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California Mosquito and Vector Control Association, Inc.

Volume 56 January 28 - 31, 1988

SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1987

Richard W. Emmons[1], Dale V. Dondero[1], Cynthia S. Chan[1], Marilyn M. Milby[2], John D. Walsh[3], William C. Reeves[2], Lucia T. Hui[3], Franklin Ennik[3], Jill Griffin[1], Edmond V. Bayer[4], and Robert A. Murray[5]

This 18th report, in a series of annual reports presented to the California Mosquito and Vector Control Association (CMVCA) since 1969, summarizes the results of cooperative efforts by local mosquito control agencies, local health departments, the California Department of Food and Agriculture, private physicians and veterinarians, and the agencies and programs represented by the authorship. Weekly reports of the surveillance program results (23 during the season) were sent to a large mailing list of participants in the program during the 1987 season, and this brief summary was prepared for presentation January 30, 1988, at the 56th annual meeting of the CMVCA.

Despite the usual extensive clinical and laboratory-test-based surveillance for human and equine cases during the year, only 1 human case of St. Louis encephalitis (SLE) was detected. This was a 2 year old boy in San Bernardino City, San Bernardino County, who apparently was infected by mosquitoes from a flood control pond close to his home. Onset of illness was September 18, 1987, and consisted of initial low-grade fever and loss of appetite; then stiff neck, headache, and fever to 103.7°F; followed by convulsions, temporary paralysis, and coma for a short period. He was hospitalized from September 23-29, but recovery was complete. Sera collected September 25 and October 26 showed significant rises in SLE antibody titers by both indirect immunofluorescence and complement fixation tests, with high IgM antibody in the acute phase serum. Antibody tests were done at both the San Bernardino County Health Department Laboratory and the State Health Department's Viral and Rickettsial Disease Laboratory.

No human or equine cases of western equine encephalomyelitis (WEE) were found, although 37 clinically suspect equine cases from 16 counties were tested for WEE antibody, in addition to routine screening of approximately 100 humans. There were 5,840 mosquito pools tested, largely in Vero cell cultures this year, rather than in suckling mice (Table 1). We are now utilizing an *in situ* enzyme immunoassay (EIA) system for detection of isolates in cell culture (Vectastain (TM) Peroxidase System). There were 122 strains of WEE

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virus isolated from various northern and central counties (Shasta, Butte, Yuba, Sutter, Yolo, Sacramento, Merced); and from Riverside and Imperial Counties in southern California. Only 2 strains of SLE virus were isolated: from Sepulveda in Los Angeles County, and from Seeley in Imperial County. Twelve strains of California encephalitis virus (CEV) were isolated from pools of *Aedes melanimon*: 7 from Lost Hills in Kern County and 5 from Butte County sites. Also, the usual large assortment of Turlock, Hart Park, and a few Bunyamwera group isolates were found (Table 2). A detailed listing of positive mosquito pools and the viruses isolated from them are shown in Table 3.

The 63 sentinel chicken flocks (20-25 chickens in each) were distributed throughout the state and were bled monthly during most of the year. In 7211 sera tested by EIA for WEE and SLE antibodies, there were 83 seroconversions for WEE (Table 4). The Table and footnotes must be read carefully to interpret the number of seroconversions in each area or flock, since some chickens could not be rebled each month or were not tested each month. The distribution of WEE positive birds ranged from Shasta to Fresno Counties in the north and central areas, and the Coachella Valley, Imperial County and along the Colorado River in the south. Fourteen SLE seroconversions occurred in Los Angeles County, Long Beach City and Imperial

Table 1.-Number of mosquitoes and pools tested during 1987 by the Viral and Rickettsial Disease Laboratory by county and species.

