. You can expect that the provisions of Big Green will become regulations and law since the problems are not going to go away and neither are those citizens who move public opinion.

Who are these actors? Certainly not every person in California. Only about 36% voted and approximately the same percentage of a random sample expressed concern about pesticides and food. Are these citizen roles only to be played by those who supported Big Green? Opinion movers will come from the other groups as well; from those opposed to Big Green and many from the 70% group. However, the percentage of citizen actors who will play this role may be in the neighborhood of only 30 to 40%.

What do you have to think about in relation to this pubic role? I think you must consider what and who impacts their values, opinions and actions. And, ultimately you have to consider how you might impact them.

I already included an abbreviated litany of

memorable events that have impacted perceptions of California publics. Research findings on Californians' perceptions, attitudes, beliefs and trusted sources of information are also instructive. Findings reported in ground breaking studies by Hawkes and Stiles (1986a, 1986b) and based upon research in Mediterranean Fruit Fly, Mexican Fruit Fly and Japanese Beetle infested communities concluded that risk perception and acceptability associated with pesticides vary with the situation and the perceived benefits. For example, nearly 60% of the respondents believed, as home users of chemical pesticides, they were very safe in applying pesticides. Yet an equal percentage feared that agricultural workers were at risk in using chemical pesticides. Also, acceptance tended to increase when apparent benefits accompanied the pesticide use (e.g., eliminating insects in food). In addition, 77% of these citizens indicated the public should be willing to eat insect-free food sprayed with pesticides, although nearly 70% would not feel safe living next to cropland sprayed with pesticides (or land sprayed for mosquitoes?). In the mid-1980's a majority (57%) of this group of Californians acknowledged the necessity of continuing chemical pesticides for agriculture, but nearly 100% believed that biological controls should be used first and pesticides as alternatives.

The publics' perception of risk also influenced their trust and confidence in those persons responsible for managing hazards, including the use of pesticides. As reported by Hawkes and Stiles (1986a, 1986b), respondents, when asked to rate their trust of various actors, rated university scientists highest (a 48% "complete trust" rating and a 50% "some trust" rating) followed by state government such as CDFA (22% complete trust and 65% some trust); the federal government such as EPA (15% complete trust and 59% some trust); industry (9% complete trust and 41% some trust); advocacy groups such as PIRG's (22% complete trust and 59% some trust); local leaders (4% complete trust and 52% some trust); and the mass media (3% complete trust and 54% some trust).

Chances are that shifts in levels of trust have occurred in the intervening five years. Given recent events, ongoing debates and conflicting information from scientists and government, it is probable that trust in scientific and governmental experts (like yourselves) has been lowered. Depending on where the political lines are drawn and where the spraying boundaries are set, trust in expert groups will vary. With the events of the last ten years, one conclusion in relation to pesticides and the public must be, as Wildavsky and Dake (1990) argue:

> ". . . the great struggles over the perceived danger of technology in our time are essentially over trust and distrust of societal institutions, . . ."

You, as experts in mosquito abatement, are advised to be sensitive to the issue of trust and the three public roles and of your responsibilities to them. Possibly 30 to 40% of citizens are concerned enough with pesticides to express that concern to legislators, to the media, and to their fellow citizens. They will take action, they will seek and spread information, they will form groups, they will protest and lobby, and they will vote. The majority may not express such concern or take action for reasons of disenfranchisement, interest in other issues, or skepticism. But, then again they may, since perception of danger is selective and the objects of their attention may shift to pesticides.

Your perceptions of the problems of pesticides may differ dramatically from those of the active citizens, as will the criteria you use to assess and evaluate potential risks. You, as scientists, are trained to focus on numbers and statistical probability to calculate levels of risk, of safety, of costs, and of benefits of technologies like pesticides. Citizens may use other probabilities in combination with other criteria to calculate potential danger. In short, different world views are operational. Let me discuss a few of the criteria.

Is the risk voluntary? If citizens feel that they have little or no choice, they rebel. If they believe they are coerced into accepting risks from spraying, you can count on a negative reaction. Events and risks that are involuntary tend to be less accepted.

Is the risk controllable? Homeowners who use pesticides in their garden often believe they are at risk but since they are in control the danger is perceived to be reduced or even eliminated. However, if the citizen feels he or she is powerless to control potential hazard from spraying, then the reaction will be different. For example, the study participants mentioned earlier felt safe spraying at home, yet unsafe living next to sprayed crops.

Is the risk fair? Are costs and benefits equally distributed? Many residents of Los Angeles did not believe they would obtain any benefit from the spraying for the Med Fly. But they knew and

were told that major beneficiaries would be those in agriculture.

Is the risk natural? Few citizens will hold Mother Nature responsible for earthquakes or tornadoes for risk or harm. However, as has been seen, citizens are less accepting of risks from pesticides sprayed on Med Flies. A question: Which is more acceptable, the threat from mosquitoes or the threat from spraying?

Is the source of risk information trustworthy? This criterion brings us back home. Are you a trustworthy source of risk information on mosquitoes and on risks associated with the use of pesticides for mosquito abatement? Since many of you represent government and you may impose involuntary, uncontrollable risks on citizens, what makes you so trustworthy?

Your responsibilities include those of communicating with these citizens and effective communication is possible. Three points are noteworthy.

First, effective communication is much more than "talking at" people. It involves listening, comprehending, and responding. Effective communication is created and sustained as a two-way exchange process. People pay selective attention to risk and danger and to those who communicate with them. Beliefs, once formed, change slowly. Opinions and understanding are influenced by past experiences and events and by the views of persons important to them. There are audiences with whom you should talk. You would be ill advised to attempt to persuade them that risks do not exist or that there is nothing to worry about. Your attempts to "educate" the public with your "scientific facts" to make them change their beliefs and opinions will not be successful. Public relation efforts, using more facts, or talking louder will not work (Hance et al. 1988).

Second, research on what risks are perceived and how citizens react to risk messages related to mosquitoes is needed. You have unique opportunity to do firsthand information gathering every time you encounter your publics. Those encounters are also opportunities to carry on effective communication.

And third, you need to act if you believe that the public (especially that 30% public) is not a single group. There are different "species" of publics as there are of mosquitoes. Publics vary on sociodemographic, personality, and cultural variables. And they will also differ on the relative weight they assign to risk criteria (Kraus and Slovic 1988).

It is well known that cultural symbols trigger responses (Fitchen 1987). The apple, the mosquito and pesticides are each cultural symbols (I have yet to encounter anyone who views the mosquito as something positive and valuable). Chemical pesticides, as symbols, can be either positive or negative. But, if you mix apples and pesticides (as did the wicked witch in Snow White and in the Alar case) a negative symbol is created and a danger results. Now, if you mix mosquitoes and pesticides, what is the resulting symbol? Is it negative or is it positive? You have an intriguing symbol. Your enemy, the mosquito, is also your friend; at least it can be in the context of your efforts to effectively communicate with citizens.

The mosquito, as with much of your work, has been hidden away in California. Periodic mosquito-related human health problems crop up in urban areas and a flurry of concern is expressed. But as rural California urbanizes, the mosquito and you will become more prominent. Both your mission and the public will be ill-served if you communicate only the dangers of the mosquito while emphasizing the miracle of chemical pesticides for controlling the mosquito. Scare tactics may work in the short term and with some citizens, but not with all and not for long. Some of you may enter neighborhoods, play sites, and work sites to do your job. Citizens you encounter need to know you are on their side. They need to know about biological and non-chemical methods for control. Many of you are aware of something called integrated pest management (IPM). You can inform them. You also need to pursue integrated practices for the control of the mosquito. You need to both do good and to communicate well.

Even though your approaches may be distinct from those used in agricultural pest control and the pesticides you utilize are truly different, you will nevertheless be cast into the same boat as others using chemical pesticides. Consequently, you will find yourself confronting more icebergs. As professionals you may want to take a close look at those icebergs before trying to dodge them. Those icebergs may be piloted by citizens who are not questioning your knowledge. But they may be questioning what confidence they can have in you and the credibility of your information. Your answers and responses to those questions may well determine your survivability.

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CHANGES ON THE HORIZON

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A great deal of attention is being focused on the year 2000 because it signals the turn of the next century. Planning processes have started for Year 2000 Health Objectives; but before we get there, we must cross the decade of the 1990's. I believe significant changes will transpire during the 1990's that will have a profound impact on how we perform our work tasks.

The year 1990 is behind us, most of the pages for 1991 are blank, and it is that way throughout the rest of this decade. We have the opportunity to fill in those pages through an aggressive and active approach of carrying out our responsibilities, or we can sit back and react to the hand that is dealt to us. I'm here to encourage the former because for too long we have been victimized by the latter. To a great extent, the future is what we choose to make of it. For many, this will be a change, but change is all about us, and that may be the certainty of the early years of this new decade in which we find ourselves. I want to touch on a few issues that I see impacting our programs and offer some thoughts on changes we may need to make.

At the federal level, we concluded on September 30, 1990 the federal fiscal year with a deficit of $220.4 billion, the second highest ever. This also marked the twenty-first straight year in which the government spent more than it took in. At the state level, we have current revenue projections that place the State in a deficit mode somewhere between $6 and $10 billion. Locally, several counties are struggling to survive.

Change, and I don't mean the pocket variety, is needed. Governor Pete Wilson, in his release of his first proposed state budget, showed that changes are taking place. He has offered a new spirit of cooperation in working with the Legislature, and this is a healthy first step. He has also provided new ideas to deal with the bleak job of balancing the budget for next year, and this has won him praise from members of both parties, even though not everyone is happy with his proposals. One important change is his stated focus on prevention. This, I believe, will have a tremendous impact on our field of environmental health.

At the heart of environmental health service delivery is the prevention of disease or illness and the enhancement of the public's well being. No matter where environmental health programs are located in the structure of government, from state services to local mosquito or vector control districts, they are part of the public health family.

Mosquito and vector control programs, as well as all environmental health services, are designed to promote health protection rather than treatment and, as such, constitute the first line of defense in a preventative approach to public health protection. We must take advantage of the new governor's awareness of prevention and emphasize the preventative nature of our programs. It is generally recognized that a preventative strategy is less expensive in the long-term than is a strategy based upon treatment methodologies. We must promote this linkage of environmental health service delivery to prevention because there is no way we can effectively compete for funding with the other health issues of the day such as "drug babies" and AIDS. We must act on the change in focus that this governor has proposed in his first weeks in office.

I envision that this year or next will bring about shifting of some services from the State to local governments and new methods of funding local programs. Governor Wilson has proposed allowing counties to raise their sales tax by one-half a cent. Across the state, this could raise approximately $1.5 billion in new funding revenues. Special districts must not be left at the starting gate but actively campaign for their share of these revenues should this proposal be enacted.

Tight fiscal times require us to use our

resources to the best of our abilities. Private industry has been faced with a similar problem in competing with foreign industry. The California Department of Health Services (CDHS) has taken the cue from private industry and begun a program of quality service delivery. I'm pleased to say that the Environmental Health Division (EHD) is recognized as one of the leaders in quality within CDHS. You may have seen the new Cadillac commercials stating that they are the 1990 Malcolm Baldridge Quality Award winners in the United States. More and more, companies are emphasizing their move to total quality management (TQM) that stresses satisfying the customer. This focus on quality does not belong only to industry but is being implemented at the federal and state levels of government as well. Most federal agencies have begun TQM efforts and, at the state level, the Departments of Motor Vehicles, General Services, and Health Services have initiated quality programs.

I mention this new focus on TQM because studies have shown that re-work accounts for 25 to 60 percent of our work effort. Total quality management focuses on the process and in empowering staff to do their job right the first time and every time. In EHD, we have developed a two-day TQM training course that is proving to be very effective. Managers in both private and public organizations are catching the "quality bug", and those that don't may not survive. I look at all of you as some of our customers, and if we can help any of you in this quality arena, please let me know. It is the process that an American, W. Edwards Deming, took to Japan in 1950, and it has made the Japanese the industrial power they are today. This then marks a second area of change, the change to quality in the services we provide to our customers.

Many of you are familiar with setting goals and objectives, some may have even created mission statements for your organizations. What has been lacking for most, however, is a vision. A vision statement provides the core values for those involved in delivering the services we provide. The vision provides a definition of our customers and what services, in a broad context, are to be provided. It further provides a litmus test for proposed new services and existing activities. If the services don't contribute towards accomplishment of the vision, they should not be conducted. Whereas mission statements can change as conditions change, a vision should remain constant and project into the future for decades to come. The vision should also serve as a source of pride for those involved in its delivery, and it can be used to inspire others to assist in making it a reality.

The following is the vision for EHD, but I urge you to consider adopting it or something similar. This, then, could serve as the vision statement for environmental health service delivery throughout California:

> To provide for us, for our parents, for our children, for all Californians' health. Health in our foods, our industries, our homes, our recreation, and our environment. Health to enjoy the never-ending miracle that is California.

You and agencies throughout California could focus your activities towards meeting this vision. Service providers in traditional health departments, comprehensive environmental health agencies, building departments, mosquito abatement districts, planning agencies, and state departments could all contribute towards achieving this vision. We need to design specific missions and goals and objectives that will contribute towards its accomplishment. The recognition of a need for a vision and the adoption of a vision statement for the environmental health service delivery system in California is the third change I see on the horizon.

It must be recognized that there is a multiplicity of governmental agencies that provide some form of service that could be classified as being in the environmental health service realm. The questions which must be asked are whether these agencies maintain a health conscience in setting and delivering their services and whether coordination with the public health family exists. Some agencies such as mosquito and vector control districts can proudly answer in the affirmative to these questions, while others have subordinated the health aspects of their programs to the back burner.

You have a proud heritage of working on prevention of plague illness since the early years of the 1900's and in mosquito-borne illness prevention for 75 years. Your efforts have contributed to the increase in the average life span of Californians from 49.8 years at the turn of the century to the present life span of 75.6 years. Environmental health practitioners should feel proud of our accomplishments and that we are part of the delivery team that has been improving the quality of life in California since 1850.

The coordination issue is one that all agencies

need to concentrate on in order to improve the environmental health delivery system. Governor Wilson has taken what could be a bold first step in the direction of coordinating service with his announcement of plans to form a California Environmental Protection Agency (Cal-EPA). An environmental health presence within Cal-EPA must be at a high level within the organization to ensure that the public health voice is heard. This lack of a health protection need was a problem in the early days of the federal EPA, and steps need to be taken to ensure that it is not repeated in Cal-EPA.

One of the driving forces behind the creation of Cal-EPA is the concern about pesticide registration, its use and monitoring. This will be a point of focus in the early days of Cal-EPA. Again, this change impacts most of your organizations. We must ensure that there is a recognition of the need and importance for public health pesticide use by governmental agencies. Use of these pesticides must be allowed when the risk from the public health threat is greater that the risk from pesticide use.

The change in organizational structure at the state level through the creation of Cal-EPA is the fourth change that I envision taking place in the near future. If done properly, this change can strengthen our environmental health delivery system through improved coordination and focus on environmental health.

I have provided you with four areas of change that, if they come to fruition, will impact your organizations for years into the future. The four changes, in summary, are:

1. A focus on prevention and the need for those providing environmental health services to get their message of being the first line of a preventative health program into the minds of key decision makers and the public.
2. A change to quality-based service. We can no longer compete with an attitude of "business as usual". We must move to provide quality service to our customers that goes beyond satisfying them, but delights them with service far greater than they were expecting.
3. The need for a vision to guide our programs into the future. I would like you to join me in adopting the vision:

   > To provide for us, for our parents, for our children, for all Californians' health. Health in our foods, our industries, our homes, our recreation, and our environment. Health to enjoy the never-ending miracle that is California.

   You are important in making that vision a reality. It is about us and those we love. It is about our work environment, our recreation, and the environment of California. It is about the society we serve and the quality of life we enjoy.
4. The creation of Cal-EPA could shift our working arrangements while allowing improved coordination of the state agencies involved in the environmental health delivery system. The environmental health program must be at a high level in Cal-EPA so that the public health voice on issues is heard.

These are the changes on the horizon. They have the potential for changing the way environmental health services are conducted in California for years to come. Change often brings about anxiety, but I think we should view it as an opportunity to take action and improve our methods of providing services to our customers. This is a time of excitement as we fulfill the vision of providing health services to allow the enjoyment of the miracle that is California.

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KEEPING UP WITH A CHANGING ENVIRONMENT THROUGH COMMUNITY AND PROFESSIONAL GROUP EDUCATION PROGRAMS

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When I ask some district managers if they have a community education program, I usually get one of three responses. The first group will give me a look of fear or confusion. They say "We don't have anyone who knows anything about community education", or "We don't have any idea of what to do". A community education program can start simply by writing a new brochure or updating an old one. Our "blue sheet", which tells what a vector is, lists our services and the telephone numbers of other related county agencies (i.e. agriculture commission and county animal control) and is mailed or handed out to everyone who request our services. This has been a great way to introduce ourselves to the public.

The next group of managers will look at me like they just ate a bowl of cold, smashed peas with a warm buttermilk chaser. They have a real distaste and dislike of "community education". They will tell me they don't need a community education program; they only need to deal with the public when they answer service requests. Silence isn't golden anymore. Taxpayers are more informed consumers today. They are aware of the tax dollars that are going out, and they want to know where the dollars are going and what they are getting for those dollars.

The third group are the managers who smile at me and say "Oh yes, we have someone who goes to the schools when they call". That's terrific, but it's only a beginning. The kids of today are the adults of tomorrow, and I believe by educating them now, they can make our jobs easier by having a more knowledgeable understanding of what we do and how we do it.

I am a firm believer that community education must go beyond the schools.

A comprehensive community education program must also include the community and professional groups in your county (Table 1). Since June of last year, our district has given over 90 presentations. Only 21 of these presentations were to schools. The rest were given to medical personnel (such as hospitals and clinics), city and county agencies (like the park rangers and code enforcement officers) and other community service groups (including wildlife rescue and the people from the open space districts).

The above numbers do not include the displays we had at the various health, science and children fairs, including our county fair. Our first participation at the county fair was in 1989. We had over 7,000 people visit our booth. The following year (1990) we almost tripled that number with over 20,000 people visiting our booth. Many of the visitors were returnees from the previous year and commented they had utilized our services throughout the year. One gentleman walked up, looked at the sign and said "Vector Control, I've heard of you. You're one of the few county agencies not on the taxpayers' hit list".

Our district presentations cover many areas of vector control, including mosquito and rodent control, head lice, and lyme disease prevention. Most importantly, these presentations let the public know who we are, our purpose and what services we offer.

Our district started a community education program because we evaluated our total program and realized we had the technical expertise, but we had no way to get information out to the public. Few people knew who we were. Even people within our own county government didn't know who we were.

A community education program can help address and dispel many myths, misunderstanding

Table 1. Types of community education and targeted audiences of the Santa Clara County Vector Control District's Community Education Program.

Type of Education	Target Audiences									
	City Councils	Health Depts.	Parks Depts.	Other County Agencies	Hospitals /Clinics	Schools	Civic Groups	Neighborhood Organizations	Industry	General Public
General Vector Control Brochures	X	X	X	X		X	X			X
Lyme Disease Presentation		X	X	X	X	X	X		X	X
Head Lice Presentation		X				X		X		X
TV, Radio, Newspaper	X	X	X	X	X		X	X	X	X
Door-Hung Brochures								X		
Rats & Mosquitoes in Mobile Homes								X		
Stand-up Visual Displays		X						X		X
County Fair Exhibit		X	X	X			X	X	X	X
Vector Control Awareness Weeks or Months	X	X					X	X		X

and worries people have regarding vectors. Just a couple of weeks ago, I received a call from a school Health Aide, who was putting out a newsletter on head lice. She called to verify some of the things she had heard. She had been told by the principal of the school that head lice do jump, and they can be transmitted by students being outside on a windy day! Can you imagine the panic that newsletter would have caused if it had gone to all the parents in that district with that information?

People are also very concerned about over-exposure to pesticides and other chemicals. Residents want to know what we're using to control mosquitoes. Is it harmful to them, their pets or the environment? If this information is available to them, many fears and misunderstandings can be corrected or even avoided.

If you contact other county and city agencies and let them know of your services, you may find some resources that could make your job easier. One example is the water district. By working with the water districts, waterways can be kept clear of debris which may minimize, and even eliminate, mosquito sources. Code enforcement is another good resource. If you teach them what constitutes rodent and mosquito sources in residential areas, they may be able to eliminate many problems for you when they do their inspections.

Whether you district chooses to be proactive or reactive, you really need to have some type of community education program. In this day of informed consumers, maintaining a low profile will not work.

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MARK-RELEASE-RECAPTURE STUDIES WITH *CULEX* MOSQUITOES ALONG THE KERN RIVER, 1990

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An outbreak of St. Louis encephalitis (SLE) occurred in Kern, Kings and Tulare Counties in 1989. Detailed data on this outbreak were presented at the 1990 CMVCA conference. Briefly, the first evidence of SLE activity was the finding that six chickens in a flock at Belridge on the west side of Kern County had antibody to SLE virus when bled on July 11. This was an area from which no pools of mosquitoes had been submitted for testing. The following week, SLE virus was detected in three pools of *Culex tarsalis* Coquillet collected on July 19/20 along the Kern River at the southwest edge of Bakersfield. By the end of the season, 71-100% of the chickens in nine Kern County sentinel flocks had been infected with SLE virus and SLE virus had been detected in 70 pools of *Cx. tarsalis*, 14 pools of *Culex quinquefasciatus* Say, and 1 pool of *Culex stigmatosoma* Dyar collected in Kern County (Table 1).