	Aedes melanimon		*Culex peus*		*Culex pipiens*		*Culex tarsalis*		Others*		TOTAL	
	Mosq	Pools	Mosq	Pools	Mosq	Pools	Mosq	Pools	Mosq	Pools	Mosq	Pools
Butte	1700	34	50	1	300	6	5625	113	50	1	7725	155
Imperial					287	11	10428	222	2211	58	12926	291
Inyo	190	5					249	7			439	12
Kern	4055	94					4593	104			8648	198
Kings							27	2			27	2
Lake	258	6					830	18	50	1	1138	25
Los Angeles			1874	80	17495	441	8704	220	15253	336	43326	1077
Merced	146	3			150	3	1017	25			1313	31
Orange			897	40	14106	369	6023	164	1496	37	22522	619
Riverside			6002	146	10501	248	36102	812	10064	254	62669	1456
San Bernardino			3720	91	13717	291	5727	130	179	8	23343	520
Sacramento			135	8	856	18	19663	421	126	3	20780	450
San Diego			257	8	2702	60	3449	76	1383	29	7791	173
Santa Barbara							317	7			317	7
Shasta							990	22			990	22
Sonoma			441	9	26	2	75	3			542	14
Stanislaus	37	1	16	1	409	12	1826	40			2288	54
Sutter	3638	74					7370	164	229	6	11237	244
Tulare							10	1			10	1
Ventura					344	10	890	21			1234	31
Yolo	44	3	80	2			20053	423			20177	428
Yuba	28	1					1199	29			1227	30
TOTAL	10096	221	13472	386	60893	1471	135167	3024	31041	733	250669	5840

*Primarily *Culex erythrothorax*. Also includes *Culiseta particeps, Anopheles freeborni, Culiseta incidens*, and *Aedes taeniorhynchus*.

County, from August to early January. Of special interest was the recognition of October 1987-January 1988 SLE seroconversions in the El Dorado Park (Long Beach City) and the LaBrea (Los Angeles County) flocks (7 chickens total). Previously, not many bleedings were done this late in the year. The finding indicates the need to do more extensive surveillance during the winter period, to assess its importance in the annual cycle.

Plans for the surveillance program during 1988 include somewhat expanded sentinel chicken serosurveillance, and an effort to increase the intensity of human disease surveillance for WEE, SLE and CEV infections.

Acknowledgments.

We thank Patricia Weber, Kate Haber, Lenore Pitstick and Doris Hollander for special assistance in the mosquito and chicken sera testing program. We also thank many other staff members of the Viral and Rickettsial Disease Laboratory; the Environmental Management Branch (formerly Vector Surveillance and Control Branch) of the California State Department of Health Services (CSDHS); the Arbovirus Field Station in Bakersfield and other staff of the School of Public Health, University of California; the Infectious Disease Branch (CSDHS); all participating local mosquito control agencies; local public health departments;

Table 2.-Number of viral isolates from mosquitoes tested during 1987 by the Viral and Rickettsial Disease Laboratory by species, county and agent isolated.

Species	County	WEE	SLE	Turlock	Calif. Group	Hart Park	Bunya Virus	Total
Aedes melanimon	Butte				5			5
	Kern				7		1	8
	Yuba	1						1
Culiseta particeps	Riverside						1	1
Culex pipiens	Riverside	1				1		2
complex	Los Angeles					1		1
Culex tarsalis	Butte	6				1		7
	Imperial	14	1					15
	Kern					8		8
	Los Angeles		1					1
	Merced	1						1
	Orange					1		1
	Riverside	45		17				62
	Sacramento	15						15
	San Diego					1		1
	Shasta	2		1		1		4
	Stanislaus					1		1
	Sutter	19		2		2		23
	Yolo	16		1		1		18
	Yuba	2				1		3
TOTAL ISOLATES		122	2	21	12	19	2	178

the California Department of Food and Agriculture; private physicians and veterinarians who submitted samples for testing; the Microbiology Reference Laboratory, Cypress, CA, for assisting in serological surveillance for human cases; and many others who assisted with the program.

We especially thank the California Mosquito and Vector Control Association for providing funds to purchase special equipment and supplies needed for testing of chicken sera and mosquito pools.

Table 3.-Viral isolates from mosquitoes tested during 1987 by the Viral and Rickettsial Disease Laboratory, compiled chronologically and by Mosquito Abatement District.