Although the minimum infection rates per 1,000 mosquitoes for the year look similar for the three species, *Cx. quinquefasciatus* and *Cx. stigmatosoma* were not submitted for testing until after the first SLE-positive *Cx. tarsalis* pool was detected, and therefore do not include negative pools collected from April through mid-July. There were 15 human cases of SLE in Kern County, with dates of onset from August 22 to October 8. An SLE outbreak of this magnitude had not occurred in the San Joaquin Valley since 1954.

The initial detection of SLE virus infections in mosquitoes and sentinel chickens in the Bakersfield area was associated with increases in mosquito abundance along the Kern River channel, even though this portion of the river remained dry throughout 1989 (Fig. 1). Infected mosquitoes also were collected from residential areas of suburban Bakersfield adjacent to the river, indicating that the riparian vegetation along the river channel may have functioned not only as a resting refuge and a hunting area for avian bloodmeals, but also as a flight corridor to suburban and urban environments for female mosquitoes produced in nearby irrigated agricultural sources.

To test this hypothesis, we carried out a series of mark-release-recapture experiments in 1990 to study the population ecology and dispersal of *Culex* mosquitoes along the Kern River and into associated housing areas.

The study area consisted of a 12 km section of the Kern River channel southwest of Bakersfield (Fig. 2). The immediate surrounding area primarily was undeveloped riparian habitat used for aquifer recharge during wet years. Residential areas and the California State University campus bordered the river at the eastern end of the study area, and additional housing was located north of Stockdale Highway. Mosquito production was mainly from adjacent cotton and alfalfa fields and from wastewater ponds to the southwest. The Kern River was dry throughout 1990.

Five mark-release-recapture experiments were carried out from May through September. Most mosquitoes for release were collected as pupae or late instar larvae at sources on the valley floor, but supplemental collections of *Cx. tarsalis* were made at a foothill site during all months except June. Adults emerged into marking containers, were counted by the strip method, marked with date- and site-specific fluorescent dust and released in the late

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Table 1. SLE-positive mosquito pools, Kern County, 1989.

Species	Mosquitoes tested	Pools tested	SLE-positive pools
Cx. tarsalis	14,917	329	70
Cx. quinquefasciatus	2,978	65	14
Cx. stigmatosoma	59	4	1

afternoon when less than 24 hours old. Subsamples of each release cohort were anesthetized with CO_2, sorted to species and counted to estimate the proportion of each species released. Up to 50 *Cx. tarsalis* females from each cohort were held for at least five days on 10% sucrose and then dissected to determine autogeny status. Autogeny rates varied from 0 to 84% for populations from the valley floor and from 3 to 100% for those from the foothills. This extreme variability was unrelated to ambient temperature or time of year.

The majority of the mosquitoes released were *Cx. tarsalis*, except in September when approximately 90,000 *Cx. quinquefasciatus* were released (Table 2). Male and female mosquitoes were released in equal numbers, but our recapture methods were aimed primarily at the recovery of females. Overall, 109,110 *Cx. tarsalis* and 112,547 *Cx. quinquefasciatus* were released, of which 1,451 and 384 were recaptured, respectively. Releases were made at two points in the river channel (R1 and R2 in Fig. 2). In September, nearly 25,000 *Cx. quinquefasciatus* were released in the center of the northern residential area (R3 in Fig. 2).

Detailed analyses of the data are still in progress. Preliminary horizontal estimates of daily survivorship, based on the recapture of marked females, range from 60 to 74% for *Cx. tarsalis* and from 65 to 81% for *Cx. quinquefasciatus*. Parity rates, based on the dissection of unmarked females collected during the recovery efforts, were 58 to 77% for *Cx. tarsalis* and much lower (11 to 35%) for *Cx. quinquefasciatus*. Lower parity rates for *Cx.*

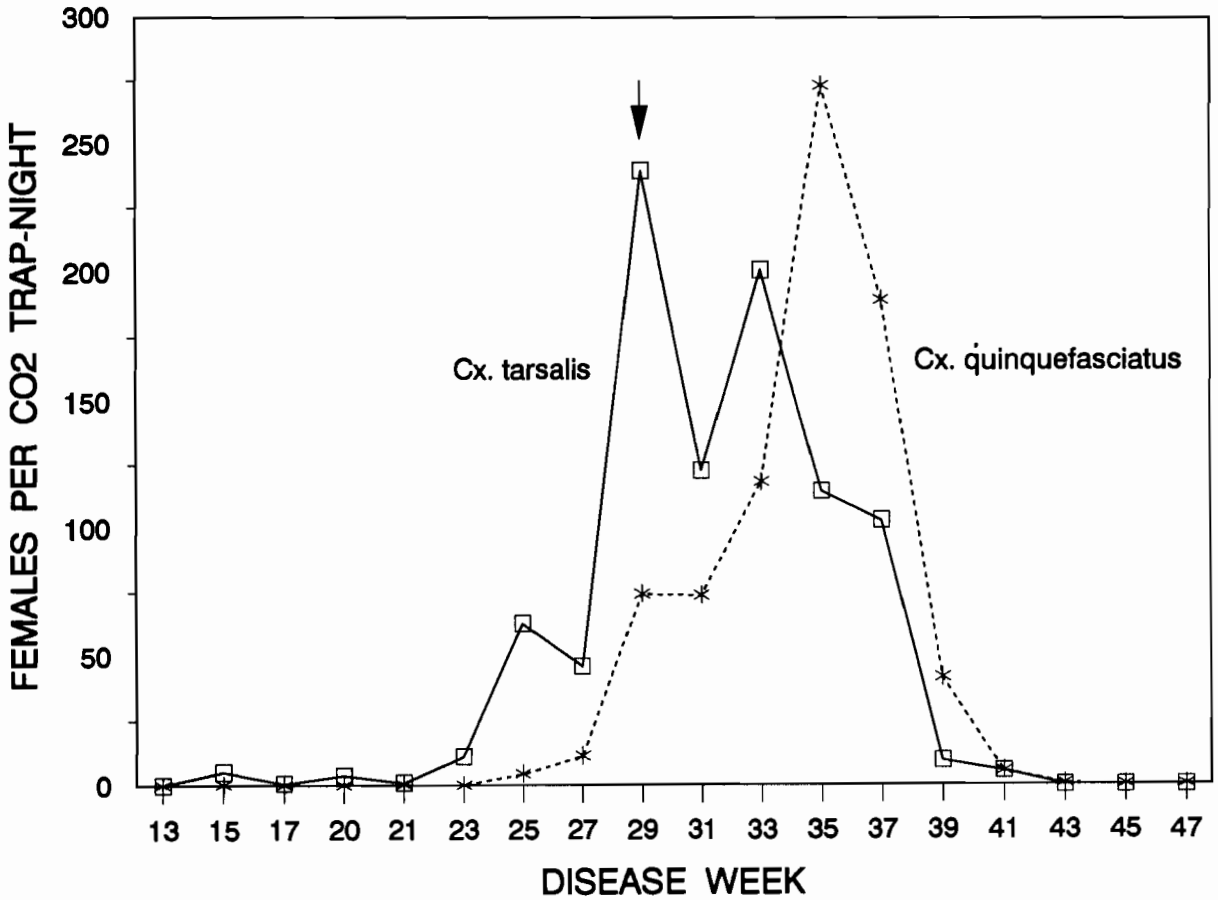

Figure 1. Abundance of *Culex* mosquitoes along the Kern River during 1989 with the week of the first SLE-positive mosquito pool indicated (arrow).

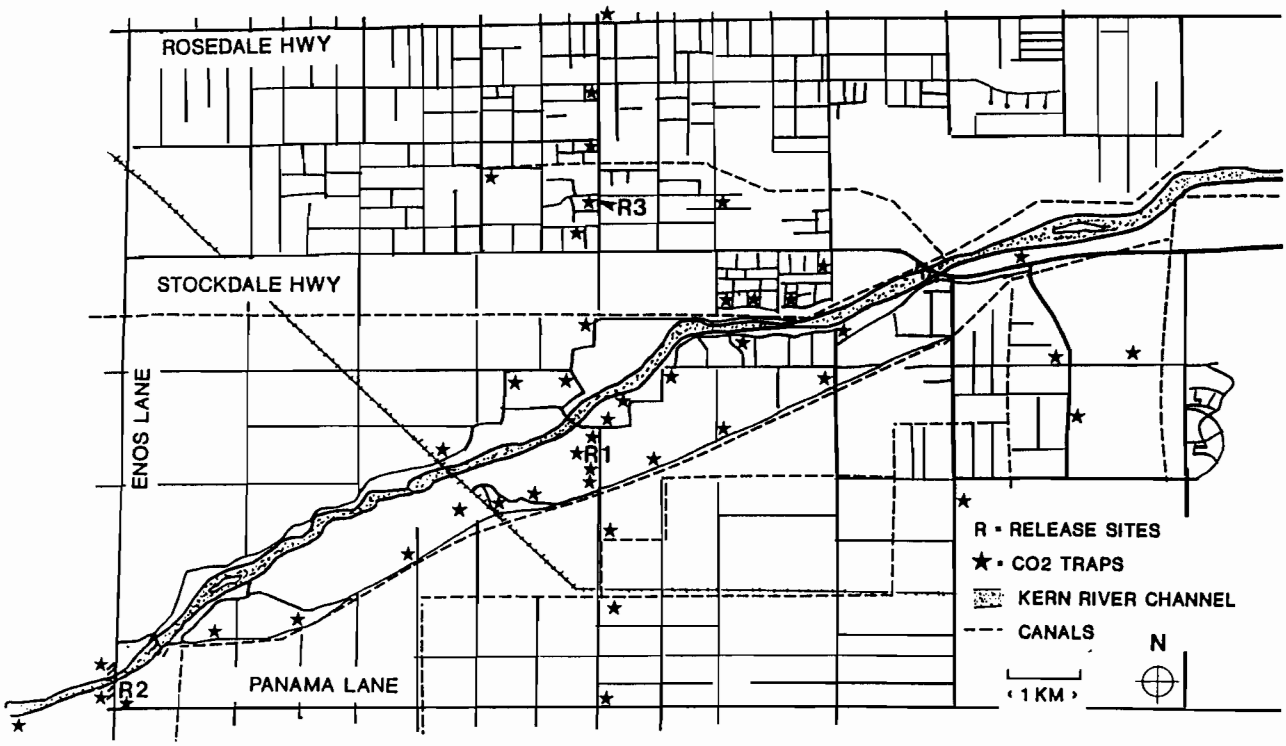

Figure 2. Map of the study area southwest of Bakersfield. R1, R2 and R3 indicate sites where mosquitoes were released. Stars indicate the locations of CO_2 traps.

quinquefasciatus may indicate significantly lower survivorship, unless their gonotrophic cycles were much longer than the 4-5 days usually estimated for *Cx. tarsalis*. It would appear that this, in fact, was the case because only 2 of 348 *Cx. quinquefasciatus* females were parous when recaptured on days 8 and 10 post-release in June. In contrast, parous *Cx. tarsalis* were recaptured frequently in all months and as early as day 4 after release in July when autogeny rates were highest. The monthly pattern of parous recaptures for *Cx. tarsalis* indicated that the gonotrophic cycle may have taken longer at cooler temperatures in May and September than in midsummer.

Preliminary population estimates indicated a peak of 150,000-200,000 *Cx. tarsalis* females in the

Table 2. Results of *Culex* release studies at the Kern River, May through September, 1990.

Species	Sex	Number released	Number recaptured	Percent recaptured
Culex tarsalis	Female	55,673	1,343	2.4
	Male	53,437	108	0.2
Culex quinquefasciatus	Female	56,060	348	0.6
	Male	56,487	36	0.1
Culex stigmatosoma	Female	466	0	--
	Male	723	0	--
	Totals	222,846	1,835	0.8

vicinity of R1 and R2 (Fig. 2) in July and August, with a major decline in numbers in September. On the other hand, the population of *Cx. quinquefasciatus* females in the same area peaked at about 200,000 in September. Our sampling emphasized the attraction of host-seeking females to CO_2 traps, and appeared to be most effective when populations were most dense.

As anticipated, dispersal tended to be along the river channel. The maximum recapture distance for a *Cx. tarsalis* female was 11.9 km from R2 upstream to trap 19 at housing near the Stockdale Highway bridge two days after release. The maximum recapture distance for *Cx. quinquefasciatus* was 10.9 km from R3 to R2 at the downstream end of the river channel six days after release. Fewer than 1% of the *Cx. tarsalis* recaptures were made in the residential areas. In contrast, nearly 8% of the recaptures of *Cx. quinquefasciatus* released in the river channel were made at the traps located in the suburban housing areas. However, when *Cx. quinquefasciatus* females were released in the residential area, 4% of the subsequent recaptures were made in traps along the river channel. Traps in or adjacent to irrigated agricultural fields accounted for 5% of *Cx. tarsalis* recaptures and 7% of *Cx. quinquefasciatus* recaptures. We anticipate that further analyses will clarify the rates of migration for both species between residential, agricultural and river channel settlement areas.

Acknowledgements.

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THE EFFECT OF *PLASMODIUM FALCIPARUM* INFECTION ON THE FEEDING BEHAVIOR OF WILD, NATURALLY INFECTED ANOPHELINE MOSQUITOES IN KENYA[1]

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ABSTRACT

The feeding behavior of wild *Anopheles* mosquitoes was observed in the field. Wild mosquitoes were collected by using humans as bait when the mosquitoes came to bite at night. Anesthetized hamsters were exposed for a period of ten minutes to individual mosquitoes held in specially designed cages while an observer described their feeding activity using a tape recorder. Mosquitoes were dissected the following morning and the salivary glands were examined for malaria sporozoites. The feeding behavior was compared between infected and uninfected groups of *Anopheles gambiae sensu lato* and *Anopheles funestus*. The number of probes and total probing time was significantly greater for the *Plasmodium falciparum*-infected *An. gambiae s.l.* (mean = 4.0 probes and 277 seconds probing time) than their uninfected counterparts (mean = 2.4 probes and 214 seconds probing time). Results for the smaller number of *An. funestus* which actually fed, followed the same trend. A higher proportion of infected *An. gambiae s.l.* than uninfected individuals of that same species landed on and attempted to feed on hamsters.

These findings show that natural malaria infection modifies feeding behavior of *Anopheles* females. Increased duration of host-vector contact by infected *Anopheles* may enhance malaria transmission, and our results may have important implications with respect to the epidemiology of human malaria.

[1]An expanded text of this study has been submitted for publication in the American Journal of Tropical Medicine and Hygiene.

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ADULT POPULATION DYNAMICS OF *AEDES DORSALIS* IN A NORTHERN CALIFORNIA TIDAL MARSH

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ABSTRACT

The dynamics of adult female *Aedes dorsalis* (Meigen) were studied in a tidal salt marsh in Contra Costa County, California during July and August of 1990. Changes in the relative abundance of host seeking females were monitored for 23 consecutive days using CDC traps. Parity rates were estimated from subsamples of females which were dissected and their parity state determined using the tracheal skein method. The estimated numbers of nulliparous and parous females collected per day were used to estimate the population parity rate, gonotrophic cycle length and daily survivorship using two methods.

The total number of females collected per day fluctuated greatly, from a low of 261 to over 74,400. Numbers of nulliparous females rose sharply on day 5 and remained high until day 12. This increase was followed 5-7 days later by an increase in numbers of parous females; suggesting that these parous females were members of the same cohort resuming host seeking after completion of their first gonotrophic cycle. The gonotrophic cycle was estimated to be 5-7 days long based on the lag between peak numbers of nulliparous and parous females in the CDC traps. The study-long population parity rate was estimated to be 0.14 with daily survivorship estimated to be 0.67-0.75.

Duration of the gonotrophic cycle and daily survivorship were also estimated using time series analysis. Large fluctuations in the numbers of parous and nulliparous females per day resulted in the correlations used to estimate these parameters being extremely low. In spite of the low correlations, this method yielded identical estimates of gonotrophic cycle length, population parity rate and daily survivorship suggesting that this method may yield valid estimates of these parameters in spite of large fluctuations in population abundance.

The 5- to 7-day gonotrophic cycle length estimates for this study are similar to previous estimates of 5 days obtained for the closely related species *Aedes melanimon* Dyar in the Sacramento Valley of California. However, the daily survivorship estimates for *Ae. dorsalis* (0.67-0.75) are substantially lower than survivorship estimates obtained for *Ae. melanimon* using similar methods. These data suggest that in spite of having similar gonotrophic cycle estimates, the *Ae. dorsalis* population has a substantially shorter adult life expectancy than *Ae. melanimon*. This would likely result in *Ae. dorsalis* having a lower capacity to serve as a vector of arboviruses than *Ae. melanimon*.

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LAKE VERA REVISITED: STUDIES ON THE POPULATION BIOLOGY OF *ANOPHELES PUNCTIPENNIS* IN THE SIERRA NEVADA FOOTHILLS OF CALIFORNIA

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ABSTRACT

The population biology of *Anopheles punctipennis* (Say) was studied at Lake Vera, Nevada County in the Sierra Nevada foothills of California during 1990 as part of an ongoing research project investigating the biology, ecology and genetics of potential malaria vectors in California. Lake Vera was the site of an outbreak of *Plasmodium vivax* malaria involving a single source with 35 subsequent cases acquired during the summer of 1952. *Anopheles punctipennis* and *Anopheles freeborni* Aitken were both present when the outbreak was investigated so one or both species may have been responsible for transmission. The objective of the current study was to examine the adult population dynamics of *Anopheles* spp. present at Lake Vera and assess their potential to serve as malaria vectors based on relative abundance, blood feeding frequency, female survivorship and the prevalence of parous individuals in the population.

Human bait landing collections were conducted for twenty consecutive evenings during August and September, 1990, with tracheal skein and ovarial dilatation methods used to determine the parity state of individual mosquitoes. Parity rate estimates were used to estimate numbers of parous and nulliparous females in each collection which were subsequently used in a time series analysis to estimate the duration of the gonotrophic cycle, adjust the parity rate to account for differences in rates of recruitment and estimate daily survivorship.

Anopheles punctipennis was the most abundant species in the landing collections, constituting over 94% of all individuals with only three *An. freeborni* females collected. The parity rate of *An. punctipennis* was estimated to be 0.82 during the study period. Time series analysis yielded an adjusted parity rate of 0.79, a gonotrophic cycle estimate of 3 days and a daily survivorship of 0.92. Over 48% of females on which the ovarial dilatation method was used to determine parity state were found to be multiparous with females having up to eight dilatations per ovariole; suggesting that females had completed up to eight gonotrophic cycles prior to being collected. These results suggest that this population had a high biological potential to serve as malaria vectors based on high population parity rates, the high prevalence of multiparous individuals in the population, a high daily survivorship estimate and a 3-day gonotrophic cycle length estimate. These findings are consistent with the hypothesis that *An. punctipennis* may have played an important role in the transmission of malaria in California.

Larval collections were made from August 27 to November 6, 1990 in Lake Vera and Rock Creek which feeds and drains the lake. *Anopheles punctipennis* was the most abundant anopheline mosquito present in both the lake and creek (98% of all adults reared from the larval collections). The remaining 2% were identified as *Anopheles franciscanus* McCracken. First instar larvae were present in all collections including the collection made on November 6; suggesting that gonoactive females were present in the adult population as late as November.

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WING-SCALE PATTERN VARIATION IN *ANOPHELES PUNCTIPENNIS*

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ABSTRACT

The pattern and color of scales on the wings of anopheline mosquitoes are important characters for distinguishing species. As with many anatomical characters, much intraspecific variation may be encountered when numerous specimens are sampled throughout a species' range. During an investigation into the population biology of *Anopheles punctipennis* (Say) at Lake Vera in Nevada County, California, we observed substantial variation in wing-scale patterns. In several instances, the specific identity of some individuals was inconclusive, since they lacked wing-scale patterning associated with this species. Many individuals matched the description of a closely related species, *Anopheles perplexens* Ludlow. *Anopheles perplexens* is distinguished from *An. punctipennis* by a difference in the relative size of the sub-costal pale spot (SCP). The ratio of the length of the SCP to the length of the dark scaled area between SCP and the apical pale spot (AP) is reported to be ≥ 0.50 for *An. punctipennis* and ≤ 0.33 for *An. perplexens*. The eggs of both species differ in appearance and 4th instar larvae differ by the number of splits of seta 2 on abdominal segments IV and V.

Variation in SCP ratios of anopheline mosquitoes at Lake Vera raised the possibility that two or more species were represented in our study on the biology of *An. punctipennis*. The objective of this investigation was to determine whether the SCP ratio was a useful character for indicating the presence of *An. perplexens*, or other cryptic species, in collections of *An. punctipennis*.

Anopheles punctipennis were collected from three locations in California and from Allerton Park near Monticello, Illinois. Only the left wing of females was used to determine SCP ratios. To assess intraspecific variation, SCP ratios were also determined for individuals from single family lines.

Since variation in wing scale patterns appeared to be greatest in individuals collected from Lake Vera, an enzyme electrophoretic analysis was done on samples from this location in order to identify any population substructuring or cryptic species.

The SCP ratio was highly variable in all four samples of field collected *An. punctipennis* with most individuals having ratios that were between 0.33 and 0.50. From 12-23% of the mosquitoes had SCP ratios that corresponded to those expected for *An. perplexens*. The SCP ratios obtained from single family progeny were as equally variable as those obtained from individuals collected in the field, demonstrating that the variability observed in the field could be ascribed to intraspecific variation.

There was no evidence from the examination of eggs or larval chaetotaxy to support the hypothesis of two or more species at Lake Vera. Although the range of SCP values was greatest for samples from Lake Vera, the expected frequencies of genotypes at eight polymorphic enzyme loci were not significantly different from those expected for a single randomly mating population.