DIST	POOL	SPECIES	NO. MOSQ.	VIRUS	DATE COLL	PLACE	COUNTY
BUCO	033	*Ae. meln*	50	CEV	6/22	Gridley	Butte
	034	" "	50	CEV	6/22	Gridley	Butte
	037	" "	50	CEV	6/22	Gridley	Butte
	039	" "	50	CEV	6/22	Gridley	Butte
	048	" "	50	CEV	6/29	Gridley	Butte
	102	*Cx. tars*	50	WEE	7/20	Nord	Butte
	104	" "	50	WEE	7/20	Nord	Butte
	112	" "	50	WEE	7/20	Nord	Butte
	125	" "	50	WEE	7/27	Honcut	Butte
	129	" "	50	WEE	8/03	Gridley	Butte
	131	" "	50	WEE	8/03	Nord	Butte
	135	" "	50	HART	8/24	Chico	Butte
CHLV	338	*Cx. tars*	50	WEE	5/12	Mecca	Riverside
	347	" "	50	WEE	5/19	Mecca	Riverside
	363	" "	26	WEE	5/27	Mecca	Riverside
	384	" "	50	WEE	5/27	Mecca	Riverside
	387	" "	50	WEE	5/27	Desert Beach	Riverside
	389	" "	50	WEE	5/27	Desert Beach	Riverside
	391	" "	50	WEE	5/27	Desert Beach	Riverside
	409	" "	50	WEE	6/03	Mecca	Riverside
	418	" "	50	WEE	6/03	Mecca	Riverside
	432	" "	50	WEE	6/10	Mecca	Riverside
	433	" "	50	WEE	6/10	Mecca	Riverside
	434	" "	50	WEE	6/10	Mecca	Riverside
	436	" "	50	WEE	6/10	Mecca	Riverside
	440	" "	50	WEE	6/10	Mecca	Riverside
	441	" "	50	WEE	6/10	Mecca	Riverside
	453	" "	50	WEE	6/22	Mecca	Riverside
	455	" "	50	WEE	6/22	Mecca	Riverside
	457	" "	50	WEE	6/22	Mecca	Riverside
	459	" "	50	WEE	6/22	Mecca	Riverside
	464	" "	50	WEE	6/22	Mecca	Riverside
	468	" "	50	WEE	6/22	Mecca	Riverside
	479	" "	50	WEE	6/22	Desert Beach	Riverside
	486	" "	50	WEE	6/22	Mecca	Riverside
	492	" "	13	WEE	6/24	Mecca	Riverside
	493	" "	50	WEE	6/24	Indio	Riverside
	500	" "	14	WEE	7/08	Thermal	Riverside
	513	" "	32	WEE	7/15	Mecca	Riverside
	535	" "	50	WEE	7/22	Indio	Riverside
	536	" "	50	WEE	7/22	Indio	Riverside
	537	" "	23	WEE	7/22	Indio	Riverside
	540	" "	50	WEE	7/23	Mecca	Riverside
	550	" "	45	WEE	7/23	Mecca	Riverside