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GENETIC DISTANCE AND POLYMORPHISM OF *ANOPHELES PUNCTIPENNIS*

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ABSTRACT

The purpose of this investigation was to study the population genetics of *Anopheles punctipennis* (Say) in order to determine the presence of cryptic species and measure intraspecific genetic variability. Since there is the possibility that *An. punctipennis* is a complex of cryptic species in North America, we have obtained, for comparative purposes, samples from Texas, Washington, Maryland, Illinois, Mississippi, Wisconsin, Mexico and various locations throughout California. Genetic profiles within and between samples were made by comparing polytene chromosome banding patterns, electrophoretic zymograms, and rDNA sequences.

To date we have found only 2-3 polymorphic inversions in samples of *An. punctipennis* collected in California. Both of these inversions are on the right arm of chromosome III. A small sample of *An. punctipennis* from Illinois, however, had at least five of the eight polymorphic inversions that have been described for this species.

Genetic variability has been estimated for 20 enzyme loci. For all samples, the mean number of alleles per locus is approximately 2 and the percentage of loci that are polymorphic ranges from 20-40 percent. Overall, the samples from California have fewer polymorphic loci than samples from other areas of North America. Samples from Lake Vera and Willits, California had the lowest heterozygosity values (0.083-0.109), whereas a sample from Capitola, California had the highest (0.141).

Genetic distance coefficients of 0.01 between samples within California indicate conspecific genetic variability. However, Genetic distance coefficients between samples from different geographic regions of North America ranged from 0.17-0.35. Such high coefficients suggest that *An. punctipennis* is more than one species. A cluster analysis, based on genetic distance values between all pairs of samples, groups *An. punctipennis* into three distinct geographic regions of genetic similarity. Samples from Mexico and Texas form one cluster that is more similar to a second cluster including Maryland, Wisconsin, Illinois and Mississippi. The third cluster includes samples from California and Yakima, Washington.

The sequence of 600 bp in the 28S region of rDNA from *An. punctipennis* (sequenced by Frank Collins and Chuck Porter at the CDC in Atlanta, Georgia) differs by 11 base-pairs between samples from California and Wisconsin. This amount of nucleotide substitution is consistent with that found interspecifically in the *maculipennis* complex.

In summary, there is good chromosomal, enzyme electrophoretic and DNA sequencing evidence that suggests that *An. punctipennis* is more than one species. The cluster analysis suggests that at least three species are present in North America. Since we have only analyzed the zymograms of 4 populations within California, we are not yet sure how many, if any, cryptic species are represented in this state.

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SNOW POOL MOSQUITOES: AN ESTIMATE OF ADULT LONGEVITY AND SURVIVAL

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ABSTRACT

The objective of the 1990 study was to obtain an estimate of the survival and longevity of adult snow pool *Aedes* mosquitoes. This work was conducted in El Dorado County, California at a source located near the edge of the mosquito abatement district's boundaries and above a developed area.

Collections of fourth-instar larvae and pupae from the site were allowed to emerge into large cages, then were transferred to 1-gallon containers to determine numbers. Both larval and adult subsamples were examined for species identification. A total of 1,304 female and 1,262 male *Aedes communis* (DeGeer) were transported back to the study site and released into a 12' by 12' tent which had screened sides, a solid roof and no floor so vegetation was accessible. The edges of the tent were securely sealed to prevent escape of the mosquitoes. Both water and raisins were provided on a continuous basis for nourishment. Estimation of the adult population was done every other day by counting both sexes of mosquitoes resting within sample areas on the side of the tent.

Female *Ae. communis* longevity was estimated at 18 days and daily survivorship at 0.67. Males were detected for 16 days. This survivorship estimate is comparable to those established by others for a known arboviral disease vector, *Culex tarsalis* Coquillett (0.68 to 0.86).

Previous isolation of Jamestown Canyon (JC) virus from *Ae. communis*, *Aedes hexodontus* Dyar, and *Aedes cataphylla* Dyar suggests the possibility that these species could be of public health importance. Information on distance and direction of dispersal to be collected from mark-release-recapture studies this next year will be combined with the above information in an effort to define the range of control that is necessary to sufficiently suppress biting mosquito populations in alpine areas.

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THE OCCURRENCE OF *PSOROPHORA SIGNIPENNIS* IN SAN BERNARDINO COUNTY, CALIFORNIA

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This is the first report on the occurrence of *Psorophora signipennis* (Coquillett) in San Bernardino County, California. Both adult and larval stages of this mosquito were collected at different times and locations in the lower Mojave Desert areas of the county.

The first larval collections of *Ps. signipennis* were made from a breeding source near Nipton, California on August 18, 1977 by R. Prochaska and M. Madon. The breeding source resulted from a series of summer storms and consisted of a number of transient ground pools scattered over several acres along Highway 68 about four miles northwest of Nipton. At the time of collection, the larval counts ranged between two and ten 3rd and 4th instar larvae per dip. The adult population was studied for landing and biting activity during the following week. Very little daytime activity was noted around the upper body area when one stood upright. However, landing and biting activity was quite apparent when one kneeled down in the bushy area for two to three minutes; suggesting that smaller animals, especially rodents, would be the main source of bloodmeal for this species. Later, on June 17, 1987, one female adult *Ps. signipennis* was collected and identified from about the same area near Nipton. Very lately, an adult male was collected on September 17, 1991 and a single female one week later in a New Jersey light trap operated in the City of Needles.

Although the occurrence of *Psorophora signipennis* is recorded for the first time in San Bernardino County, the distribution of this species has been reported in neighboring Riverside and Imperial Counties (Chew and Gunstream 1970), lower Colorado River area, Arizona (Richards et al. 1956) and Clark County, Nevada (Hicks 1974).

Acknowledgement.

The authors thank Gail Grodhaus (deceased) and Lucia T. Hui, California Department of Health Services, for their help in the larval identification of these species.

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EFFICACY AND PERSISTENCE OF SUSTAINED-RELEASE METHOPRENE PELLETS IN AN IRRIGATED PASTURE[1]

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ABSTRACT

The insect growth regulator, methoprene, has recently been formulated as sustained-release Altosid® pellets. The purpose of this study was to evaluate the efficacy and persistence of the pellets against *Aedes* mosquitoes through several flood cycles in an irrigated pasture.

Every other check in an irrigated pasture in eastern Contra Costa County, California, was randomly assigned one of three treatments: 3.4 kg/ha (3 lbs/acre) methoprene pellets, 9.0 kg/ha (8 lbs/acre) methoprene pellets, or control. There were three replicates of each treatment, and the pellets were applied four days prior to flooding.

Emergence rates of *Aedes* mosquitoes were monitored through seven flood cycles (126 days). During each flood cycle, approximately 150 pupae were collected from each check and placed in laboratory emergence cages. The adults were counted, sexed and identified as they emerged, and the mortality rate calculated as the number of pupae dying (not emerging)/number of pupae in the sample. The mortality rate was corrected using Abbott's formula.

Aedes melanimon Dyar, *Aedes nigromaculis* (Ludlow), and *Aedes vexans* (Meigen) were found in the irrigated pasture. Emergence was minimal in the treated checks during the first two flood cycles (4 and 20 days post-treatment, respectively), with mortality rates exceeding 98 percent. Mortality then decreased to 86.9 and 81.0% at the low and high treatment rates, respectively, during the third flood cycle (34 days post-treatment). During the fourth flood cycle, insufficient irrigation resulted in the pasture drying up before the larvae reached the fourth instar.

During the fifth flood cycle (69 days post-treatment), mortality increased to 92.6 and 96.8% for the low and high treatment rates, respectively. The water in each check had drained into low lying areas, and thus the pupae, and perhaps the methoprene, were highly concentrated. This may explain the increase in mortality rates from the third flood cycle. Mortality decreased during the sixth and seventh flood cycles (87 and 115 days post-treatment, respectively) to between 64.6 and 53%.

Mortality in the control checks for flood cycles 1-3 and 5-7 averaged ca. 14, 3, 1, 6, 12, and 26%, respectively. During each flood cycle, rates in the checks treated with methoprene were significantly higher (ANOVA, $P<0.05$) than in the control checks, but mortality rates between the low and high treatment levels were not significantly different.

In summary, both application rates of methoprene sustained-release pellets provided greater than 98% control of *Aedes* mosquitoes in an irrigated pasture through two flood cycles, or 20 days post-treatment, and greater than 80% control through five flood cycles, or 69 days post-treatment.

[1] An expanded version of this paper has been published in the Journal of the American Mosquito Control Association 7(4):646-648.

1991 Proc. Calif. Mosq. Vector Control Assoc. 59: 70-74

COMPARATIVE EFFICACY STUDY OF ALTOSID®, ALTOSID® XR AND BACTIMOS® *BTI* BRIQUETS AGAINST *CULEX QUINQUEFASCIATUS* BREEDING IN CATCH BASINS

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ABSTRACT

A study was conducted in 1990 comparing the efficacy of Altosid®, Altosid® XR and Bactimos® *Bti* briquets against populations of the Southern House Mosquito, *Culex quinquefasciatus*, breeding in catch basins in the Fresno area. Six monthly treatments with Altosid® standard 30-day briquets reduced adult emergence approximately 82% for the season. A single application of Altosid® XR extended briquets suppressed emergence approximately 49% for a 150-day period. Applications of Bactimos® *Bti* briquets markedly reduced 3rd and 4th instar larval and pupal populations.

Introduction.

The approximately 5,000 catch basins located throughout the boundaries of the Fresno Mosquito and Vector Control District provide an ideal breeding habitat for the Southern House Mosquito, *Culex quinquefasciatus* Say. However, control strategies of these mosquitoes in this particular habitat are not well established (Stewart 1977, Hazelrigg and Pelsue 1980, Mulligan and Schaefer 1982).

Prior to 1979, our mosquito control efforts in catch basins were strictly limited to treatments with Golden Bear 1356 Larviciding Oil on a treatment interval of 10 days. Since 1979 we have used methoprene (Altosid®) standard 30-day briquets without quantifying their control efficacy. *Bacillus thuringiensis* var. *israelensis* (*Bti*) de Barjac briquets were preliminarily tested on these sites in 1989.

The objective of this study was to document the effectiveness of Altosid® standard, Altosid® XR extended residual, and Bactimos® *Bti* briquets on controlling populations of the Southern House Mosquito breeding in catch basin habitats.

Materials and Methods.

We used three briquet formulations: Altosid® standard briquets, a 30-day formulation; Altosid® Extended Residual (XR) briquets, a 150-day formulation; and Bactimos® *Bti* briquets, a 30-day formulation.

In order to minimize location effect on the control, three sections of Fresno were chosen. They were: (1) Old Fig Garden, a residential section with an abundance of mature trees; (2) Valley Medical Center, an area with mature trees interspersed with lawns, streets and parking lots; and (3) Carrozza Park, a recreational park.

We selected 35 catch basins as test sites in these three areas. Three sites were set aside as controls (one for each section). Twelve sites were treated with Altosid® 30-day briquets at approximately one month intervals throughout the season (for a total of six treatments). Ten basins were treated with Altosid® XR 150-day briquets a single time and ten were treated with Bactimos® *Bti* briquets. The basins were of various design, however most of the catch basins we used were dead wells which hold water for extended periods.

Four dip samples were taken weekly from each basin with a white enamel dipper. Contents of each dip were placed in two shallow white enamel pans

(18 x 29 x 5 cm). Each larval stage was counted separately and recorded. In those sites treated with Altosid® briquets, 50 pupae were removed into 16 ounce disposable plastic cups which were brought to the lab for observation. Dead pupae and successfully emerged adults were recorded at each observation.

Bactimos® *Bti* briquets were anchored in the catch basins as a preventative measure to keep the briquet from floating away. Water depth at each site was checked and recorded weekly.

Results and Discussion.

Of the 35 sites studied, four were lost because of drying and one was used as a disposal site for motor oil. The other 30 sites continually produced mosquitoes. An average of 46 larvae and pupae/dip/site/collection (range: 10-100/dip) was collected from sites during the test period. The larval mosquito population peak occurred during the first and second week in July (Fig. 1). The average weekly air temperature during the course of study was approximately 72° F, ranging from 64-91° F (Fig. 1). Water volumes in basins ranged from 6 to 210 gallons.

Some of the other resident organisms (beside mosquito larvae) noted in the catch basins were rotifers, nematodes, oligochaetas, leeches, cladocerans, copepods, dragonfly nymphs, dytiscid beetles, psychodids, tabanids, pond snails and spiders (Black Widow).

First and second instar larvae were predominantly collected from the control basins (Fig. 2). Only 14 percent of collections were pupae.

During the first two of the six tests performed with the Altosid® 30-day briquets, the resulting emergence reduction was nearly 100 percent (Fig. 1). As the study continued, the reduction decreased slightly. The average emergence reduction throughout the study period was approximately 82 percent.

In contrast, the results of the treatments with

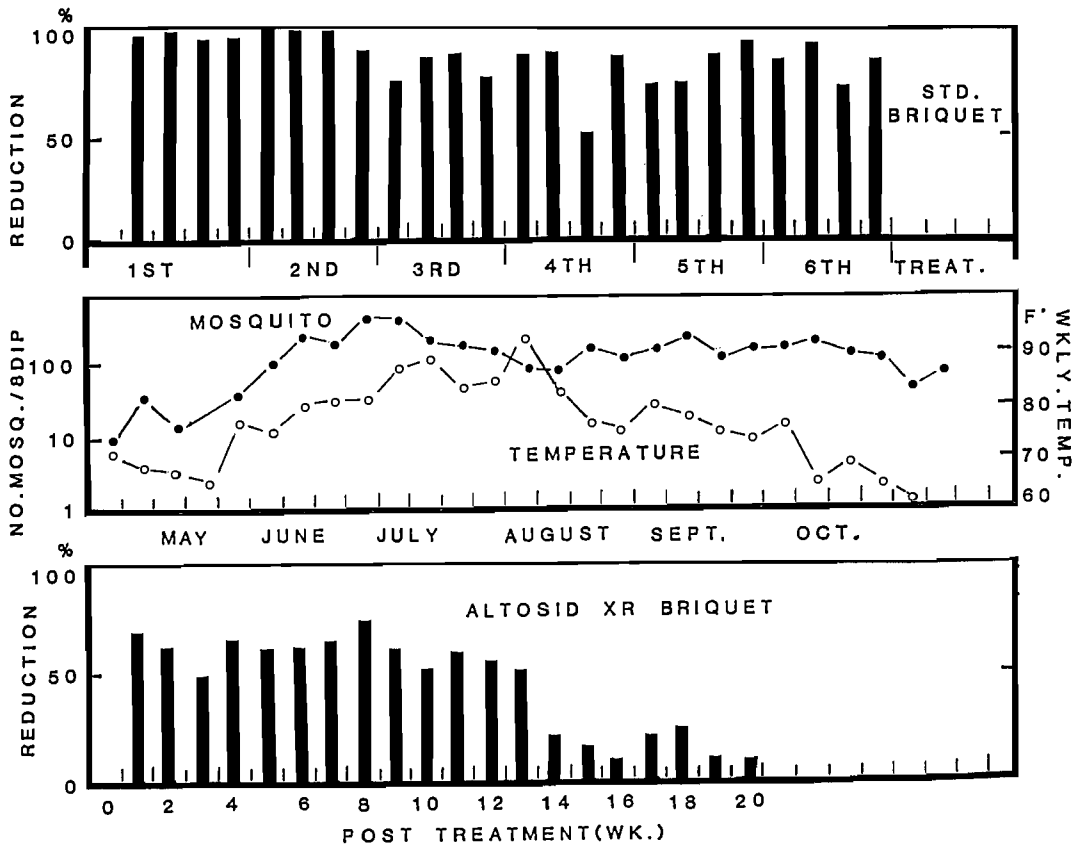

Figure 1. Effects of Altosid® 30-day (top) and 150-day (bottom) briquets on emergence of *Cx. quinquefasciatus* breeding in catch basins. Also shown (middle) are the seasonal distribution of immature mosquitoes and average weekly air temperature.

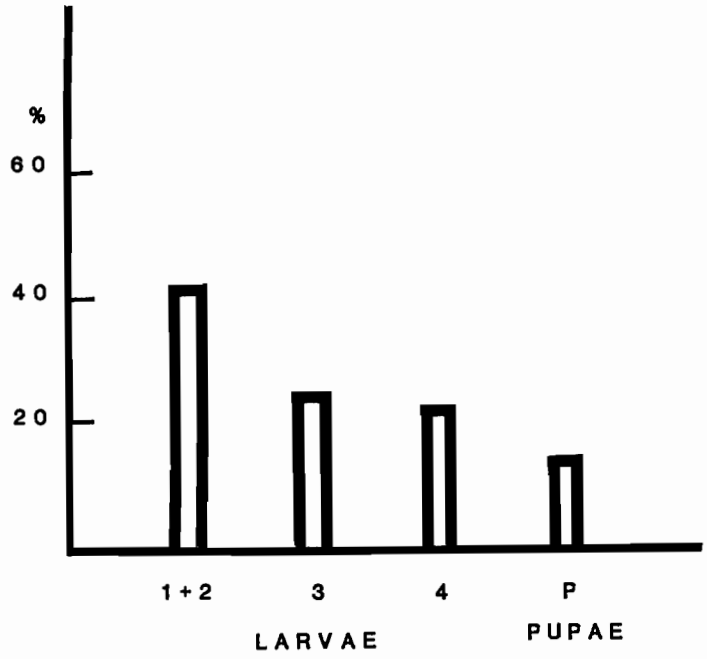

Figure 2. Proportion of immature *Cx. quinquefasciatus* breeding in the Fresno area catch basins.

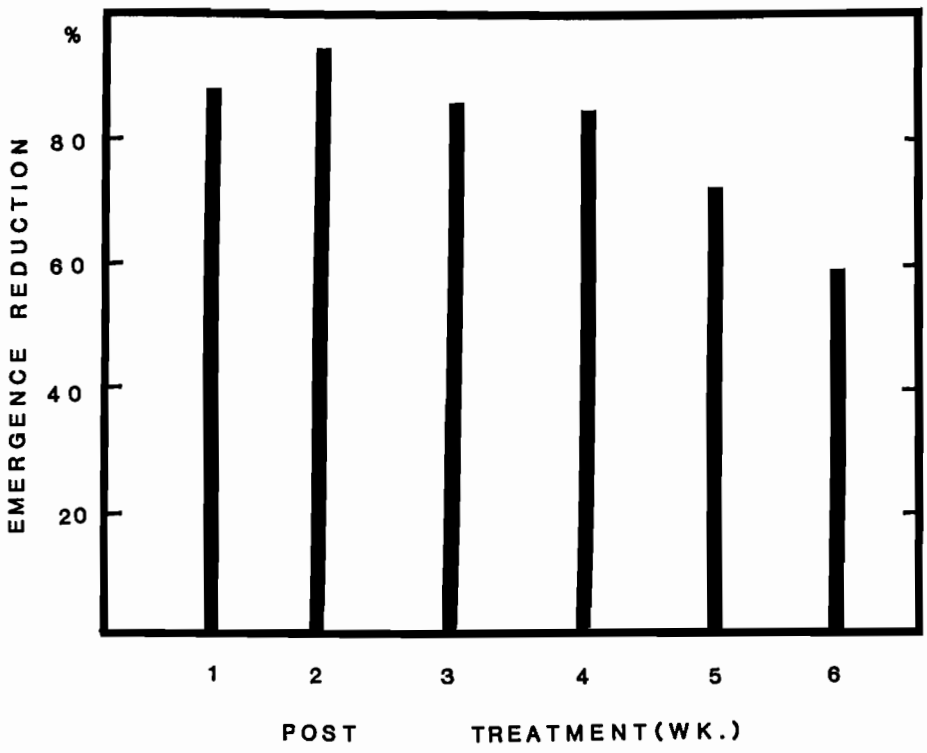

Figure 3. Effects of Altosid® 30-day briquet on *Cx. quinquefasciatus* emergence; showing extended effects.

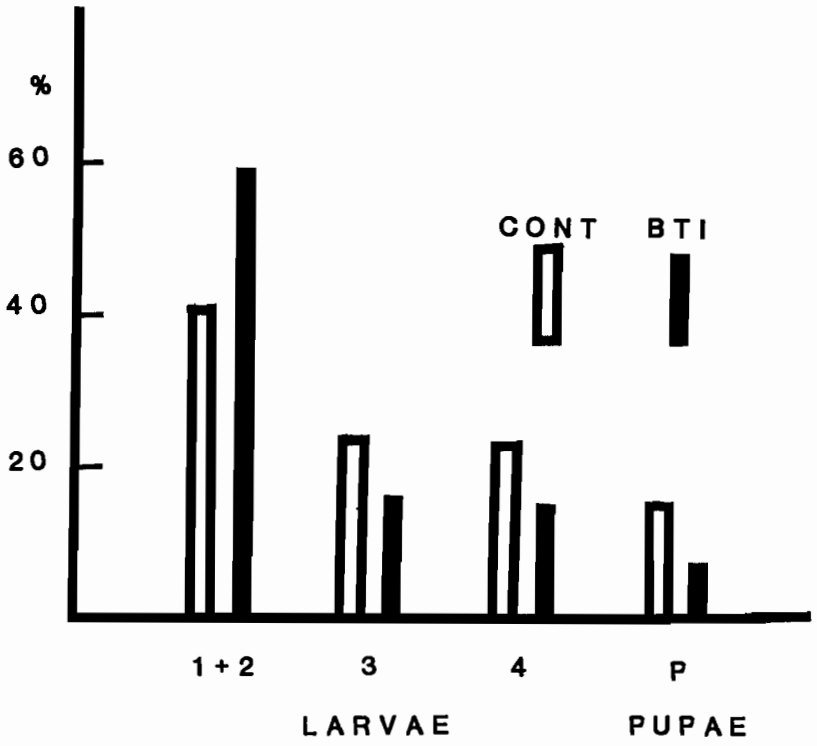

Figure 4. Bactimos® *Bti* briquet effects on immature mosquito populations by comparing the age distribution of control and treated basins.

a single application of the Altosid® XR 150-day briquets were not as favorable. Although some reduction in emergence was observed for five months or more, the average emergence reduction throughout the 150-day study period was approximately 49 percent (Fig. 1). Even in the initial 90-day period, during which they achieved the best results, emergence reduction was only 62 percent.

With those results in mind, we thought that temperature, water volume or formulation might be affecting efficacy. We have examined the relationship between temperature vs. emergence reduction rate and water volume vs. reduction rate. There seems to be a negative effect on emergence by high temperatures, however, the correlation coefficient is not significant ($r = -0.1$; $P = 0.05$). The relationship between water volume and emergence reduction, also had a negative effect but was not significant ($r = -0.3$; $P = 0.05$).

To estimate the amount of chemical released, some of the catch basins treated with the standard 30-day briquet were left untreated for longer than recommended. The data indicates that standard briquets persisted more than one month; after a 42-day period they still exhibited an average of 76% reduction in emergence (Fig. 3).

In view of the standard briquet persisting longer than expected (at least 42 days), we thought that the XR briquets were not releasing active ingredient (Methoprene) at rates high enough to provide us with acceptable control.

Our study of treatments using *Bti* briquets was limited to monitoring the larval and pupal populations at each site. In the *Bti* treated sites, the population proportion of 1st and 2nd instar larvae were 20% greater and the proportions of 3rd, 4th instar larvae and pupae were markedly lower than that found in the control sites (Fig. 4). This is probably due to greater mortality in younger instars as it is known that younger instars are more susceptible to *Bti* toxins (Sebastien and Brust 1981).

Summary.

In summary, one Altosid® 30-day briquet treatment per month in each catch basin gave an average of 82% reduction in adult *Cx. quinque-*

fasciatus emergence for the season. However, with a single application of one Altosid® XR briquet only 49% reduction in emergence was obtained for a 150-day period. Our study with *Bti* briquets was limited, and further defined study is needed.

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A SUMMARY OF RESEARCH ON S-31183:

A PROMISING NEW MOSQUITO LARVICIDE

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Introduction.

S-31183 is a juvenile hormone-type larvicide. It does not cause direct larval toxicity but exposed larvae die in the pupal stage or as abnormal adults. During the past four years the Mosquito Control Research Laboratory has conducted comprehensive studies on S-31183 as a mosquito control agent. These include evaluations of efficacy and nontarget effects as well as chemical persistence in the habitats where this control agent would be used. These studies are summarized in this report.

Efficacy.

The late fourth-instar larvae are the most susceptible stage, as is typical for juvenile hormone-type compounds. In laboratory studies, LC_{50}'s varied from 0.01 to 0.05 ppb against *Aedes* and *Culex* larvae. There was no indication of cross-resistance from tests against organophosphorus-susceptible (OP-S) and organophosphorus-resistant (OP-R) strains. On pasture plots in western Kern County, mixed larval populations of *Aedes nigromaculis* (Ludlow) and *Aedes melanimon* Dyar were effectively controlled at doses of 0.005 lb (AI)/acre and above. In ponds on the U.C. Kearney Agricultural Center (Parlier), *Culex quinquefasciatus* Say larvae were controlled from 2 to 14 days following applications of 0.005 to 0.04 lb (AI)/acre (Schaefer et al. 1988). In dairy wastewater lagoons, treatments of 0.09 lb (AI)/acre resulted in the control of *Culex* larvae for periods ranging from 6 to 68 days. The length of control period varied with the degree of pollution; the greater residual occurring in the most polluted sources (Mulligan and Schaefer 1990).

Nontarget Effects.

When populations of copepods and cladocerans in aquaria were treated with 0.01 ppm S-31183, there were no adverse effects. Also, in the pasture plots treated with 0.005 lb (AI)/acre, no adverse impacts were noted on a variety of nontarget organisms (Schaefer et al. 1988). Tests were also conducted on rice plots, at Parlier, at doses eight times (0.04 lb (AI)/acre) and twenty times (0.1 lb (AI)/acre) of that effective against pasture mosquitoes (0.005 lb (AI)/acre). Each dose was applied twice, one month apart, and all treatments were made in triplicate. Nontarget, aquatic organisms were sampled over a 52-day period (starting just before the first treatment) by dipping, drag-netting and trapping. Most organisms were not affected by the treatments but there was a temporary cessation of reproduction in daphnids and some dragonfly adults emerged having wing deformations. Overall, S-31183 was safe to aquatic, nontarget organisms including mosquito predators (Schaefer and Miura 1990).

Chemical Properties and Persistence.

S-31183 has a maximum water solubility of 0.15 ppm at 24° C. While it is very stable in water, stability decreased slightly as temperature increased and to a greater extent in the presence of sunlight; however, aqueous stability increased slightly with increasing pH. On the pasture plots, the active ingredient disappeared from the water after 24 hours.

In the laboratory, fish were continually exposed to water treated with the maximum water solubility (0.15 ppm) for up to 96 hours. During the exposure period, tissue residues of S-31183 increased for approximately 72 hours (as would be expected for most organic chemicals). However, once the fish were placed in untreated (rinse) water, the residues rapidly declined.

On all of the treated rice plots, the residues in

the water disappeared after two days and none were detected in the mud. S-31183 residues on rice vegetation declined to below detectable limits within seven days. Bluegill sunfish, held in the rice plots, accumulated residues from the treated water into their tissues for approximately 48 hours but then these steadily declined and were completely dissipated after seven days. Thus, no potential for long-term bioaccumulation was found (Schaefer and Miura 1990).

In laboratory leaching trials, soil columns of four different soil types were treated with S-31183 and water was passed through under pressure. Over 50% of the active ingredient remained in the upper 6 cm of the 30 cm (length) columns. Thus, no indication of rapid potential for downward migration in soil was apparent.

In dairy wastewater lagoons, S-31183 rapidly leaves the water and adsorbs onto organic debris. It remains on the organic matter, where it decays at an exponential rate while providing biological activity against mosquito larvae for up to two months (Schaefer et al. 1991).

Selection for Resistance.

It is especially important to consider how quickly insecticide-resistant strains of mosquitoes might be able to become tolerant to a new chemical control agent. Therefore, an OP-R strain of *Culex quinquefasciatus* was pressured with S-31183 for 17 generations. Egg viability began declining in the F_7 generation and became lower as the selection process continued. By the F_{17} generation, egg viability was too low to allow continuation of the study. Susceptibility tests of the F_5, F_{10}, F_{15} and F_{17} generations showed no indication of increased tolerance to S-31183 (Schaefer and Mulligan 1991).

Commercial Outlook for S-31183.

S-31183, also known by the common name pyriproxyfen, is owned by Sumitomo Chemical Company. It has shown commercial potential against several insects of public health importance including fleas, cockroaches and mosquitoes. It is now being sold in Japan as a mosquito larvicide under the name Sumilarv®. It will be commercially developed by McLaughlin Gormley King Company under the name Nylar® for distribution in the United States. Hopefully, it will be registered in the U.S. by 1993 or 1994. Commercial development in some other countries is also progressing.

Acknowledgements.

We gratefully acknowledge the assistance of the Consolidated and Kern Mosquito Abatement Districts and the Delta Vector Control District. The studies were supported with funds from USDA Cooperative Agreement No. 82-CRSR-2-1010, from a special California appropriation for mosquito control research and from grants-in-aid from Sumitomo Chemical Company and McLaughlin Gormley King Company.

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1991 Proc. Calif. Mosq. Vector Control Assoc. 59: 77-80

EFFECTS OF RECOMBINANT JUVENILE HORMONE ESTERASE ON *AEDES AEGYPTI*

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ABSTRACT

Recombinant juvenile hormone esterase (JHE), cloned from *Heliothis virescens*, was injected into *Aedes aegypti* larvae resulting in a dose-dependent decrease in survival. In addition, ovariole maturation in mosquitoes was impaired by injection of JHE into larvae or pupae.

Introduction.

One of the most promising strategies for mosquito control entails the use of recombinant DNA technology to disrupt the insect endocrine system. Juvenile hormone (JH) is a promising target since it regulates a variety of physiological processes in mosquitoes, including ovariole maturation (Masler et al. 1980) and blood feeding (Meola and Petralia 1980) in females. Juvenile hormone titer may also play a role in controlling initiation of mosquito metamorphosis, and reduction of JH titer at the larval stage has the potential to cause premature pupation or block development. There are a number of evolving molecular technologies which may allow us to exploit such effects for mosquito control.

Juvenile hormone titer in insects is controlled by a balance between biosynthesis and degradation, the latter primarily by two classes of hydrolytic enzymes, juvenile hormone esterase (JHE) and juvenile hormone epoxide hydrolase (Hammock 1985). Juvenile hormone esterase was cloned from *Heliothis virescens* (Lepidoptera: Noctuidae) (Hanzlik et al. 1989) and expressed in a baculovirus vector (Hammock et al. 1990). The expressed recombinant juvenile hormone esterase, acting as an anti-JH enzyme, is potentially useful for mosquito control. The goal of this study was to evaluate the effect of recombinant JHE on *Aedes aegypti* (L.) by injecting larvae or pupae with varying amounts of JHE and monitoring larval and pupal survival as well as adult ovariole maturation.

Methods.

Juvenile hormone esterase, for microinjection of larvae, was produced by a previously developed protocol for baculovirus expression in insect cell culture (Hammock et al. 1990). Triton X-100 (0.05%) was added to the cell culture medium to kill baculoviruses after JHE expression. To concentrate and purify the enzyme, baculovirus cell culture medium containing secreted JHE was loaded onto a DEAE ion exchange column and eluted with a salt gradient, 50 mM to 200 mM NaCl in 10 mM Tris-HCl at a pH of 8.5 (Ichinose et al. 1991). Before the DEAE column, the protein concentration was 0.16 mg/ml and the activity of JHE was 80 nmol JH III/min/ml. After the DEAE column, the protein concentration was 4.1 mg/ml and the activity of JHE was 10,000 nmol JH III/min/ml (approximately 75% pure JHE). The dose of JHE administered to mosquitoes will be presented in JHE activity units, defined here as 40 pmol of JH III hydrolyzed per minute. Before injection, JHE was stored at -80° C for 1-4 months in sodium azide. Control injections employed the same concentration of sodium azide that was present in the JHE treatments.

The Rock strain of *Aedes aegypti* was used for all injection experiments. Juvenile hormone esterase was introduced using a stereo dissecting microscope and micromanipulator to carefully control the site of injection. A finely drawn microcapillary tube was coupled to a Narishige microinjector to dispense small volumes of JHE into the side of the thorax. A cold light source was used for illumination during the injection process. For each insect, 1 μl of JHE was added to a finely-drawn calibrated microcapillary tube and the volume completely discharged into the body. This procedure was employed to ensure that a consistent volume was administered to each insect. Extensive methods development and practice was required to avoid excessive mortality resulting from injection of larvae or pupae. Tris-HCl buffer (pH 8.5) or tissue culture media (EX-CELL 400, J.R. Scientific, Woodland CA) were used for control injections.

Determination of ovariole development in treated and control female mosquitoes was done four days after adult eclosion, by placing them on clean microscope slides and dissecting ovarioles in drops of physiological saline under a stereoscopic microscope. After disrupting ovarioles with dissecting needles and covering them with a cover slip, individual follicles were measured under a compound microscope at 40X magnification by means of a squared reticle. Ovariole development was also scored using the scheme of Kawai (1969).

Results and Discussion.

When examined at two days after the injection of 4th stage larvae with JHE, the survival of treated larvae decreased in a dose-dependent manner (logistic regression, $p < 0.0001$)(Fig. 1). Survival to the imago stage was significantly lower for treated larvae than for control larvae (Fisher's exact test, $p = 0.0018$). Another consequence of exposure to recombinant JHE was partial eclosion, which was only observed among treated larvae and pupae (11% incomplete eclosion). A hypothesis to explain the JHE effects observed in this study would include the supposition that recombinant JHE activity has

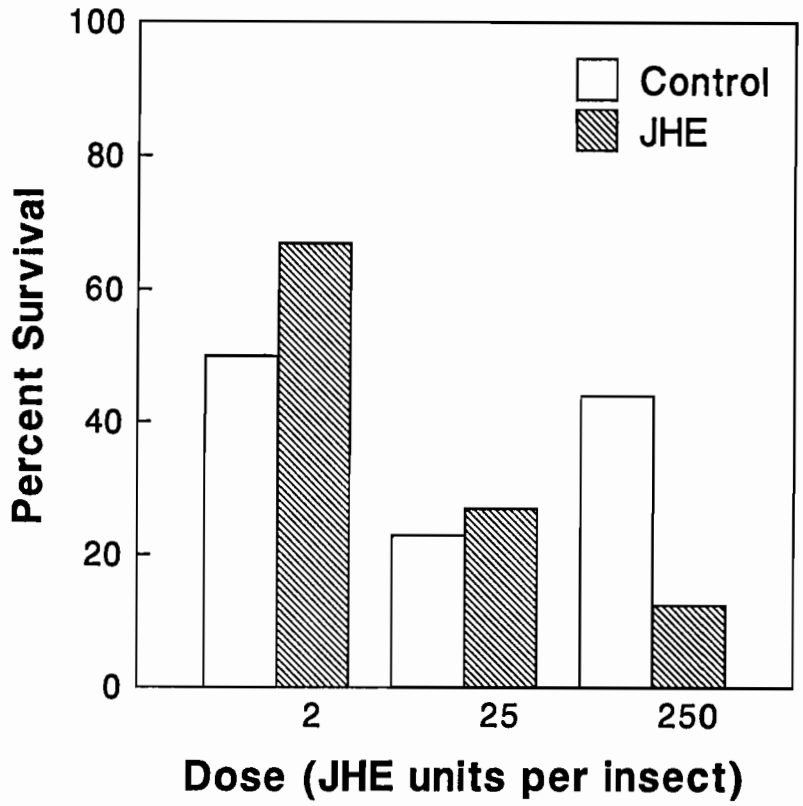

Figure 1. The effect of JHE dose on survival of *Aedes aegypti* larvae. Forty control and forty treatment last instar larvae were injected to evaluate each JHE dose.

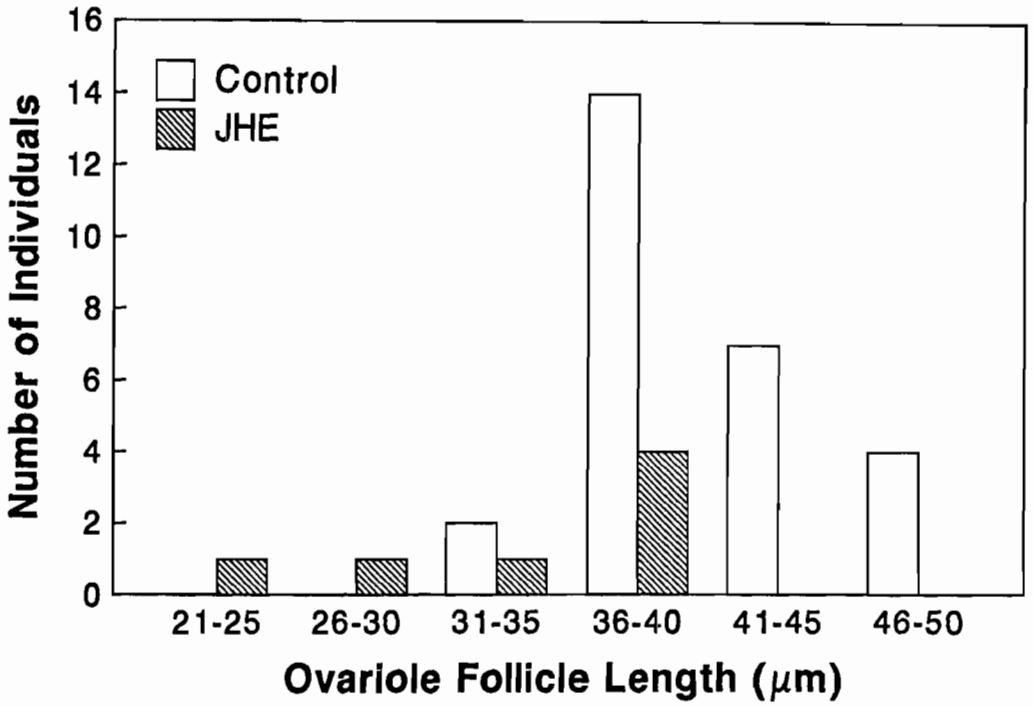

Figure 2. Distribution of mean ovariole follicle size (μm) measured in 4-day old *Aedes aegypti* females after injection of last instar larvae or pupae with JHE (pooled results from injection of 25 or 250 units of JHE per insect).

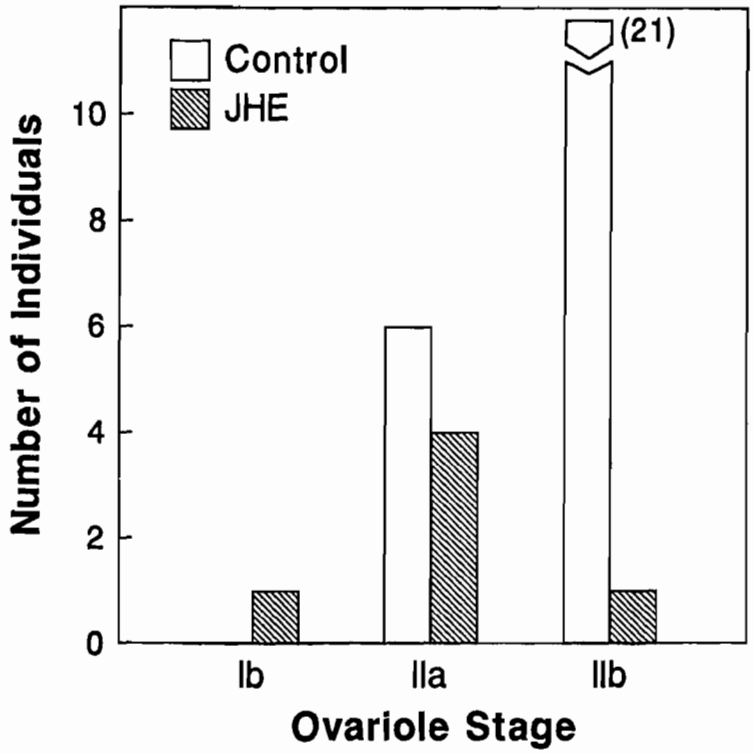

Figure 3. Number of individuals per ovariole stage after injection of last instar larvae or pupae with JHE (pooled results from injection of 25 or 250 units of JHE per insect).

been introduced in excess of analogous endogenous enzymatic activity on JH in the mosquito hemolymph and the assumption that JH titer drops accordingly. In the future, these studies can be extended to evaluate life-stage dependent physiological responses (Eldridge 1968) after perturbation of the endocrine system.

Evidence for female reproductive dysfunction following injection of larvae and pupae is presented in Figures 2 and 3. As shown in Figure 2, a significant reduction in ovariole size was observed in treated animals (Mann Whitney test, $p < 0.01$). All the treated larvae had a mean ovariole follicle length of 40 μm or less, whereas 93% of the control larvae had a mean follicle length of 36 μm or more (Fig. 2). In addition, the reproductive effect of introduced JHE was indicated by ovariole stage classification (Fig. 3). The less developed ovariole stages (Ib, IIa) were relatively prevalent among individuals exposed to JHE as larvae or pupae (Fisher's exact test, $p = 0.0046$). A noteworthy observation from this study is that perturbation at the larval stage can effect adult reproduction. In summary, JHE expressed by an appropriate mosquito vector would be useful for control, both as a mosquitocide and through deleterious effects on reproduction.

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American Cyanamid has been involved in mosquito control for over 40 years. Our products Cythion® and Abate® have been and continue to be important tools used in mosquito control programs not only in Florida or the rest of the United States, but throughout the world.

The addition of Acrobe® to our product line allows Cyanamid to provide more tools for the mosquito control workers allowing them to do their jobs better and allowing Cyanamid to support in a very real way an integrated approach to mosquito control.

The addition of Acrobe® and other products now under development, allows Cyanamid to provide a number of mosquito control products to meet the needs of our customers and to actively support integrated pest management of mosquitoes.

Acrobe® is a high potency biological larvicide containing a minimum of 1200 ITU/mg of *Bacillus thuringiensis* var. *israelensis*. It is formulated as an aqueous suspension with unique characteristics which give Acrobe® and advantage over other *Bti* products currently on the market.

Acrobe® has been under development by Cyanamid for several years. Results of field trials conducted throughout the United States are summarized in Table 1. In each of these trials, Acrobe® exceeded the expectations of cooperators doing the study. In many cases, cooperators were impressed with the control of third and early fourth instar larvae. These comments (and others) led us to review what characteristics of this product were responsible for this performance advantage.

We found that several physical characteristics of Acrobe®, including a specific gravity closer to that of water and an unique viscosity profile, contribute to Acrobe's® outstanding performance. These characteristics result in improved suspension in mosquito larval habitats over the existing *Bti* products.

Results of a viscosity study comparing Acrobe® to Vectobac® and Teknar® showed dramatic differences in formulation viscosity over operational temperature ranges. This characteristic allows Acrobe® to be applied Ultra-Low Volume without fear of reduced flow rates or errors in calibration.

We believe Acrobe® has several advantages over current *Bti* products. These advantages include:

1. More activity on third and early fourth instar mosquito larvae.
2. Lower specific gravity increasing suspension time within larval habitats.
3. Product of choice for Ultra-Low Volume *Bti* applications.

Table 1. Results of field trials with Acrobe® biolarvicide.