continued -

Table 3.-continued

DIST	POOL	SPECIES	NO. MOSQ.	VIRUS	DATE COLL	PLACE	COUNTY
CHLV	566	*Cx. tars*	50	WEE	7/29	Indio	Riverside
	618	" "	50	WEE	8/19	Indio	Riverside
	621	" "	50	WEE	8/19	Indio	Riverside
	622	" "	50	WEE	8/15	Indio	Riverside
	623	" "	50	WEE	8/11	Indio	Riverside
	624	" "	50	WEE	8/19	Indio	Riverside
	625	" "	50	WEE	8/19	Indio	Riverside
	626	" "	50	WEE	8/19	Indio	Riverside
	627	" "	50	WEE	8/19	Indio	Riverside
	630	*Cx. pip*	50	WEE	8/11	Indio	Riverside
	375	*Cx. tars*	50	TRLK	5/27	Mecca Duck Club	Riverside
	392	" "	50	TRLK	5/27	Desert Beach	Riverside
	678	" "	50	TRLK	9/03	Mecca/Indio	Riverside
COLO	242	" "	50	WEE	6/10	Bard	Imperial
	244	" "	50	WEE	6/10	Bard	Imperial
	250	" "	50	WEE	6/10	Bard	Imperial
	254	" "	50	WEE	6/10	Bard	Imperial
	261	" "	36	WEE	7/07	Bard	Imperial
IMPR	095	" "	50	SLE	8/25	Seely	Imperial
KERN	089	*Ae. meln*	50	CEV	6/15	Lost Hills	Kern
	104	" "	50	CEV	6/23	Lost Hills	Kern
	105	" "	50	CEV	6/29	Lost Hills	Kern
	109	" "	50	CEV	6/29	Lost Hills	Kern
	125	" "	50	CEV	7/13	Lost Hills	Kern
	127	" "	50	CEV	7/13	Lost Hills	Kern
	138	" "	50	CEV	7/27	Lost Hills	Kern
	071	*Cx. tars*	34	HART	6/12	Buttonwillow	Kern
	117	" "	50	HART	7/13	John Dale	Kern
	119	" "	50	HART	7/13	John Dale	Kern
	120	" "	25	HART	7/13	John Dale	Kern
	130	*Ae. meln*	18	BUNYA	7/13	Buttonwillow	Kern
	132	*Cx. tars*	50	HART	7/27	John Dale	Kern
	175	" "	50	HART	8/24	John Dale	Kern
	195	" "	50	HART	8/26	Kern River Bottom	Kern
	134	" "	28	HART	7/27	John Dale	Kern
LOSA	041	*Cx. pip*	21	HART	6/30	Belair	Los Angeles
MERC	008	*Cx. tars*	50	WEE	8/04	Merced	Merced
NWST	289	*Cs. part*	46	BUNYA	7/21	Mira Loma	Riverside
	318	*Cx. pip*	50	HART	8/11	Prado Corona	Riverside
ORCO	498	*Cx. tars*	24	HART	9/11	Irvine Duck Club	Orange
PALO	293	" "	50	WEE	6/09	Palo Verde	Imperial
	338	" "	50	WEE	6/09	Palo Verde	Imperial
	342	" "	50	WEE	6/09	Palo Verde	Imperial
	343	" "	50	WEE	6/09	Palo Verde	Imperial
	345	" "	50	WEE	6/09	Palo Verde	Imperial