State	Dominant Species	Percent Mortality		
		24 hours	48 hours	96 hours
Florida	*Culex nigripalpus*	100	--	--
Maryland	*Aedes sollicitans*	>95	--	--
Massachusetts	*Culex pipiens*	>85	>90	--
Michigan	*Culex pipiens*	99	--	>95
Utah	*Aedes dorsalis*	>85	96	--
Washington	*Culex tarsalis*	99	98	--

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FORMIDABLE POSITION OF TURBELLARIANS AS BIOLOGICAL MOSQUITO CONTROL AGENTS

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Interest in biological control of medical pests and vectors dates prior to the turn of the last century (Lamborn 1890). At that time dragonflies were recognized as potential natural enemies for the control of mosquitoes. However, the enormous difficulties associated with the colonization and management of these insects quickly extinguished any idea for their practical use in mosquito control. Shortly thereafter, the mosquitofish, *Gambusia affinis* (Baird and Girard), was stressed for biological control. This small fish, being much easier to deal with than dragonflies, was quickly utilized and transported throughout the world during the early decades of this century in attempts to control mosquitoes.

The mosquitofish, *G. affinis*, along with several other natural controls, was utilized with increasing intensity during the first 40 years of the century. The use of all biological controls was curtailed sharply with the introduction of synthetic organic insecticides after World War II. The convenience and rapid action of these chemicals was so dramatic for such insects as mosquitoes, flies and lice, that other control tactics were quickly reduced to a minor role. Nevertheless, interest in alternative methods of control, especially biological, was to arise again when the succession of chemicals developed during the 1940's and 1950's began to fail due to the development of widespread genetic resistance in vector and pest populations. Although the biological control of medically important pests and vectors has made some progress since its revival, it has been rather slow and is still well behind that which has occurred in agricultural systems (Service 1983). This disparity is partly due to the problems of fixing pest tolerance levels, but more importantly because of the temporary, unstable habitats exploited by medically important pests (Legner and Sjogren 1984, Garcia and Legner 1992).

As Service (1983) pointed out, the successful widespread use of biological control agents against mosquitoes will require a much better understanding of the ecology of predator/prey and pathogen/host relationships. The opportunistic characteristics of many species (i.e. their ability to exploit temporary habitats, coupled with their short generation time, high natural mortality, great dispersal potential, and other R-strategist characteristics) pose difficult problems for any biotic regulatory mechanism. Mosquitoes, in general, exploit a wide breadth of different aquatic habitats. Consequently, under many conditions a biological control agent will have a much narrower range of environmental activity than that of the target species. Thus, in many situations a number of different biological control agents and/or appropriate methods will be necessary if we expect to control even a single mosquito species across its range of exploitable breeding sources.

The most important non-arthropod invertebrate predators to draw attention for mosquito control are the turbellarian flatworms and a coelenterate. Flatworm species which were shown experimentally to be excellent predators of mosquito larvae in a variety of aquatic habitats are *Dugesia dorotocephala* (Woodworth) and *Dugesia tigrina* (Girard) (Legner and Medved 1974; Yu and Legner 1976; Collins and Washino 1978; Case and Washino 1979; Legner 1977, 1979; Ali and Mulla 1983; George 1978; Meyer 1981a, 1981b; George et al. 1983). Several biological and ecological attributes of flatworms would seem to make them ideal candidates for manipulative use. Among them are ease of mass production, an overwintering embryo, effective predatory behavior in shallow waters with emergent vegetation, on-site exponential reproduction following inoculation (Medved and Legner 1974, Tsai and Legner 1977, Legner and Tsai 1978,

Legner 1979) and tolerance to environmental contaminants (Levy and Miller 1978, Nelson 1979).

Collins and Washino (1978) and Case and Washino (1979) suggested that flatworms, particularly *Mesostoma*, may play an important role in the natural regulation of mosquitoes in some California rice fields because of their densities and their predatory attack on mosquito larvae in sentinel cages. Preliminary analysis using extensive sampling showed a significant negative correlation between the presence of flatworms and population levels of *Culex tarsalis* Coquillet and *Anopheles freeborni* Aitken (Case and Washino 1979). However, these workers cautioned that an alternative hypothesis related to the ecology of these species may have accounted for the correlations. Later investigations by Palchick and Washino (1984), employing more restrictive sampling, were unable to confirm the negative correlations between *Mesostoma* and mosquito populations. However, the enormity of the problem associated with sampling in California rice fields, coupled with the complexity of the predator/prey interactions (Collins and Washino 1979), make further studies necessary before the role of this group of flatworms in rice fields can be clearly established.

Nevertheless, in a detailed study by Legner (1977), which was later corroborated by Ali and Mulla (1983), the relationship between the number of *Dugesia dorotocephala* applied per m^2 of water surface and the average density of mature *Culex* spp. larvae was highly significant and lineal, the latter assuming densities of <1 per standard dipper at the higher *Dugesia* application rates. It was emphasized that the use of *D. dorotocephala* for direct mosquito suppression as an alternative to insecticides or *Gambusia* is desirable because natural predator densities appeared unaffected and a single application resulted in a prolonged and increased suppression of mosquito larvae as the planarians reproduced in the environments to which they had been introduced.

Although *D. dorotocephala* is widespread in North America (Kenk 1972, Garcia and Legner 1992), some strains are very cannibalistic and might be unsuitable for mass rearing. The production of adequate numbers of the non-cannibalistic strain is possible if cultures are stockpiled during winter months (Legner et al. 1976) and rapid mass production can be obtained through carefully controlled culture with filtration, optimum temperature and dissolved oxygen and food (Legner et al. 1976, Tsai and Legner 1977, Legner and Tsai 1978). Progeny require about one month to reach maturity.

The important attributes for manipulative use of flatworms mentioned above raises the question of why they have not been developed further for use in mosquito control. Perhaps the contemporary development of *Bacillus thuringiensis* var. *israelensis* DeBarjac (H-14), a highly selective and easily applied microbial insecticide, may have been at least partially responsible for slowing further work and development of these predators (Garcia and Legner 1992). The mass culture of flatworms must be continuous and demands skilled technical assistants (Legner and Tsai 1978). Their persistence in field habitats may also depend on the presence of other organisms which can be utilized for food during low mosquito abundance, such as ostracods (Legner et al. 1976).

The coelenterates, like the flatworms, showed great promise for further development and use in selected breeding habitats. *Chlorohydra viridissima* (Pallas) is efficient in suppressing culicine larvae in ponds with dense vegetation and is also capable of being mass produced (Lenhoff and Brown 1970; Yu et al. 1974a, 1974b, 1975). However, like the flatworms, work on these predators has waned, perhaps for similar reasons as speculated for the flatworms. Other reasons may be that microbial pesticides, unlike these non-arthropod predators, can be employed over an extensive range of different mosquito breeding habitats. Also, commercial production of coelenterates would be much more costly, and storage of viable cultures all but impossible.

Recent emphasis on the fungal genus *Lagenidium* which is capable of infecting and killing several genera of mosquito larvae (e.g. *Anopheles, Culex, Aedes,* and *Psorophora*) encourages the continued quest for biological control agents as alternatives to pesticides (McCray et al. 1973; Christensen et al. 1977; Glenn and Chapman 1978; Washino and Fukushima 1978; Washino 1981; Axtell et al. 1982; Domnas et al. 1982; Jaronski and Axtell 1982, 1983a, 1983b). The potential of such fungi for operational mosquito control is nevertheless no greater than for some of the flatworms or hydra. This recent switch in attention to fungi may be due to the existence of a greater number of mycologists in the research force than specialists in the other groups. Problems of mass production, dissemination of an acceptable fungal stage and adaptability

to polluted water habitats have placed their immediate deployment in doubt. Similar problems were either nonexistent or minimal with the *Dugesia* flatworms, so that their integrity as effective and available biological control agents is undiminished.

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CARBON DIOXIDE: AN ALTERNATIVE TO ETHER AS AN ANESTHETIC IN A PLAGUE SURVEILLANCE PROGRAM

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ABSTRACT

A comparative study was conducted by the Los Angeles County Vectorborne Disease Surveillance Program using carbon dioxide gas and ethyl ether (from a commercially available starting fluid) as an agent of anesthesia and euthanasia. In September and October of 1990, thirty-six *Spermophilus beecheyi* were captured from three sites in Los Angeles County during routine plague serology activities. The captured ground squirrels were split into two equally numbered groups. One group was given ether and the other carbon dioxide gas to achieve anesthesia, euthanasia, and ectoparasite immobilization. Carbon dioxide gas proved to provide a clean, safe and inexpensive alternative to ether.

Introduction.

In California, most if not all county funded plague surveillance programs use ethyl ether (ether) as an inhalation anesthetic for *Spermophilus* spp. and other small rodents during plague serology projects. The Los Angeles County Vectorborne Disease Surveillance Program tests an average of 470 *Spermophilus beecheyi* yearly; ether from pressurized cans has been used since 1979. Although ether is widely accepted because of its anesthetic effect on mammals and their ectoparasites, there are significant risks associated with its use.

Concerned with the volatility and toxicity of ether (Mackison and Partridge 1981) and the potential danger to staff and the public during its use and transportation, an alternative was sought. Other inhalation anesthetics were either flammable, toxic, expensive, or had no documented information on their effects to ectoparasites.

The analgesic and anesthetic properties of carbon dioxide (CO_2) were first discovered by Leake and Waters in 1929 (Booth 1982). Its use as an accepted euthanasia agent for dogs, cats, swine and laboratory rodents is well documented (Ibid). Before investing in a CO_2 delivery system, we wanted to assess its usefulness in our program. We used a crude method of obtaining CO_2 gas by placing dry ice in a clean Hudson-type sprayer containing a pressure release mechanism. We compared the efficacy of CO_2 and ether as anesthetizing and euthanizing agents. After preliminary studies gave promising results, a refined system was purchased.

Materials and Methods.

A study was conducted comparing the use of CO_2 gas to ether in our plague surveillance program. Ether was obtained from a commercially available starter fluid which contains ether as the primary ingredient. The CO_2 delivery system consisted of a tank filled with five pounds of gas, a standard pressure regulator (set at 20 psi) attached to 137 cm length of high pressure hose and a flow

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gun nozzle.

During September and October of 1990, day trapping was conducted at three separate locations in Los Angeles County (Knapp Ranch Park, Leo Carillo State Park, and Chantry Flats in the Angeles National Forest) as part of the routine plague serology activities. Tomahawk™ traps were set for an average of three hours and resulted in the collection of 36 *S. beecheyi*.

Animals were separated into two groups at each site; each group was given one of the two anesthetics. Individual *S. beecheyi*, still caged, were placed into transparent plastic bags (50.8 cm x 76.2 cm x 1 mm). Ether (one 8-second burst) or CO_2 (continuous flow) was administered until the animal became unconscious. The anesthetized animal was then removed from the trap, placed back into the bag and additional anesthetic was given to maintain unconsciousness. Whole blood was drawn by cardiac puncture and additional anesthetic was given to achieve euthanasia and ectoparasite immobilization. A stopwatch was used to measure the elapsed time to achieve anesthesia and euthanasia.

When ether was used, the carcass was removed from the bag and the ectoparasites were brushed from the carcass into a pan. The fleas were collected with forceps and placed into a vial containing physiological saline.

When CO_2 was used, the carcass remained in the bag and additional anesthetic was added to inflate the bag. Masking tape was wrapped around the twisted top of the bag to prevent leakage. The inflated bag was placed in a shaded area for a period of at least 9 minutes, which was found to be the minimum time required for flea immobilization. After this period, the bag was opened and the ectoparasites were brushed from the carcass into the bag. To facilitate flea collection, the bag was again inflated and held at an angle, causing fleas to accumulate in a corner. Using scissors, the corner containing the fleas was removed and placed in a vial of physiological saline.

Results.

Results of the three trials that were conducted are summarized in Table 1. One way ANOVA with a block design (Dunn and Clark 1974) was used to evaluate differences between agents and trials. Differences in the mean time necessary to achieve anesthesia and euthanasia were not significant between trials (α = 0.01). Differences between agents, however, were highly significant (p = 0.0001).

Discussion.

Carbon dioxide achieved anesthesia and euthanasia in a significantly shorter period of time than ether in all three trials (Table 1). The use of CO_2 gas provides a number of additional advantages over the use of ether.

Carbon dioxide, unlike ether, is inexpensive,

Table 1. Comparison of carbon dioxide and ether as an agent for anesthesia and euthanasia for *Spermophilus beecheyi* at three locations in Los Angeles County, California.

	CARBON DIOXIDE				ETHER			
TRIAL	1	2	3	TOTAL	1	2	3	TOTAL
Sample size	3	11	4	18	3	11	4	18
ANESTHESIA								
Mean time (seconds)	50.3	45.2	46.2	46.3	93.0	74.9	59.0	74.4
Standard error	0.9	2.4	3.1	1.6	13.4	4.8	25.9	4.5
EUTHANASIA								
Mean time (seconds)	206.6	190.0	214.5	198.2	351.3	314.4	316.2	321.0
Standard error	15.8	13.2	25.9	10.0	61.5	28.3	18.3	21.6

relatively safe for the operator in the field, and does not cause irritation of eyes, nose, and throat. When using ether, an Organic Vapor Canister Mask (OVCM) is an essential safeguard; it requires periodic filter changes and fit tests to ensure proper function. During the use of CO_2, the OVCM is not required as this anesthetic is odorless and does not cause irritation. Additionally, CO_2 appears to be a more suitable euthanasia agent because it apparently produces less stressful behavior by the animals.

The benefits of using CO_2 over ether can also be seen during its transportation and disposal. Ether has long been known to be a volatile substance and has been documented as a cause of fires and explosions. When used in our operations, special precautions during its transportation were required. Carcasses euthanized with ether also present problems associated with odor and disposal. Historically, the fur of a rat carcass that was impregnated with ether caused an explosion in a refrigerator resulting in a major fire (Pitt and Pitt 1985). The potential for a similar accident exists for any operation that refrigerates carcasses that were euthanized with ether.

Routine surveillance activities frequently became the subject of public scrutiny when ether was used. The odor of ether created public apprehension and complaints were received. When CO_2 was used, no such concerns were detected.

Although immobilization of ectoparasites with CO_2 required nine additional minutes, the lost time was recovered during the overall procedure. Use of the CO_2 procedure did not leave any liquid residue, thus facilitating the collection of ectoparasites. Since the starter fluid also contains other petroleum distillates, the use of CO_2 eliminates the potential effects these distillates have on attempts to culture organisms that are present in the ectoparasites.

It is our experience that CO_2 provides a safe, economical and efficient alternative to ether as an agent of anesthesia and euthanasia for small rodents during routine plague surveillance. Further investigations are necessary to determine whether the use of CO_2 enables more efficient collection of ectoparasites, and whether attempts to isolate *Yersinia pestis* from ectoparasites collected in this manner are facilitated by the absence of petroleum distillates.

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A PORTABLE CAGE AQUACULTURE SYSTEM FOR THE SUPPLEMENTAL PRODUCTION OF MOSQUITOFISH

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Introduction.

Of the many existing locations in California where permanent bodies of water may support populations of the mosquitofish, *Gambusia affinis* (Baird and Girard), few are routinely utilized to their fullest extent to contribute to biological mosquito control programs. Various extenuating conditions often prevent mosquito abatement districts from advantageously employing potential incidental fish production sites. Typically, many of these bodies of water do not lend themselves to easy harvest of fish populations due to abundant aquatic weeds, competing or predatory fishes, or obstacles to practical seining activities. Consequently, the Sutter-Yuba Mosquito Abatement District began a study in 1989 at the municipal wastewater facility for the City of Live Oak to see if mosquitofish raised in an experimental cage culture system could help rehabilitate a once productive source of fish that has been more recently under-utilized. This would provide control technicians a mosquitofish source within their zone of operation easily accessible on an as-needed basis. Unfortunately, the size of the experimental cage culture system was unwieldy and therefore, operationally impractical.

In 1990, much of 1989's research was repeated, broadened, and expanded to include two additional sites. The original Live Oak site was again chosen, as well as ponds at the Marysville and Sheridan Wastewater Treatment Facilities. At each site, three cage culture units were installed. Although several aspects of the 1989 study remained the same, the 1990 study was broadened to better gain an understanding of the mosquitofish's immediate environment. With the influence that a sewage oxidation pond's huge phytoplankton blooms have on dissolved O_2, CO_2 and pH levels, the effect of fluctuating weather conditions upon phytoplankton populations were recognized as having an important role in quickly modifying environmental conditions for the fish. With a maximum distance of approximately 25 miles between the two most widely separated study locales, it was possible that local weather and site-specific physical factors could differentially influence environmental conditions at the three impoundment facilities. Also, the term of the 1990 study was extended somewhat over that used in 1989, which would presumably better accommodate the normal span of the mosquitofish reproductive season in the Sacramento Valley.

Materials and Methods.

In 1989, difficulties were encountered because the cage unit's immense size made transport and deployment an arduous and delicate operation. Unacceptably protracted fry harvest labor required the efforts of three men. In contrast, the 1990 complete cage system units were scaled down to fit inside the bed of a pickup vehicle. This reduction in overall size was very important in deployment at the three widely separated experimental sites. Also, the decrease in cage size reduced the number of needed technicians from three to two. However, harvests were possible, but not practical with one technician, given the total number of cage units involved in this study.

The experimental cage culture systems tested in 1990 were designed using essentially the same basic concept employed in 1989. Each cage culture system utilized an inner brood fish cage and an outer fry enclosure (Figure 1). The inner cage consisted of a framework of wood having two endplates measuring 24" by 12" (61 x 30.5 cm) and four, 1" x 2" x 40" (2.5 x 5.1 x 101.6 cm) crossmembers. Secured to each of the endplates was styrofoam float material cut to the same endplate dimensions. The assembled brood structure was wrapped in a

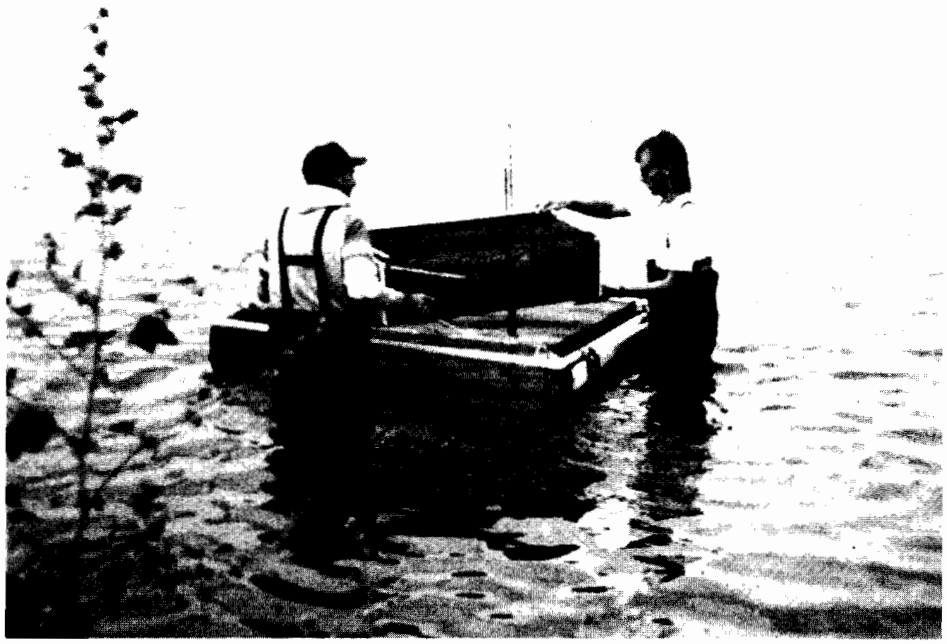

Figure 1. Installation of the first of three cage culture units at the Live Oak Wastewater Treatment Facility.

plastic 1/8" (3.2 mm) mesh (Internet #XV-1670 - InterNet Inc., Minneapolis, MN 55427) and was fastened to it with electrical tape and metal staples. A simple flap lid was created at the top of the cage for removal and restocking of brood fish.

The outer fry cage consisted of a rectangular 5' x 4' (152.4 x 121.9 cm) frame, constructed of schedule 40 PVC pipe and included a 0.04" (1 mm) mesh fiberglass/PVC screening (Aquascreen® - Menardi-Criswell Corp., Augusta, GA 30903) box cut and fabricated to fit inside this outer framework. This outer enclosure was attached to the PVC support piping to serve as a containment area for newborn fish. Two pontoon-style floats cut out of poly-iso-cyanurate foam insulation were fastened to the PVC pipe frame with stainless steel U-shaped clips and provided buoyancy for the outer enclosure. A total of nine complete systems were constructed for installation at the three sewage oxidation sites selected for this study.

Each of the inner cages were stocked with 100 gravid female mosquitofish. When these females released their fry, the young had the potential to escape through the mesh of the inner brood cages, while the outer cage enclosures, as confinement barriers, kept all the fry within the culture system. Seclusion of the fry within a protective environment alleviated problems with potential predators. Zooplankton and other forage microorganisms were able to pass through the fine mesh netting and allow the young fry and adults to feed upon the vast food source existing in the surrounding waters of the wastewater impoundment.

At each of the three pond sites, three cage culture units were floated in the water. All of these small systems, regardless of situation, were anchored some distance offshore in water at least 2.5 - 3.0' (76.2 - 91.4 cm) in depth to discourage vandalism and maximize pond water circulation through the culture enclosures. To decrease the influence of sewage loading on pond water quality, the cage units were placed in those wastewater ponds farthest from the primary sewage treatment plant effluent source, wherein more consistent dissolved oxygen levels occurred and mosquitofish were known to have already survived in past years.

Each of the three cages installed at the pond sites were treated differently with respect to the experimental protocol. The first cage system was set up as a control. Only fish that were dying or had already died were periodically replaced. The second regime, called Treatment #1, was conducted so that only the gravid females that had expelled their fry and dead or moribund fish were replaced.