continued -

Table 3.-continued

DIST	POOL	SPECIES	NO. MOSQ.	VIRUS	DATE COLL	PLACE	COUNTY
PALO	389	*Cx. tars*	50	WEE	6/23	Palo Verde	Imperial
	390	" "	50	WEE	6/23	Palo Verde	Imperial
	392	" "	50	WEE	6/23	Palo Verde	Imperial
	174	" "	50	WEE	5/06	Blythe	Riverside
	320	" "	50	WEE	6/09	Blythe	Riverside
	399	" "	50	WEE	6/30	Palo Verde	Imperial
	396	" "	44	WEE	6/30	Blythe	Riverside
	420	" "	50	WEE	7/21	Blythe	Riverside
	369	" "	50	TRLK	6/18	Blythe	Riverside
	370	" "	50	TRLK	6/18	Blythe	Riverside
	371	" "	26	TRLK	6/18	Blythe	Riverside
	385	" "	33	TRLK	6/23	Blythe, 10th Ave.	Riverside
	394	" "	19	TRLK	6/30	Blythe, 6th Ave.	Riverside
	440	" "	50	TRLK	8/11	Blythe, 10th Ave.	Riverside
	442	" "	50	TRLK	8/11	Blythe, 10th Ave.	Riverside
	443	" "	50	TRLK	8/11	Blythe, 10th Ave.	Riverside
	476	" "	50	TRLK	9/02	Blythe, 10th Ave.	Riverside
	479	" "	50	TRLK	9/02	Blythe, 10th Ave.	Riverside
	491	" "	50	TRLK	9/08	Blythe, 10th Ave.	Riverside
	542	" "	50	TRLK	9/29	Blythe, 10th Ave.	Riverside
	223	" "	50	TRLK	5/18	Blythe, 10th Ave.	Riverside
	291	" "	31	TRLK	6/02	Blythe	Riverside
SACR	382	" "	50	WEE	8/03	Herald	Sacramento
	423	" "	50	WEE	8/10	Wilton	Sacramento
	497	" "	50	WEE	8/17	Rio Linda	Sacramento
	506	" "	50	WEE	8/17	Galt	Sacramento
	576	" "	50	WEE	8/24	Wilton	Sacramento
	578	" "	50	WEE	8/24	Wilton	Sacramento
	585	" "	50	WEE	8/24	Wilton	Sacramento
	590	" "	50	WEE	8/24	Rio Linda	Sacramento
	711	" "	50	WEE	8/31	Rio Linda	Sacramento
	717	" "	50	WEE	8/31	Rio Linda	Sacramento
	770	" "	16	WEE	9/10	Natomas	Sacramento
	787	" "	50	WEE	9/14	Wilton	Sacramento
	790	" "	50	WEE	9/14	Wilton	Sacramento
	796	" "	50	WEE	9/14	Elk Grove	Sacramento
	845	" "	50	WEE	9/21	Elk Grove	Sacramento
	156	" "	50	WEE	7/13	Davis	Yolo
	193	" "	50	WEE	7/20	Merritt	Yolo
	194	" "	50	WEE	7/20	Merritt	Yolo
	195	" "	50	WEE	7/20	Merritt	Yolo
	259	" "	40	WEE	7/27	Davis	Yolo
	335	" "	50	WEE	8/03	Woodland	Yolo
	366	" "	50	WEE	8/03	Woodland	Yolo
	448	" "	50	WEE	8/10	Woodland	Yolo

continued -

Table 3.-continued

DIST	POOL	SPECIES	NO. MOSQ.	VIRUS	DATE COLL	PLACE	COUNTY
SACR	452	*Cx. tars*	50	WEE	8/10	Woodland	Yolo
	545	" "	50	WEE	8/17	Davis	Yolo
	572	" "	50	WEE	8/19	Woodland	Yolo
	609	" "	50	WEE	8/24	Zamora	Yolo
	648	" "	50	WEE	8/24	Yolo	Yolo
	735	" "	50	WEE	8/31	Davis	Yolo
	816	" "	50	WEE	9/21	El Macero	Yolo
	874	" "	50	WEE	9/20	Davis	Yolo
	170	" "	50	HART	7/14	Woodland	Yolo
	564	" "	50	TRLK	8/19	Woodland	Yolo
SAND	115	" "	50	HART	7/08	San Elijo Lagoon	San Diego
SHAS	015	" "	47	WEE	8/18	Redding	Shasta
	020	" "	50	WEE	8/25	Anderson	Shasta
	010	" "	50	HART	8/18	Redding	Shasta
	019	" "	50	TRLK	8/25	Anderson	Shasta
SOUE	473	" "	23	SLE	8/31	Sepulveda	Los Angeles
SUYA	046	" "	46	WEE	7/24	Sutter	Sutter
	052	" "	59	WEE	7/24	Sutter	Sutter
	055	" "	55	WEE	7/29	Yuba City	Sutter
	058	" "	50	WEE	8/05	Sutter	Sutter
	059	" "	50	WEE	8/05	Sutter	Sutter
	060	" "	50	WEE	8/05	Sutter	Sutter
	061	" "	55	WEE	8/05	Sutter	Sutter
	064	" "	50	WEE	8/07	Sutter	Sutter
	065	" "	50	WEE	8/07	Sutter	Sutter
	066	" "	50	WEE	8/07	Sutter	Sutter
	067	" "	50	WEE	8/07	Sutter	Sutter
	068	" "	50	WEE	8/07	E. Nicolaus	Sutter
	069	" "	50	WEE	8/07	E. Nicolaus	Sutter
	070	" "	29	WEE	8/07	E. Nicolaus	Sutter
	071	" "	50	WEE	8/10	Sutter	Sutter
	072	" "	50	WEE	8/10	Sutter	Sutter
	074	" "	50	WEE	8/10	Sutter	Sutter
	098	" "	50	WEE	8/19	Robbins	Sutter
	144	" "	50	WEE	9/04	Pleasant Grove	Sutter
	262	*Ae. meln*	50	WEE	10/08	Loma Rica	Yuba
	040	*Cx. tars*	50	WEE	7/22	Marysville	Yuba
	086	" "	50	HART	8/13	Marysville	Yuba
	112	" "	50	WEE	8/25	Arboga	Yuba
	024	" "	50	TRLK	6/29	O'Banion Corner	Sutter
	024	" "	50	HART	6/29	O'Banion Corner	Sutter
	025	" "	50	HART	6/29	O'Banion Corner	Sutter
	087	" "	53	TRLK	8/17	East Nicolaus	Sutter
TRLK	013	" "	18	HART	7/28	Crow's Landing	Stanislaus