The third regime, or Treatment #2, called for periodic replacement of all the gravid females regardless of condition.

On May 10, 150 gravid females were placed in each of the three culture units at the Marysville site. Seven days later almost all of the stocked fish had died, therefore one hundred replacements were immediately restocked in each unit. Approximately 25 live fish per cage remained the next time the cages were checked on June 2, so the cage systems were relocated to an adjacent pond exhibiting better water quality. It was suspected that excessively high levels of dissolved CO_2 coupled with low nocturnal O_2 levels were the cause for the large fish kills noted in the first cage location at this particular wastewater treatment facility. On June 7, 100 gravid females were put in each of the cage systems at the new pond. Fry were harvested two weeks later on June 21.

On May 15, 100 gravid females were put in each of the cage units at the Live Oak Sewage Treatment Facility pond site. Similarly, brood fish stocking was completed at the Sheridan Treatment Facility pond site on May 16. The first fry harvest was initiated on June 7 at both the Live Oak and Sheridan sites. During the 2-week period between harvests, routine checks on the culture systems were made. The date, time, temperature of the pond water, dissolved O_2, as well as occasional dissolved CO_2 levels, and brief weather descriptions were recorded. Also, the general condition of all the cages were checked to assure that repairs were not required. When time permitted, chemical and physical data and general observations were recorded at the time of routine cage harvest activities.

The fry were harvested approximately bi-weekly, put into small containers and preserved in 10% formalin for later enumeration and examination. The brood females were then replaced according to the respective treatment protocols.

By June 28, 2" (5.1 cm) plastic mesh covers were placed over the cage units to deter birds from alighting on the cage framework and preying upon fish. Although bird depredations were never actually observed, one brood cage's flap lid had been depressed below the water line at the Sheridan site, presumably by the weight of roosting birds. Some exposed brood fish could have possibly been consumed by these birds at that time.

Obtaining adequate stocks of gravid female mosquitofish for the study in early September was very difficult. It is possible that some of those obtained, which were thought to be gravid, were in actuality not and didn't expel their young within the 2-week harvest interval. On September 21, the last fry collection was made and the cage systems were recovered and returned to the district yard.

Due to the lack of necessary numerical repetition in cage culture units within and among treatments for this study, the analyses of data had to be limited to the identification of apparent trends and was not the result of any standard statistical methodology.

Results and Discussion.

After sorting and compiling collected data, it was possible to conclude which of the three sewage facility pond sites harboring the cage culture systems promoted the greatest fry production (Table 1), and which experimental brood stock management protocol afforded gravid females the best conditions for fry production (Table 2).

The Live Oak wastewater pond site yielded an average of 1,400 fry per harvest, which was greater than the individual yield of the pond sites at Marysville or Sheridan. In contrasting the three brood stock management treatments any resultant differences were much less discernable. The control cages produced only 0.68% more fry than from the regime described as Treatment #1 and 6.06% more fry from the regime called Treatment #2. A possible explanation for the 6.06% difference seen between the cage units comprising the controls and Treatment #2 was that all of the females were replaced every two weeks despite their actual reproductive readiness.

Furthermore, collected evidence suggested the existence of a direct correlation between water temperature and dissolved oxygen to provide the innate cue that actually stimulated gravid females to expel their young. As water temperatures and dissolved oxygen levels increased, fry production during those periods was greater (Figure 2).

With respect to the numbers of fry propagated, the results from the 1989 and 1990 studies were very similar. The total number of fry harvested from the three cage stockings in 1989 was 4,438 (Coykendall et al. 1991). Combining the mean fry produced from three of the 1990 brood fish stockings would yield 4,200 fry, which was only 238 less fry than from the three brood stockings made in 1989. Furthermore, the 1,500 brood females stocked in 1989 had a mean yield amounting to 2.96 fry per

Table 1. Fry harvests grouped according to cage site.

Site		No. harvests	No. fry harvested	Mean no. fry/harvest
Live Oak		8	11,200	1,400
Marysville		7	6,268	895
Sheridan		8	4,712	589
	Totals	23	22,180	964

Table 2. Fry harvests (from all three sites) grouped according to treatment protocol.

Treatment	Brood fish replaced	No. fry harvested	% of all fry harvested
Control	Dead and dying	7,892	35.58
Treatment #1	Spawned out, dead, dying	7,740	34.90
Treatment #2	All fish	6,548	25.52
	Totals	22,180	100.00

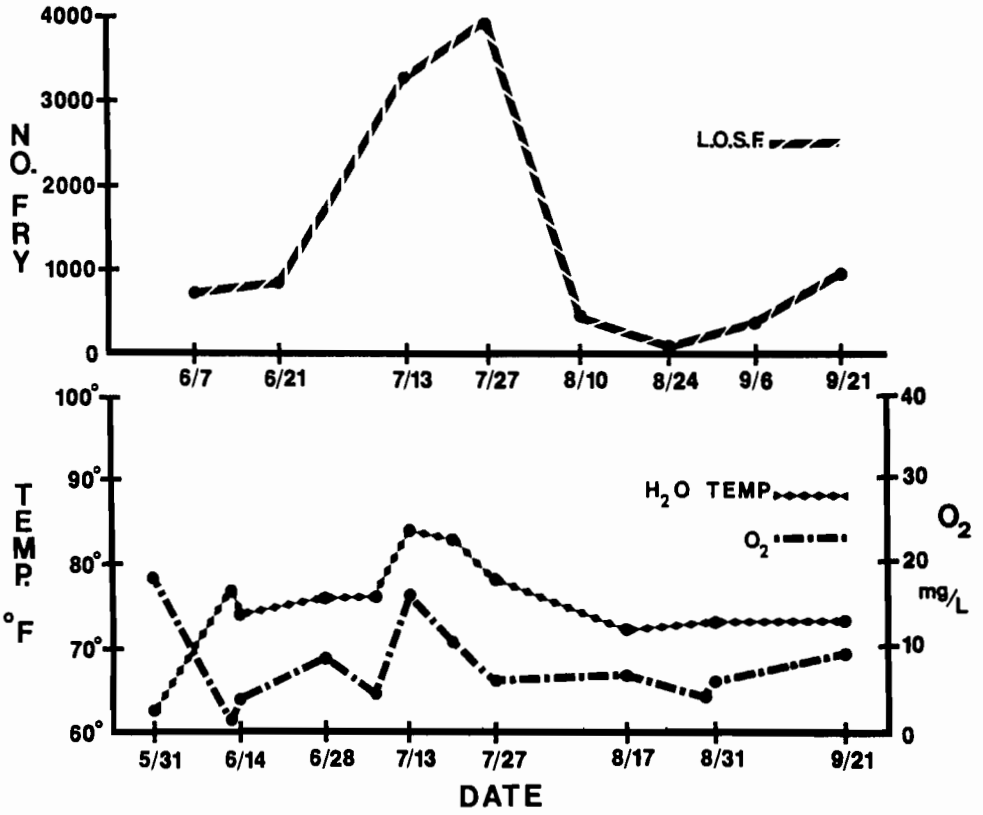

Figure 2. Influence of water temperature and oxygen levels on fry production in the Live Oak Wastewater Treatment Facility oxidation pond.

female. In 1990, data reflecting the actual number of female brood fish periodically restocked was not recorded, thus an exact ratio could not be calculated. However, after each harvest it was a certainty that less than 300 brood fish were replaced. With less than 900 females from three brood fish stockings, and with 4,200 offspring harvested, the mean fry yield for each female could be expected to exceed a minimum value of 4.67.

A massive quantity of notonectid eggs and egg cases had been oviposited on the outside of all the outer cage enclosures by late July. This accumulation hindered the free exchange of outside pond water through the cage systems, which very likely limited forage zooplankton availability. The notonectic oviposition occurring on the fine mesh netting of the outer enclosure diminished sharply after placing one Altosid® (methoprene) XR Extended Release Briquet (Zoecon Corp., Dallas, TX 75234) in each of the brood cage units. By early August, aquatic weed growth in the Live Oak pond site had become a hindrance to the fry harvesting process. The outer netting supported large quantities of filamentous algae and also enclosed substantial amounts of duckweed (*Lemna* sp.). Ortho Diquat® Herbicide (Chevron Chemical Co., Ortho Agricultural Chemicals Division, San Francisco, CA 94120) was applied as a single treatment over the entire pond in an attempt to control these weeds. While much of the filamentous algae was successfully controlled, the duckweed infestations remained largely unaffected; however, the chemical treatment was deemed beneficial in that subsequent harvests were easier to perform. The effect the methoprene treatments or the single Diquat® treatment had upon the adult females, their reproductive processes, or to the fry was assumed to be negligible; thus no adjustments were made to the data in any compensatory effort.

Recommendations.

The 1990 cage culture methodology has value as it achieved some measure of success since numerous fry were continually produced throughout the summer. In many presently un-utilized ponds, a technician could install and periodically harvest fry from this type of culture system.

It is clear that some of the difficulties experienced in 1989 were successfully overcome in 1990; however, the design and/or management techniques could certainly be further refined. For example, designing smaller cages for the 1990 study was an immense improvement with respect to cage portability and manageability; although an even narrower construction width in the outer cage would have aided in the harvesting of fry when only one person would be conducting the harvest procedure.

With regard to management, there was no pronounced difference in the number of fry propagated in the control and Treatment #1. However, operationally, the control protocol would be the most practical to adopt because the tiresome task of removing and replacing brood females that had released their young, which was required in the Treatment #1 protocol, would be too time consuming. Contrasting the control with Treatment #2, the noticeable 6.06% difference would signify the control protocol as again the more productive regime.

It was fortunate that the cage culture units themselves did not require much maintenance because the distances between the three sites were substantial and required too much travel time. With the exception of the aquatic weed control activities required at the Live Oak site, the culture system installed there required very little maintenance and continually produced modest, but usable quantities of young fish, and succeeded as a potential supplemental mosquitofish source for the Sutter-Yuba Mosquito Abatement District. Other agencies could easily adopt this system; although some modifications in cage configuration and management/harvest protocol may be necessary for optimization.

References.

Coykendall, R.L., E.E. Kauffman, and S.E. Abshier. 1991. A versatile cage culture system for the mosquitofish, *Gambusia affinis* (Baird and Girard). Proc. Calif. Mosq. Vector Control Assoc. 58:107-110.

INTENSIVE CULTURE TECHNIQUES FOR OVERWINTERING MOSQUITOFISH, *GAMBUSIA AFFINIS*

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ABSTRACT

As the District developed more earthen ponds over the last six years for the purpose of mosquitofish production, predation has significantly reduced overwintering survival of our mosquitofish stocks. The purpose of this study is to find alternative methods of overwintering mosquitofish where they will not be available to predators. In this preliminary study, three different overwintering techniques were evaluated over the course of a couple of seasons (1988-89 and 1989-90). The traditional earthen ponds, cement raceways and aluminum raceways were all compared for the percentage of overwintered fish stock. Reproductive condition of the overwintered brood fish from the various techniques was examined after they were stocked in rearing ponds by comparing that pond's production to other ponds of similar size and stocking rate.

The intensive culture overwintering techniques, cement and aluminum raceways, provided the best overwintering survival; there was a statistically significant difference in harvest data. In the 1988-89 season, the cement raceways had a 1.66% survival rate compared to the earthen ponds with a 41.3% survival rate. When evaluated in the 1989-90 season, the aluminum raceways had a 60.7% survival rate compared to the earthen ponds with a 36.5% survival rate for the same season. All the raceway fish were fed freshwater invertebrates (collected from the District's overwintering ponds) and a commercial feed ration identical to that used for the ponded fish.

In the production ponds, there was a significant difference in harvest data from a pond stocked with fish that overwintered in cement raceways compared to ponds stocked with fish overwintered in earthen ponds. Pond 14, the only pond stocked with cement raceway overwintered-fish, produced 305 pounds of mosquitofish. The most productive of the District's earthen production ponds that were initially stocked with earthen pond overwintered-fish at a rate comparable to Pond 14 (50 pounds/pond) yielded only 255 pounds of mosquitofish.

There was no significant difference in harvest data from a pond stocked with aluminum raceway overwintered-fish compared to those with earthen pond overwintered-fish, although the pond with aluminum raceway overwintered-fish did produce the highest yield for ponds of that size (0.78 acres) and similar stocking rate (70 pounds/pond).

With so few replications of each intensive culture overwintering technique, this data only represents a preliminary study which will require further investigation.

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SCAT HOVERCRAFT USE IN MOSQUITO CONTROL

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Each year the public demands increased mosquito control, but despite advances in pesticides, there are still limitations in cost, labeling, and residual control. This often requires more frequent treatment of sources, and therefore, there is a need to develop innovative and efficient new application technology to keep treatment ahead of the mosquito cycle. We are confident we have found a new and exciting tool in the SCAT Hovercraft.

The hovercraft has a 30-year history as a commercial transportation vehicle, perhaps best known for carrying people swiftly and smoothly across the turbulent English Channel. More recently, it has caught on as a recreational toy, and SCAT of Florida is the leading company in the mass production and sale of small craft for the general public.

The craft is essentially a boat/aircraft hybrid with an engine that produces both lift and thrust. It flies on an air bubble created by forced air contained by skirts around the bottom. It is steered by a combination of thrust rudders and adjustments of the operator's body position.

How we came to be the first to use it for mosquito control requires that I subject you to a geography and history lesson. The Northwest Mosquito Abatement District (NWMAD) encompasses approximately 155 square miles of northwest Riverside County. Formed in 1959, the District is responsible for 25 miles of the Santa Ana River and the 8,000-acre Prado Flood Control Basin which protects Orange County from floods. Duck club ponds comprise six hundred acres of this basin and are filled in the Fall with reclaimed water from the river. These ponds present a substanitial mosquito-breeding problem for the District and require considerable amounts of time, manpower, equipment and pesticides to control properly.

In 1989, while discussing duck pond problems with a resident plagued by mosquitoes, it was suggested that we look into the SCAT Hovercraft as a possible solution. We quickly contacted the company for further information. Soon thereafter we set about arranging a demonstration for the purpose of evaluation and possibly convincing management of its potential. SCAT's commercial representative brought a model to test in four selected sites: vegetated duck ponds, water treatment percolation ponds, dairy ponds and a flooded pasture.

Our impressions were overall positive. The machine is simple, rugged, versatile over wet and dry terrain, fast and light. It flies over berms, up and down steep banks, over dairy pond floatage and through small stands of tules.

Some limitations of the craft are:

1. Diminished maneuverability in tight spaces.
2. Pronounced turbulence, flying dust and chaff.
3. Inability to fly over rocky, uneven terrain, wide ditches, and woody or thick vegetation.

These drawbacks, however, are overshadowed by its remarkable speed in treating wet, open areas in a short amount of time. The efficiency of the craft allows treatment of 6-8 acres per hour in duck ponds depending on density of growth, the number of ponds and the ability of the operator.

The SCAT AC 960 Hovercraft purchased by the District last September for under $7,500 is 9-1/2 feet long, 6 feet wide, weighs just over 400 pounds and has a payload of 500 pounds. Ours is powered by a Rotax 52-horsepower, two-stroke, twin-cylinder gasoline engine similar to those that power ultra-light aircraft. These engines are specifically designed for dependability and a high power to weight ratio. The engine has only seven moving parts and minor adjustments can usually be made in the field. A 6-gallon gas tank provides about two hours of flight time.

We worked closely with SCAT's engineers to adapt their craft for mosquito control. SCAT equipped this commercial-grade model with a sprayer bar and mounting brackets for nozzles and

plumbing. We added two 12-volt liquid pumps, switches, gauges and solenoids to control pesticide flow through the five swivel-type nozzles. Two removable tanks carry a total of 12 gallons of liquid pesticide onboard.

We have used the hovercraft for control work only in duck ponds and sewage ponds, but as it showed promise over other terrain during the demonstration, we look forward to testing the craft in dairy ponds, pastures, and the meandering Santa Ana riverbed. We also will be testing other types of larvicide and adulticide delivery systems.While not entirely replacing other equipment, it is by far the most fun, and we are proud to be the first to use it successfully in a mosquito control program.

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USE OF THE ARMY INSECTICIDE MEASURING SYSTEM (AIMS) AT THE DISTRICT LEVEL

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An Army Insecticide Measuring System (AIMS), a droplet measurement and analysis device, has been used at the San Joaquin County Mosquito Abatement District (SJCMAD) for some time. This device, a product of KLD Associates, Inc. (Huntington Station, NY 11746), was purchased primarily to 1) measure the volume median diameter of droplets within an aerosol insecticide cloud, 2) assist in the calibration of our aerosol generating machines, and 3) limit liability to the District by maintaining records of our equipment's performance. However, when put to use, it was found to be instrumental in modifying our cold fogging (adulticiding) operations.

To evaluate the degree of control achieved by our District's adulticiding operations in rice fields near the City of Escalon, San Joaquin County, California, sentinel cages containing approximately ten laboratory-reared adult *Culex pipiens* L. mosquitoes each were placed on stands approximately three feet above ground level in the rice fields. The stands were located at intervals up to one mile from the point of application in a single transect parallel to the wind direction. They were simultaneously exposed to a single aerosol cloud of pyrethrin-based insecticide (Pyrenone Crop Spray, Fairfield American Corp., Newark, NJ 07105) applied at a rate of two ounces per acre from a truck-mounted Leco model HD Series D ultra-low volume aerosol generator (Lowndes Engineering Co., Inc., Valdosta, GA 31601). Measured atmospheric conditions indicated that an inversion was in existence during the treatment. A single cage of mosquitoes was held in similar atmospheric conditions but away from the fogging operation as a control.

A disappointing 14% mortality of the caged mosquitoes was noted in this single trial (Table 1). The aerosol generator (cold fogger) is typically operated with an expected 300 foot swath. The destruction of sentinel mosquitoes was not completely effective within this distance and although some control was noted past 300 feet, no control was noted past 3/4 of a mile (3,960 feet). The aerosol generator had received its routine calibration some time prior to this investigation and was found to be operating within acceptable parameters at that time. Following this trial failure, a program to assess and characterize the cloud produced by the District's generators was developed and implemented. The AIMS device was used along with a personal computer for this achievement.

Understanding the AIMS device.

The AIMS device is a microprocessor-based, field portable unit which will count and measure those droplets that impinge upon a hot wire probe held in an aerosol cloud. It will determine the size of each droplet (diameter), count the number of droplets measured, and calculate the collective volume median diameter (VMD) of those droplets in addition to some other basic but useful functions. The droplets, once measured, are assigned by the machine to one of eleven bins; each bin containing a range of droplet sizes whose diameters are close to 1.0, 1.5, 2.5, 6.5, 12.5, 22.0, 31.5, 40.0, 90.0, 170.0 or 200.0 microns. The bins' contents, the duration of the count, and the VMD of the cloud are displayed on a paper tape printed for a permanent record.

Making use of the information.

After the device collects the information about the cloud, it is up to the user to evaluate this information and adjust his/her equipment to its best advantage. We arrange the information in such a way as to be able to determine the percentage (by volume) of the pesticide falling into the various

Table 1. Results of two trial applications of Pyrenone Crop Spray against sentinel cages of *Cx. pipiens* mosquitoes using Leco U.L.V. vehicle-mounted equpiment. The first and second trials were prior to and after characterization of the aerosol cloud by the AIMS device, respectively.

Cage distance (feet)	Percent mortality	
	Trial 1	Trial 2*
Control	0.0	5.0
100	0.0	80.0
200	10.0	95.0
300	20.0	60.0
600	50.0	95.0
1,320	20.0	65.0
2,640	0.0	85.0
3,960	10.0	45.0
5,280	0.0	95.0
Mean Average	13.8	77.5

*Figures have been corrected for control deaths.

droplet sizes (bins). The approximate volume contained in the various bins may be estimated by applying the formula for the volume of a sphere ($1/6\pi D^3$, where D is the droplet diameter in microns) multiplied by the number of droplets in the bin. The resulting value is the total volume (in microns^3) of insecticide contained within that particular bin. Once this has been done for all the bins, the percentage of volume in each bin may be calculated from the total insecticide volume in all bins. Groups of bins are then formed by selecting those with the most desirable droplet sizes.

To reduce the time required to perform the mathematical calculations, a computer program was written using the commonly available Lotus 1-2-3® spreadsheet software (Lotus Development Corp., Cambridge, MA 02142). It allows information characterizing the cloud to be quickly generated (Fig. 1) with a minimal amount of initial input. Using the results of these calculations, the aerosol generating machines are adjusted to produce a high percentage of insecticide volume in the droplet sizes that are believed to have the greatest effect on target organisms, while still complying with label restrictions.

Effecting change.

With the droplet information in hand, the aerosol generating machines are then adjusted to produce a desirable cloud; normally a cloud with at least 85% of its droplet volume falling in the 6.5-31.5 micron range. However, label restrictions may require other cloud configurations. On our equipment, adjustment of the cloud droplet size is through the regulation of air pressure at the aerosol nozzle and by changing the insecticide flow rate. Inability to adjust the droplet size in this way indicates poor or defective nozzles and the need for repair of the aerosol generator. For instance, it was necessary to replace a complete nozzle on one of our generators in order to produce the desired cloud.

Once the ability to characterize the aerosol cloud and adjust the equipment accordingly was obtained, a second trial application was undertaken to evaluate the effectiveness of our program. The same aerosol generator used in the first trial was adjusted to deliver 94% of its insecticide volume in the 6.5-22.0 micron range. Sentinel cages were used again in the same manner and locations as before with the exception that the number of adult mosquitoes in each cage was increased from ten to twenty. The same application equipment, materials, and rates were also used and similar atmospheric conditions were encountered during the treatment period. The degree of adult mosquito mortality in the sentinel cages increased to 77.5% and effective control was more or less even along the full mile (Table 1).

The use of sentinel cages was discontinued at this point and the degree of control of wild mosquitoes was then used to evaluate the effectiveness of our adulticiding operations. Control effectiveness was estimated during three subsequent treatments with the use of CDC CO_2-baited traps by trapping adult mosquitoes in the same area prior to and after the treatments. No controls were used in the CDC monitoring. This trapping showed reductions similar to those indicated by the sentinel cages with reductions of 77%, 80%, and 87% (mean average of 81%). As a result of these findings, the swath of the aerosol application for adult mosquito control in rice fields has now been increased to one mile; resulting in substantial savings in both time and insecticide.

Some final remarks.

It is not the purpose of this paper to solely

Date	June 28, 1990			
Fogger number	39			
Pesticide	Crop Spray			
Pesticide flow	6.00	ounces/minute		
Air pressure	6.00	psi		

droplet size	number	volume	percent of volume	accumulated percent
1.00	641.00	335.63	1.34	1.34
1.50	223.00	394.07	1.57	2.91
2.50	90.00	736.31	2.93	5.84
6.50	37.00	5320.35	21.20	27.04
12.50	7.00	7158.58	28.53	55.57
22.00	2.00	11150.56	44.43	100.00
31.50	0.00	0.00	0.00	100.00
40.00	0.00	0.00	0.00	100.00
90.00	0.00	0.00	0.00	100.00
170.00	0.00	0.00	0.00	100.00
200.00	0.00	0.00	0.00	100.00

Percentage of Volume

bins 1-2.5	5.84
bins 6.5-31.5	94.16
bins 40-200	0.00

Figure 1. Sample output from the Lotus 1-2-3 program characterizing the aerosol cloud as analyzed by the AIMS device.

compare treatment results pre-AIMS and post-AIMS device use. It is meant to present the way this device is used at the District level by the SJCMAD. We felt that our adulticiding operations were less than adequate (this being shown by the live adult mosquitoes in our sentinel cages after treatment in the initial trial) and we utilized the AIMS device to characterize the aerosol cloud emitted by our equipment so that we could correctly adjust that equipment for optimum performance.

The AIMS device is one tool used to provide information about an aerosol cloud. Other methods are available to gather this same information. The advantages of the AIMS device are the speed of its results and its portability. However, the overall expense of this machine and the fragility of the wire probes may put this device out of the means of all but the most dedicated districts.

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, the written report, and presentation at the annual conference.

David R. Mercer was the recipient of the 1991 award at the 59th Annual Conference held in Sacramento. The other finalists were Jeffrey Beehler and Barbara Des Rochers. The three finalists' papers are printed on pages 101-114.

Previous William C. Reeves New Investigator Award Winners:
1988 - Vicki L. Kramer
1989 - Truls Jensen
1990 - Gary N. Fritz

1991 WINNER

WILLIAM C. REEVES NEW INVESTIGATOR AWARD

1991 Proc. Calif. Mosq. Vector Control Assoc. 59: 101-107

TANNIC ACID CONCENTRATION MEDIATES *AEDES SIERRENSIS* DEVELOPMENT AND PARASITISM BY *LAMBORNELLA CLARKI*

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ABSTRACT

Tannic acid concentration influenced developmental rates, survivorship and size of *Aedes sierrensis*. Concentration of tannic acid also influenced *Lambornella clarki* cell number and attack rates against mosquito larval predators; however, uninduced cultures of *L. clarki* initiated parasitic cycles at intermediate tannic acid concentrations. Thus concentration of tannins in treehole waters may influence vector potential for treehole mosquito species.

Introduction.

Tannins are polyphenolic, water soluble, high molecular weight compounds which bind proteins (Martin and Martin 1982; Hagerman and Robbins 1987). Tannins have been widely studied as defensive compounds produced by plants against herbivory. Herbivorous insects exploit, avoid or succumb to diets rich in tannins depending upon their adaptations to tannins (Bernays 1978; Bernays et al. 1983; Martin et al. 1987; Buntin and Wiseman 1990).

Little is known about the effects of tannins upon mosquito development although tannin-rich litter accumulates in treeholes inhabited by container-breeding mosquitoes such as *Aedes sierrensis* (Ludlow). Both major types of tannins (i.e. condensed and hydrolyzable) are found in oak leaves and bark; both types leach into treehole water. Tannin content varies between treehole habitats and within individual treeholes as decomposition rates and water levels vary (Bradshaw and Holzapfel 1988; D.R. Mercer unpublished data). Treehole inhabitants must tolerate a wide range of tannin concentrations throughout their distribution while individuals must tolerate variability during development.

Here I report effects of tannin concentration upon *Ae. sierrensis* and *Lambornella clarki* Corliss and Coats, a protozoan inhabitant of treeholes which serves as both prey and parasite of mosquito larvae (Washburn et al. 1988). The free-living, bactivorous form of *L. clarki* which persists in the absence of mosquitoes is known as a trophont. In response to the threat of predation by mosquito larvae, some trophonts transform into theronts, the host-seeking form of *L. clarki*. Theronts die if they cannot find and encyst upon suitable larval hosts. Successful infection by a single ciliate is often sufficient to kill the larva and result in the clonal production of numerous ciliates. The ability of free-living trophonts to switch to parasitism of larval predators may insure the persistence of *L. clarki* and may be exploited for the biological control of treehole-breeding mosquitoes.

Materials and Methods.

The effects of tannin on mosquito development. I measured adult production and size of *Ae. sierrensis* raised in tannic acid solutions of eight concentrations: 0, 0.015, 0.075, 0.15, 0.75, 1.5, 7.5 g/l

and a saturated solution. I added 10 newly hatched *Ae. sierrensis* larvae, equal amounts of ground rat chow and 50 ml of the appropriate tannic acid solution to five replicates of each treatment. I exposed larvae to long day photoperiod at room temperature and assessed developmental profiles twice weekly. When adults emerged, the number produced and mean male and female winglengths (measured at 30X) were determined for each replicate.

Similarly, mosquito survivorship was monitored in solutions of mixed tannins purified from loblolly pine foliage at five concentrations: 0, 0.1, 0.25, 0.5 and 1.0 g/l. Ten replicates with 25 newly hatched larvae in 50 ml tannin solution were used for each treatment. I determined adult production and mean male and female winglengths for each replicate. Significant differences among treatment means were determined by one-way ANOVA (α = 0.05); significant differences between pairs of means were determined by multiple comparison methods (T- or GT2-method, Sokal and Rohlf 1981).

The effects of tannin on *Lambornella clarki*.

Bioassay: I represented treehole tannins with tannic acid in laboratory experiments to test concentration effects upon *L. clarki* parasitism of *Ae. sierrensis* larvae. For each of these tests, a bioassay was used to quantify attack rates of ciliates against mosquito hosts. Laboratory cultures of *L. clarki* grown in cerophyll infusions were migrated into dilute, autoclaved treehole water. At time zero, cells were aliquoted into replicated microcosms containing different concentrations of tannic acid dissolved in dilute treehole water. Cells in half the replicates of each concentration were intentionally induced to parasitize mosquito larvae with the addition of ground larval homogenate; an identical set of control replicates received no induction cue. Induced populations of *L. clarki* form theronts at about 48 hr at which time 10 newly hatched *Ae. sierrensis* larvae were added to each replicate. Larvae were removed and stained 24 hr later; microscopic examination of these larvae indicated the induction response within the corresponding cell population by the number of cuticular cysts formed on the larvae added to that replicate.

Tannic acid concentration: The bioassay was conducted with *L. clarki* of the "DH" geographic strain (from Marin County). Tannic acid concentrations were 0, 0.025 and 0.25 g/l.

Cell numbers and morphologies: In a separate experiment, I determined cell densities at 24-hr intervals and cell morphologies at 12-hr intervals following exposure to tannic acid in addition to conducting the bioassay (48 to 72 hr). Cell densities were measured by fixing a sample of the appropriate solution with amido black stain and microscopically counting the number of cells in one milliliter. Cell morphologies were determined by spotting out cells into droplets, viewing them microscopically and categorizing 100 cells as trophonts, dividers and theronts.

Geographic strains of *Lambornella clarki*: Two additional strains of *L. clarki* ("BOT" and "MLAG" from Santa Barbara and San Diego Counties, respectively) were tested for the effect of tannic acid upon induction response. Populations of both of these strains were exposed to a wider range of tannic acid concentrations during the bioassay: 0, 0.1, 0.2, 0.3 and 0.4 g/l.

Results.

The effects of tannin on mosquito development. The concentration of both tannic acid and loblolly pine tannin in larval rearing solutions significantly affected mosquito development. The two highest concentrations tested produced no adults for either type of tannin. Mean adult production varied significantly with tannic acid concentration among those treatments which produced adults (ANOVA, $F = 20.88$, $p >> 0.01$); significantly more adults successfully eclosed from intermediate concentrations (i.e., 0.075 to 1.5 g/l, Table 1). Mean adult numbers also varied significantly among loblolly pine tannin treatments which produced adults ($F = 100.3$, $p >> 0.01$).

Similarly, tannin concentration had significant effects on adult size (Table 1). Mean winglengths were significantly different among tannic acid treatments for females ($F = 5.24$, $p >> 0.01$) but not males ($F = 2.46$, $p = 0.06$). Loblolly pine tannin concentration had a significant effect upon both mean male ($F = 70.16$, $p >> 0.01$) and female winglengths ($F = 47.59$, $p >> 0.01$); the largest adults for both sexes eclosed from 0.25 g/l pine tannin solutions.

The effects of tannin on *Lambornella clarki*.

Tannic acid concentration: The induction response of *L. clarki* populations treated with ground larval homogenate decreased with tannic acid concentration (Fig. 1). Cell populations exposed to no tannic acid during the bioassay formed 17.6 ± 1.9 (mean ± one standard error of the mean) cuticular cysts per larva, a relatively high

Table 1. Effect of tannin concentration on *Aedes sierrensis* production and size. Numbers shown are treatment means; means followed by different letters within a column and type of tannin are significantly different by multiple comparison methods (T- or GT2-method, Sokal and Rohlf 1981). Initial populations were 10 (tannic acid) or 25 (pine tannin) newly hatched larvae.

	Tannin concentration (g/l)	Number of adults	Mean male winglength (mm)	Mean female winglength (mm)
TANNIC ACID	0	3.6^{a}	2.65^{d}	$3.27^{e,f}$
	0.015	6.4^{b}	2.75^{d}	3.38^{e}
	0.075	8.8^{c}	2.75^{d}	$3.35^{e,f}$
	0.150	$8.2^{b,c}$	2.77^{d}	$3.35^{e,f}$
	0.750	9.6^{c}	2.71^{d}	3.22^{f}
	1.500	9.4^{c}	2.72^{d}	$3.29^{e,f}$
LOBLOLLY PINE TANNIN	0	3.0^{g}	2.01^{i}	2.27^{l}
	0.10	9.8^{h}	2.21^{j}	2.35^{l}
	0.25	3.6^{g}	2.44^{k}	2.80^{m}

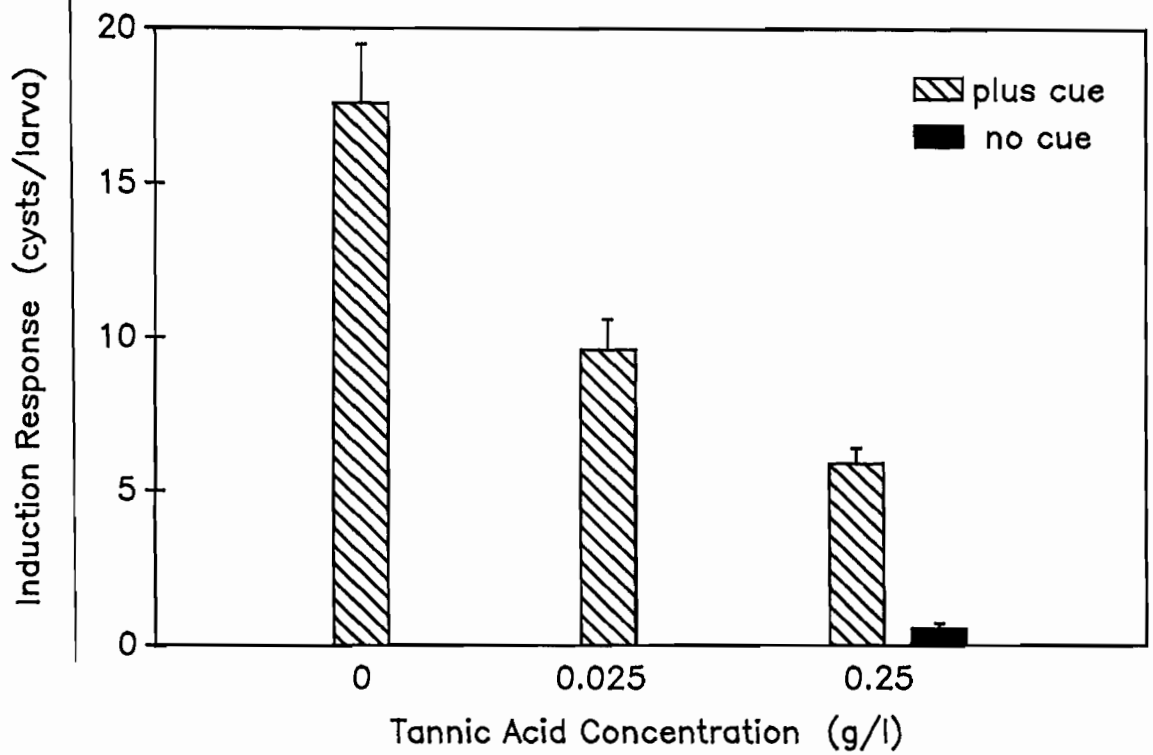

Figure 1. Effect of tannic acid concentration on "DH" induction response. Bar height represents treatment mean ± one standard error of the mean.

response. In the 0.25 g/l treatment, the mean response was only 5.9 ± 0.5 cysts per larva.

There were also 0.56 ± 0.18 cysts per larva formed in the high tannic acid replicates which received no induction cue; this low level response was significantly greater than that of the no tannic acid control (Fig. 1). Microscopic examination revealed that high proportions of cells exposed to 0.25 g/l tannic acid were lysed or misshapen; those which survived produced the unexpected encystment response even without larval cue.

Cell numbers and morphologies: The addition of tannic acid slowed theront formation in the treatments which received larval homogenate; peak theront production occurred between 36 and 48 hr for the 0 and 0.025 g/l treatments. Proportionally fewer theronts were formed in the 0.25 g/l treatment, and peak theront production occurred between 48 and 60 hours. Among the treatments which received no larval homogenate, significant numbers of theronts appeared only in the 0.25 g/l tannic acid treatment with peak formation between 60 and 72 hours.

The effect of tannic acid concentration upon *L. clarki* cell density through time is illustrated in Figure 2a for the treatments which received no induction cue; results for treatments exposed to larval homogenate were similar but are not shown. There were no differences in cell growth rates and mean cell numbers between the 0 or 0.025 g/l tannic acid treatments. However, from an initial density of 220 cells/ml, the density of *L. clarki* exposed to 0.25 g/l tannic acid decreased to 130 cells/ml during the period of the bioassay; results from the bioassay are shown in Figure 2b. There was good agreement between the proportion of theronts and the number of cuticular cysts formed during the bioassay.

Geographic strains of *Lambornella clarki*: The effects of tannic acid concentration upon induction response for the "BOT" and "MLAG" geographic strains are shown in Figure 3a and 3b, respectively. Uninduced populations of both strains produced significantly more cuticular cysts at intermediate concentrations of tannic acid than the corresponding controls. The patterns of response among the replicates intentionally induced with larval homogenate differed from previous results with "DH" strain.

Discussion.

Intermediate concentrations of both hydrolyzable tannin (i.e., tannic acid) and condensed tannin (i.e., pine foliage tannin) in the development medium of immature mosquitoes were apparently optimal for mosquito production. High concentrations of both types of tannins resulted in complete larval mortality, and control solutions with no dissolved tannins produced relatively few mosquitoes for both experiments. In the case of loblolly pine tannin, these adults were also significantly smaller than adults emerging from solutions of intermediate concentration.

These data are consistent with a field experiment in which significantly more adults eclosed from tannic acid solutions of intermediate concentrations than high or no tannic acid solutions (D.R. Mercer, unpublished data). In all three experiments, intermediate tannin concentrations slowed the rate of larval development relative to tannin-free controls. Populations which developed slowly fed longer and produced more numerous, and in some cases larger, adults. Thus, tannin concentration may directly influence vector potential for treehole mosquitoes.

Tannins may also impact natural enemies of treehole mosquitoes. The effects of tannin concentration upon populations of *L. clarki* are probably influenced by the vigor and origin of the cells. Thus, for the strains tested, there were no consistent trends between tannin concentration and the number of cuticular cysts formed by cell populations induced with larval homogenate.

The parasitic response by uninduced populations of *L. clarki* to intermediate concentrations of tannic acid is more noteworthy. Cuticular cysts were induced by tannic acid alone in all three geographic strains tested. Free-living *L. clarki* switched to parasitism when exposed to osmotically compromising concentrations of tannic acid.

There appears to be a property inherent to solutions of intermediate tannic acid concentration which lyses cells, slows cell growth rates and induces a significant number of parasitic cells. In natural treeholes, parasitized larvae may be temporary refugia for ciliates until rainfall dilutes tannin concentration or the population becomes acclimated to the tannin. Finally, since low level parasitic infections of larvae carry through to emergent adults which disperse the ciliate, *L. clarki* may escape treeholes with excessive tannins through parasitism. The response of *L. clarki* to the perceived threat of tannins may ultimately determine its utility as a manipulated biological control agent for container-breeding mosquitoes.

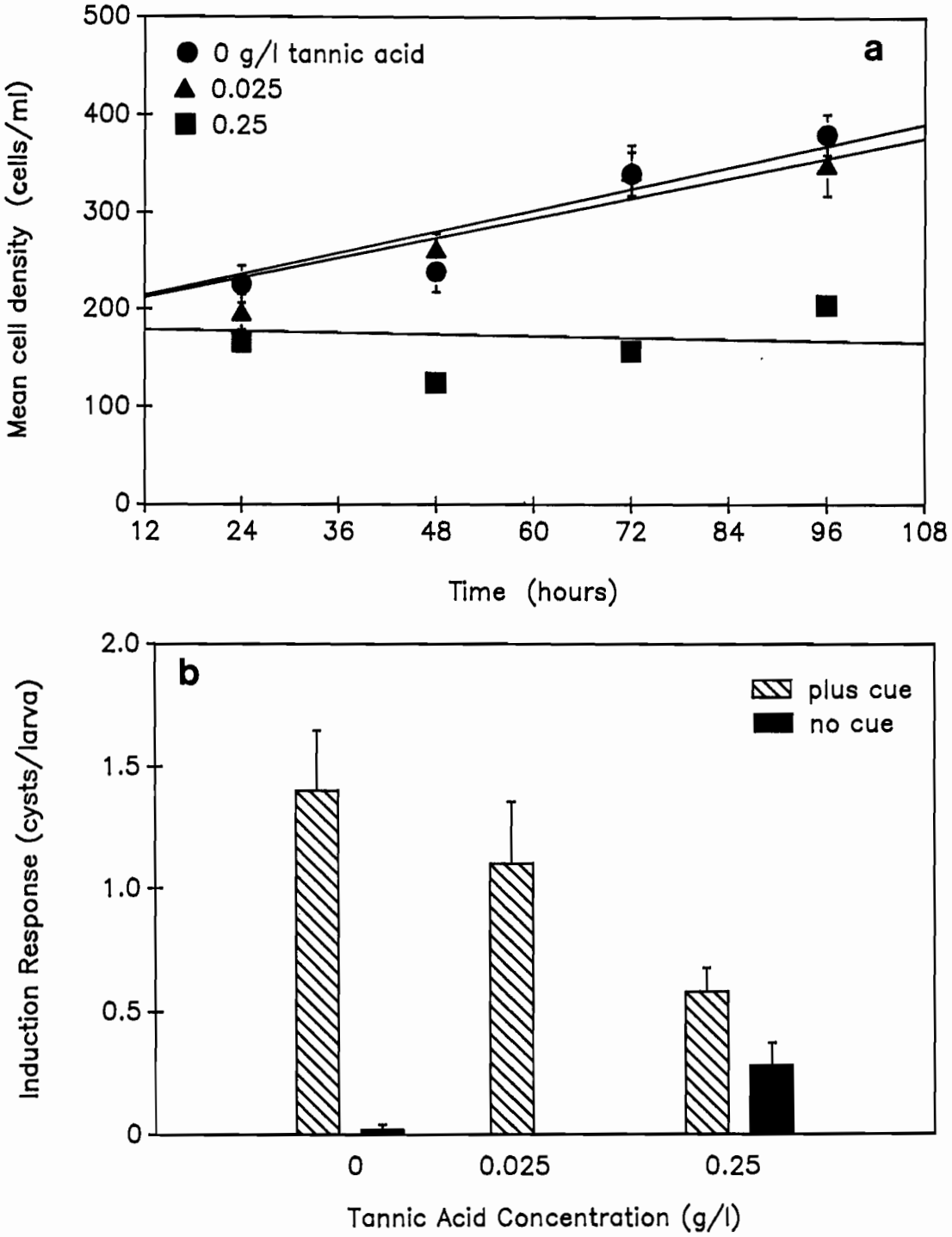

Figure 2. (a) Cell density of "DH" cells following exposure to tannic acid. Points represent treatment means ± one standard error of the mean; regression lines indicate growth rates. (b) Induction response of "DH" cells following exposure to tannic acid. Bar height represents treatment mean ± one standard error of the mean.