Table 4.-WEE and SLE seropositive chickens/number tested (percent positive), California, 1987.

Flock location	WEE positive/number tested (percent positive)						
	May 11-15	June 8-12	July 6-10	Aug 3-7	Aug 31-Sep 4	Sep 28-Oct 2	Oct 26-30
Northern California							
Shasta, Cottonwood	0/17	0/17	0/17	0/17	0/17	2/17(12)	not tested
Tehama, MAD office	0/19	0/20	0/20	5/20(25)	7/20(35)	11/20(55)	not tested
Corning, Martin Ranch	0/19	0/20	0/20	1/20(5)	1/20(5)	4/20(20)	not tested
Butte, Chico	0/20	0/20	0/19	0/19	0/19	0/18	not tested
Butte, Honcut	0/17	0/18	0/19	0/19	0/19	1/19(5)	not tested
Butte, Gray Lodge	0/20	0/20	0/20	1/20(5)	1/20(5)	6/19(32)	not tested
S-Yuba, P. V. Ranch	0/20	0/20	0/20	0/20	0/20	1/20(5)	not tested
S-Yuba, Dean's	0/20	0/19	0/19	3/19(16)	8/19(42)	8/18(44)	not tested
S-Yuba, Barker	0/20	0/20	0/20	0/20	3/20(15)	4/20(20)	not tested
Sac-Yolo, Merritt	0/20	0/20	0/19	0/19	1/19(5)	1/19(5)	not tested
Sac-Yolo, Natomas	0/20	0/20	0/20	0/20	0/20	1/20(5)	not tested
Sac-Yolo, Elk Grove	0/19	0/18	0/19	0/16	0/18	1/17(6)	not tested
Marin-Sonoma, W. Santa Rosa	0/20	0/20	0/20	0/19	1/19(5)	1/19(5)	not tested
Solano, Dixon	0/20	0/20	0/20	0/20	0/20	1/20(5)	not tested
Santa Clara, San Martin	0/20	0/19	0/18	0/18	0/16	0/16	not tested
N. CALIFORNIA WEE TOTAL	0/291	0/291	0/290	10/286(3)	22/286(5)	42/282(15)	
San Joaquin Valley							
San Joaquin, Lodi	0/21	0/21	0/20	0/20	1/20(5)	1/20(5)	not tested
Eastside, Oakdale	0/20	0/20	0/9	0/9	0/9	0/9	not tested
Turlock, Victoria	0/20	0/20	0/20	0/20	0/20	0/20	not tested
Merced, Los Banos	0/20	0/20	0/20	0/19	0/19	0/19	not tested
Fresno Westside, Mendota Ref.	0/20	0/19	0/20	0/17	0/17	1/17(6)	not tested
Consolidated, Friant Rd.	0/20	0/20	0/18	0/17	0/17	0/17	not tested
Kings, MAD office, Hanford	0/20	0/20	0/20	0/20	0/18	0/18	not tested
Delta, Kingsburg GC	0/20	0/19	0/20	0/20	0/20	0/20	not tested
Tulare, MAD office	0/16	0/16	0/16	0/15	0/16	0/16	not tested
West Side, Lost Hills	0/25	0/25	0/25	0/25	0/25	0/25	not tested
West Side, Maricopa	0/20	0/20	0/18	0/15	0/14	0/14	not tested
Delano, Teviston	0/20	0/19	0/18	0/16	0/16	0/16	not tested
Kern, Wasco	0/20	0/17	0/17	0/17	0/16	0/16	0/16
Kern, F. C. Tracy	0/20	0/20	0/20	0/18	0/18	0/18	0/18
Kern, Buttonwillow	0/19	0/19	0/19	0/18	0/17	0/17	0/17
Kern, Wildlife Refuge	0/18	0/16	0/16	0/16	0/16	0/16	0/16
Kern, Oildale	0/20	0/19	0/18	0/18	0/17	0/8	0/8
Kern, John Dale	0/20	0/20	0/20	0/20	0/19	0/19	0/19
Kern, River Bottom	0/20	0/20	0/20	0/18	0/17	0/17	0/17
SAN JOAQUIN WEE TOTAL	0/379	0/370	0/354	0/338	1/331(<1)	2/322(1)	0/111

continued -

Table 4.-continued. WEE and SLE seropositive chickens/number tested (percent positive), S. California, 1987.

Flock location	WEE positive/number tested (percent positive)						
	July 6-10	Aug 3-7	Aug 31-Sep 4	Sep 28-Oct 2	Oct 26-30	Nov 16-21	Dec 14-18
Southern California							
Santa Barbara, Goleta	0/20	0/20	not tested	0/20	not tested	not tested	not tested
Ventura, Pt. Mugu	0/20	0/20	0/20	0/20	not tested	not tested	not tested
Ventura, Simi Valley	0/20	0/20	0/20	0/19	not tested	not tested	not tested
Los Angeles, La Brea	0/19	0/19	0/19	0/19	0/19	not tested	0/19
Southeast, Balboa Golf	0/19	0/18	0/15	0/16	0/16	0/16	not tested
Southeast, Harbor Lakes	0/25	0/25	0/25	0/25	0/25	0/25	0/25
Southeast, Norwalk	0/24	0/25	0/25	0/25	0/25	0/25	0/25
Los Angeles, Cal Poly	0/21	0/14	0/23	0/20	0/20	0/20	0/20
Long Beach, El Dorado	0/10	0/10	0/14	0/10	0/10	0/10	0/7
Orange, Duck Club	0/23	0/22	0/21	0/21	0/19	0/19	0/17
Orange, Fullerton	0/24	0/24	0/24	0/24	0/21	0/24	0/24
Orange, San Mateo Point	0/16	0/16	0/16	not tested	0/16	not tested	not tested
San Bernardino, San Bernardino	0/20	0/17	0/19	0/18	0/18	not tested	not tested
West Valley, Chino	0/24	0/24	0/24	0/24	0/24	0/24	0/24
West Valley, Briano Bros.	0/24	0/20	0/15	0/14	0/13	0/13	0/13
Riverside, Lake Elsinor	0/14	0/15	0/13	0/18	not tested	not tested	not tested
Coachella Valley, Mecca	13/24(54)[a]	14/24(58)	14/24(58)	15/24(62)	15/24(62)	16/24(67)	16/24(67)
Coachella Valley, Thermal	1/25(4)	2/25(8)	2/25(8)	2/25(8)	2/25(8)	2/25(8)	2/25(8)
Coachella Valley, North Indio	3/21(14)	6/21(29)	7/21(33)	7/20(35)	7/19(37)	7/19(37)	7/19(37)
Northwest, Corona	0/21	0/20	0/18	0/13	0/12	0/10	0/9
Imperial, Palo Verde	1/20(5)	1/20(5)	1/20(5)	1/20(5)	1/20(5)	1/20(5)	1/20(5)
Imperial, Bard	4/20(20)	9/17(53)	9/17(53)	9/17(53)	9/17(53)	9/17(53)	9/17(53)
Imperial, El Centro	all dead						
San Diego, Vista	0/17	0/15	0/15	0/15	0/15	not tested	not tested
San Diego, San Ysidro	0/9	0/5	1/23(4)[b]	1/25(4)	1/25(4)[c]	not tested	not tested
San Diego, Lakeside	0/20	0/20	0/20	0/19	0/19	not tested	not tested
Colorado River, Needles	0/12	0/12	0/10	0/10	0/9	0/9	0/9
Colorado River, Havasu Refuge	0/25	0/25	0/22	0/21	0/21	0/21	0/20
Colorado River, Blythe	3/13(23)[d]	2/12(17)	2/12(17)	2/12(17)	2/12(17)	2/12(17)	2/12(17)
S. CALIFORNIA WEE TOTAL	25/550(5)	34/529(6)	37/520(7)	37/514(7)	37/444(8)	37/333(11)	37/332(11)
	SLE positive/number tested (percent positive)						
	July 6-10	Aug 3-7	Aug 31-Sep 4	Sep 28-Oct 2	Oct 26-30	Nov 16-21	Dec 14-18
Los Angeles, La Brea	0/19	0/19	2/19(11)	2/19(11)	2/19(11)	not tested	6/19(32)
Southeast, Balboa Golf	0/19	0/18	0/15	1/16(6)	1/16(6)	1/16(6)	not tested
Long Beach, El Dorado	0/10	0/10	0/14	0/10	0/10	0/10	2/7(29)[e]
Imperial, Bard	0/20	0/17	3/17(18)	3/17(18)	4/17(24)[f]	4/17(24)	4/17(18)
S. CALIFORNIA SLE TOTAL	0/550	0/529	5/520(1)	6/514(1)	7/444(2)	5/333(2)	12/332(4)