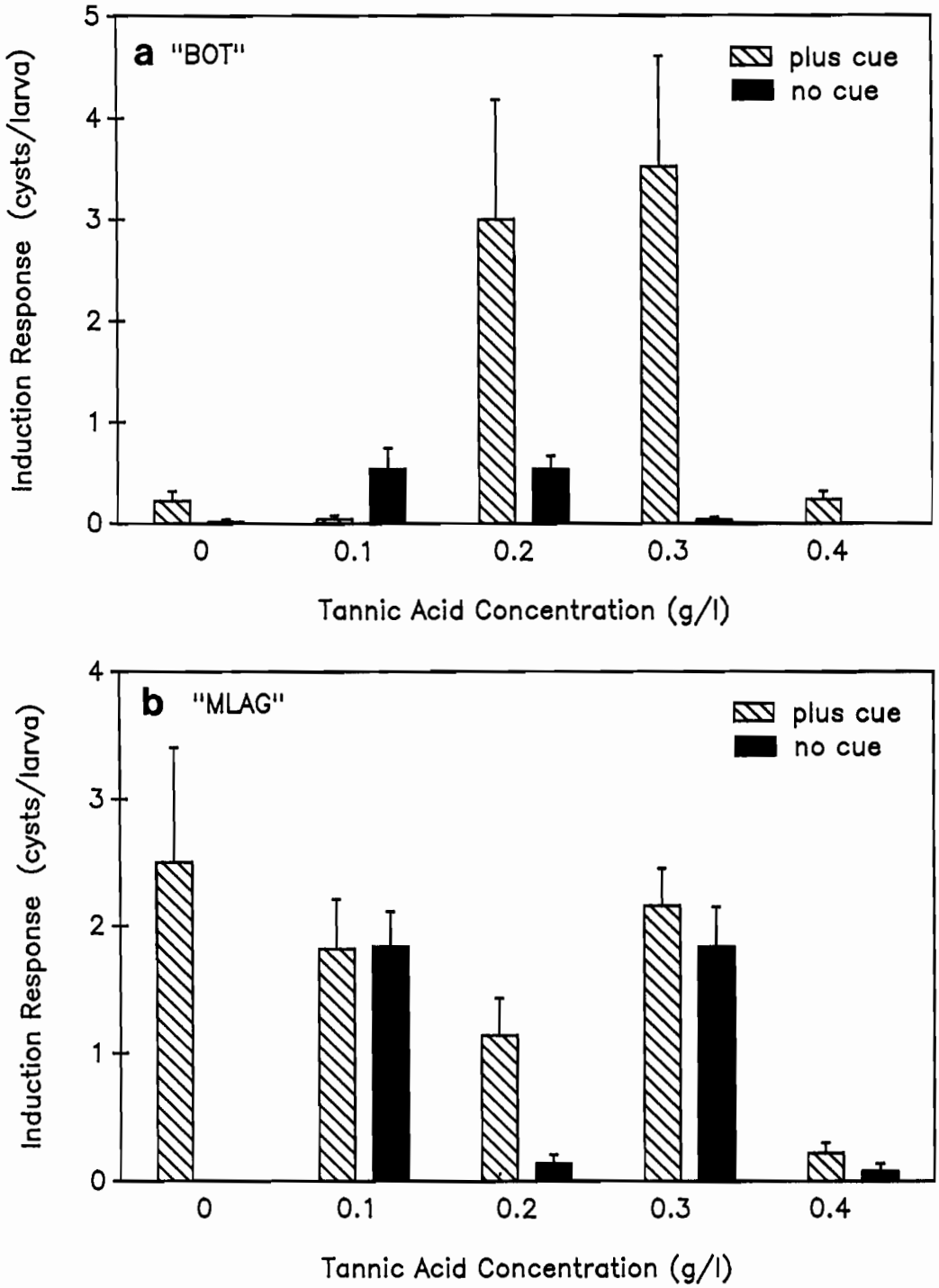

Figure 3. Effect of tannic acid concentration on migrated (a)"BOT" and (b)"MLAG" cell population induction responses. Bar height represents treatment mean ± one standard error of the mean.

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THE OVIPOSITION BEHAVIOR OF *AEDES TRISERIATUS*

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Introduction.

Aedes triseriatus (Say) is a low density woodland mosquito that breeds primarily in tree holes (rot holes in trees which are filled with rain water, decaying organic matter and stem flow). It also breeds in other small water containers, including discarded tires (Craig 1983). Females oviposit at the water line in the container and eggs hatch with subsequent flooding. *Aedes triseriatus* is primarily a species of the upper Midwest, but it has been reported in 42 states (Siverly 1972). In the northern range overwintering is in the egg stage and in the southern United States production may be continuous (Siverly 1972).

Aedes triseriatus is of public health interest as it is the principle vector of La Crosse encephalitis virus (LACV)(DeFoliart et al. 1986). LACV, a bunyavirus, was first isolated in 1960 in La Crosse, Wisconsin from the brain of a fatal case in a 4-year old girl (Thompson et al. 1965). The virus produces an acute febrile disease which may progress to encephalitis, and causes more childhood illness than any other arbovirus in the United States (Kappus et al. 1983). Since isolation, over 1,400 cases have been reported in the midwestern and eastern states, with about 400 of these occurring in Wisconsin (Thompson 1983).

As LACV is transmitted transovarially in *Ae. triseriatus* (Watts et al. 1973), new breeding sites can serve as foci for LACV infection. In Minnesota, Balfour et al. (1976) related occurrences of human LACV infection to the proximity of *Ae. triseriatus* breeding sites. LACV may also be transmitted horizontally through vertebrate amplification (DeFoliart 1983) and venereally from infected males to uninfected females (Thompson and Beaty 1978).

Aedes triseriatus responds poorly to light traps (Craig 1983), and since it breeds in containers, it is not subject to sampling by dipper. To detect its presence, Loor and DeFoliart (1969) adapted the oviposition trap previously used for monitoring *Aedes aegypti* (L.) during the eradication program. The trap consists of a black-painted, 400 ml beverage can filled with water which serves as an artificial oviposition site. A slat of balsa wood (Novak and Peloquin 1981) is clipped to the side of the container using a binder clip. Female *Ae. triseriatus* then oviposit on the balsa. The strips may be removed and the number of eggs counted. The oviposition trap is currently the major tool for those charged with monitoring *Ae. triseriatus* populations.

A complete understanding of *Ae. triseriatus* oviposition biology is necessary for the development of efficient monitoring programs. The epidemiological importance of the breeding site also makes studies of oviposition biology relevant. First, the attractiveness of five reported oviposition attractants was examined in the laboratory. Secondly, the attractiveness of fish oil emulsion, a reported *Ae. triseriatus* oviposition attractant (Holck et al. 1988), and dyed oviposition water are compared with untreated oviposition traps in the field. Finally, the spatial distribution of eggs was examined.

Factors influencing oviposition in *Ae. triseriatus*.

A number of physical and biological factors have been suggested as oviposition attractants or stimulants for mosquitoes. Most of the work is based on laboratory studies and has been recently reviewed by Bentley and Day (1989). We tested five factors reported to increase oviposition in *Ae. triseriatus* in the laboratory to determine if there were simple ways to increase oviposition trap efficiency. The five factors, 1) dyed oviposition water, 2) presence of decaying organic matter, 3) presence of a dark oviposition container, 4) presence of eggs on the oviposition substrate and 5) the presence of conspecific larval factor (LF), were compared using a replicated 2^{5-2} fractional design. Larval factor is a volatile attractant produced by fourth instar *Ae. triseriatus* larvae and is present in

larval holding water (Bentley et al. 1976).

A standard factorial design matrix and generators (Box et al. 1978) were used in the experimental design. Each of the factors were coded as present (+) or absent (-). A third replicate, using a "fold-over" design (Box et al. 1978) was used to clarify the effect of the presence of decaying organic matter from the interactions with other factors. Factorial designs have several advantages, the most important being that they allow the estimation of two factor interactions. For example, if the combination of LF and decaying organic matter are important in inducing oviposition when present in tandem, this interaction can be quantitatively considered.

The first factor, dyed oviposition water, was produced using red and green odorless vegetable dyes. Water containing organic matter was made by placing white oak (*Quercus alba* L.) leaves in distilled water before the beginning of the selection trial. The third factor, a darkened oviposition container, was made by encircling the normally gray oviposition container with black construction paper. Balsa strips with 40-100 *Ae. triseriatus* eggs were prepared before the start of the assay. Finally, LF was provided by the water in which the bioassay mosquitoes were reared. Larvae were reared at a specified density and, as with the decaying organic water infusion, particulate matter was removed with filter paper.

Thirty mated, blood-engorged *Ae. triseriatus* females were placed in cages with plastic dental cups serving as oviposition containers and a strip of balsa wood serving as an oviposition substrate. After six days, the balsa strips were removed and the eggs counted. Regression analysis was performed on the data to clarify the effects of the different factors on oviposition behavior.

The strongest attractant for ovipositing *Ae. triseriatus* was dyed oviposition water. Of 7,854 eggs laid in the three replicates, 6,959 (89%) were deposited in containers with dyed oviposition water. The regression coefficients for the model, along with their standard errors and P-values can be found in Table 1. This analysis confirmed that dyed oviposition water had the greatest effect on oviposition ($P<0.0001$).

The presence of eggs on the oviposition substrate also increased oviposition ($P<0.005$). Sixty-nine percent of the eggs were laid in oviposition cups which had eggs on the oviposition substrate, suggesting the presence of an egg pheromone. The presence of decaying organic matter ($P<0.001$) and LF ($P<0.05$) had significant negative regression coefficients. The negative effects of organic matter and LF do not necessarily imply that they deter oviposition when compared to distilled water. These two results merely reflect the great attractiveness of dyed oviposition water. Given a choice between dyed oviposition water with

Table 1. Regression coefficients (square root transformed data), standard errors and P-values for factors assayed for *Aedes triseriatus* oviposition attraction.

Factor	Coefficient	±	Standard error	P
Constant	15.21	±	0.691	<0.0001
Dyed oviposition water	8.52	±	0.733	<0.0001
Decaying organic matter	-2.48	±	0.691	<0.01
Dark oviposition container	-0.044	±	0.691	
Conspecific eggs on substrate	2.96	±	0.733	<0.001
Larval factor	-1.57	±	0.691	<0.05
Dyed water/organic matter interaction	-3.60	±	0.733	<0.001
Dyed water/eggs on substrate interaction	-2.17	±	0.733	<0.001

no organic matter and water containing organic matter and no dye, mosquitoes preferred the former.

Due to the nature of the experimental design, it was necessary to use two experimental blocks (an individual cage) for each replicate. When comparisons were made within blocks, the organic matter did prove attractive when compared to distilled water. Within each block there was not true statistical independence, and water of high optical density is such a strong attractant that fair comparisons cannot be made as these factors are not both present or absent within a block with the other factors held constant. The extremely significant attraction to dyed oviposition water indicates that it is a very important factor in the selection of an oviposition site by *Ae. triseriatus*, especially in conjunction with the presence of eggs on the oviposition substrate.

Field evaluation of two reported *Ae. triseriatus* oviposition attractants.

Two methods of improving the competitiveness of oviposition traps with natural oviposition sites were tested in the field. Although there have been few field studies, fish oil emulsion plant food has been reported to attract ovipositing *Ae. triseriatus* in the field (Holck et al. 1988) and dyed oviposition water has been shown in several laboratory studies to act as an attractant (Wilton 1968; Beehler, Lohr and DeFoliart, unpublished data). If either of these two factors are attractive in the field they could be easily incorporated into an oviposition trapping program. Oviposition traps compete with naturally occurring oviposition sites and traps placed in areas with more natural sites may have fewer eggs than those in areas with fewer tree holes or other containers. Any method of increasing a trap's competitiveness with natural sites greatly increases its sensitivity.

In July 1988, thirty oviposition traps were placed along a 120 m transect running through a second growth oak woodlot in Iowa County, Wisconsin. Traps were placed basally and were arranged in groups of three on each of ten trees. Within each trap group, the center trap served as a control and was filled with tap water. The trap on the left was filled with water which had been dyed with vegetable dye. The trap on the right was filled with a 1% solution of fish oil emulsion (Fish Oil Emulsion Plant Food, Green Light Co., San Antonio, Texas). Balsa wood strips were removed weekly from the traps. The traps were then washed and their position was reversed on the tree. After refilling with the appropriate treatment, new balsa strips were clipped into the trap. During the first week, only trees 1-3 and 9-10 had traps containing emulsion. The following week, these traps were washed and left empty on the tree. Trees 4-8 were then treated with emulsion solution. This process was repeated for the six weeks of the study in an effort to quantify any olfactory cues at a trap group.

Each week the number of eggs deposited in traps containing dyed oviposition water exceeded the number deposited in control traps (Fig. 1). Although the same concentration of fish oil as Holck et al. (1988) was used, no eggs were collected in any traps containing fish oil emulsion. Within a few days the fish oil emulsion solutions became cloudy and the balsa strips were covered with bacterial film. Laboratory oviposition bioassays showed fish oil emulsion to be repellent to ovipositing *Ae. triseriatus* (Beehler and DeFoliart 1990a). Holck et al. (1988) apparently did not encounter problems with bacterial film. Regression analysis confirmed that treating the water with vegetable dye significantly increased the number of eggs deposited in oviposition traps ($P<0.001$). The total number of eggs did not vary throughout the study period ($P=0.27$) (Beehler and DeFoliart 1990a).

Increasing the optical density of oviposition water greatly increased the number of *Ae. triseriatus* eggs deposited in oviposition traps. This simple procedure could increase the sensitivity of traps used in *Ae. triseriatus* monitoring programs.

Spatial distribution of *Ae. triseriatus* eggs.

Oviposition attractants make traps used in monitoring *Ae. triseriatus* more sensitive, but spatial distribution of eggs is also important when determining where and how many traps are to be placed when establishing a monitoring program.

Twenty-five oviposition traps were placed in an oak woodlot along a 500 m ellipsoidal transect. Traps were placed basally on the side of the tree and were checked three times weekly beginning June 6, 1988. After the first oviposition on June 20, traps were checked daily until September 30. Balsa strips with eggs were removed and replaced with fresh strips. The eggs on the strips were then counted in the laboratory. Water in the traps was maintained at a constant level throughout the study.

Several methods have been developed for

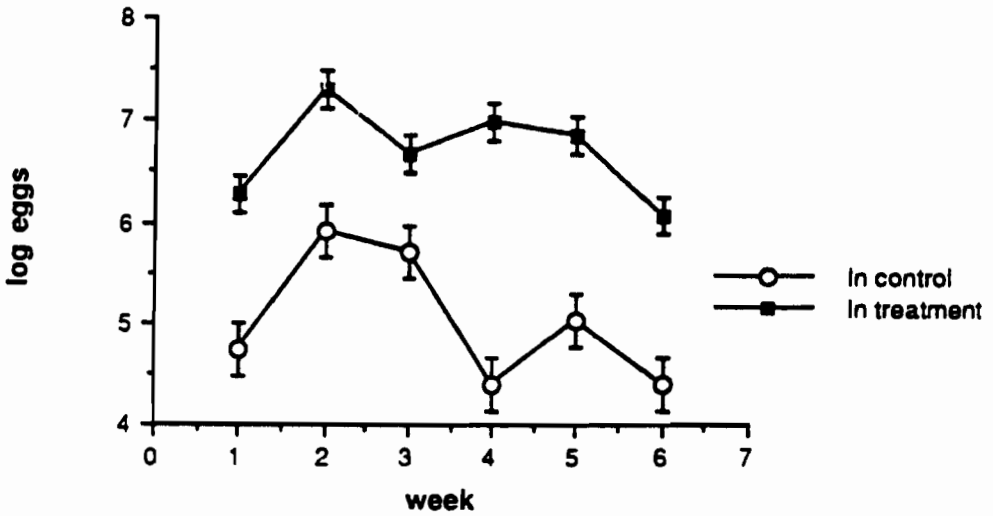


Figure 1. Total number of *Ae. triseriatus* eggs laid in paired oviposition traps in the field. Treatment traps contained dyed water and control traps contained tap water.

determining the spatial distribution of individuals in the field. The most common are those of Taylor (1961) and Iwao (1968,1970). The first (Taylor 1961) used the relationship between the daily mean egg count and the variance. The natural log of the variance of the daily count was regressed on the natural log of the mean for days that were positive for oviposition. A T-test based on the standard error estimate of the resulting regression equation ($y = 1.196 + 1.467x$) showed that the regression line had a slope significantly greater than 1 ($P<0.001$). Taylor (1961) stated that the slope of a mean/variance regression of field samples greater than 1 indicates a clumped distribution of individuals.

Iwao (1968,1970) used a similar mean variance ratio to determine clumping. With this method a crowding index (Lloyd 1967) is regressed against the daily mean egg count. The resulting regression equation was $y = 30.31 + 2.028x$. A T-test on the slope again showed it to be greater than 1 ($P<0.001$), again indicating a clumped distribution of eggs among the oviposition traps. Iwao's method also allows estimation of clump size using the estimate of the intercept. For example, an intercept of 0 would indicate that eggs were clumped individually. In this case, an intercept of 30.3 suggests that individuals are clumped in groups of 31 ± 9.8 ($P=0.05$). This parameter can be seen as an estimate of egg batch size for a single *Ae. triseriatus* female for one oviposition.

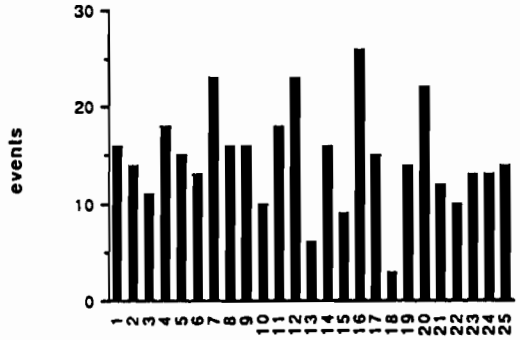
Figure 2 shows the total number of eggs laid in each individual oviposition trap, as well as the total number of days that each trap was positive for eggs. Some traps attracted many ovipositing mosquitoes and others attracted very few. These high and low values, which are based on a 95% mean confidence interval, and are not correlated to tree diameter, species of tree on which the trap was attached, degree of shading or temperature (Beehler and DeFoliart 1990b). I am unable to suggest why distribution of eggs was clumped.

Although the distribution of eggs in traps is contagious, Figure 2 shows that relatively few oviposition traps would be sufficient for detecting and monitoring populations of *Ae. triseriatus* in the field. Three oviposition traps per hectare greatly reduces the probability of placing a trap in a site which is unattractive to ovipositing *Ae. triseriatus* females. A reference distribution based on field data shows the probability of placing a trap in one of these unattractive sites to be $P<0.01$.

Conclusions.

These studies show that simple changes in trapping procedure, such as dying the water in the oviposition trap, could increase the ability of oviposition traps to compete with naturally occurring oviposition sites. This increases a trap's sensitivity in attracting ovipositing *Ae. triseriatus* thus making current monitoring programs more efficient. Although trap placement was shown to be important, a moderate number of traps adequately monitor *Aedes triseriatus*.

A)



B)

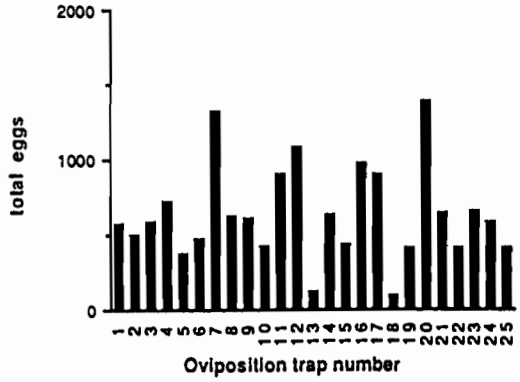


Figure 2. Distribution of *Ae. triseriatus* eggs in oviposition traps. A) Number of days each trap was positive for eggs (n=366). B) Total number of eggs in each trap (n=15,998).

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VERTICAL TRANSMISSION OF ST. LOUIS ENCEPHALITIS VIRUS IN *AEDES TAENIORHYNCHUS*, *AEDES DORSALIS*, AND *PSOROPHORA COLUMBIAE*[1]

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ABSTRACT

Collected field populations and/or colonized strains of *Aedes taeniorhynchus*, *Aedes dorsalis*, and *Psorophora columbiae* were evaluated for their ability to transmit selected strains of St. Louis encephalitis (SLE) virus to their offspring when infected by intrathoracic inoculation. Mean minimum infection rates (MMIR) of SLE virus in F-1 larvae and adults of inoculated parental females were 1:62 and ≤1:627, respectively, for a southern California field population of *Ps. columbiae* and 1:11 and ≤1:151, respectively, for a colony of that same species from Colombia, South America. Mean minimum infection rates in F-1 larvae of *Ae. taeniorhynchus* were 1:42 for newly colonized strains from California, but there was no transmission of SLE virus to F-1 adult progeny. Mean minimum infection rates in F-1 larvae and F-1 adults for a colony of *Ae. dorsalis* were ≤1:963 and ≤1:1352, respectively. Approximately 42% of the *Ps. columbiae* and 56% of the *Ae. taeniorhynchus* females transmitted SLE virus horizontally when orally infected and held at 27° C for up to three weeks.

A total of 15,492 *Ps. columbiae* and 38,210 *Ae. taeniorhynchus* were field collected and assayed for virus between 1986 and 1989. No strains of SLE virus were isolated.

The results indicate that these three mosquito species are capable of both vertical and horizontal transmission of SLE virus. Due to the lack of field evidence in areas where SLE virus is active, however, the involvement of these species in the maintenance of SLE virus in California remains speculative.

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[1] The results of this research will be submitted for publication in the American Journal of Tropical Medicine and Hygiene.

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