a. 2 chickens (8%) WEE positive in June bleeding.

b. New chicken put out 10 days earlier; prebleed was also WEE positive; came from near Lakeside.

c. Presumably the 1 seropositive chicken remained positive, but is not included in total.

d. 1 chicken (8%) positive in June bleeding, 1 July positive chicken died prior to August bleeding.

e. In Jan. 1988 bleeding, 3/10 (30%) positive for SLE (3 sera from Dec. 1987 bleeding leaked in transit and could not be tested).

f. 1 other chicken positive, but was negative in November bleeding, so not counted.

NOTE: all other chickens were negative for WEE and SLE in May and June.

MOSQUITO ABUNDANCE AND ARBOVIRUS ACTIVITY IN SOUTHERN CALIFORNIA

INTRODUCTION

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Members and guests of the California Mosquito and Vector Control Association: I am pleased to welcome you to this symposium which was organized by Dr. William K. Reisen and which will serve to present recent data from an ongoing series of research projects on the ecology of arboviruses and mosquito vectors in southern California. This is a collaborative research effort that makes maximal use of the encephalitis surveillance program and involves personnel and resources of many of the mosquito abatement or vector control districts and County Health Departments in Southern California, the Vector Surveillance and Control Branch and the Viral and Rickettsial Disease Laboratory of the California Department of Health Services, and the Arbovirus Research Program at the University of California in Berkeley and Bakersfield.

I will provide you with a brief overview of the scope of the research along with some of the questions that we are addressing. Then I will allow the other speakers to provide you the specifics on the individual studies.

Background.

The arbovirus research program at the University of California in Berkeley has initiated studies on the mosquito-borne viral encephalitides in southern California for several reasons. First, one of our primary research interests for the past 30 years has been the elucidation of mechanisms that allow western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses to persist through the winter months when mosquito vectors cease feeding on vertebrate hosts. Since the late 1960's, however, WEE and SLE viral enzootic activity has become quite sporadic in our long-term study areas in the Central Valley, and has not been detectable during the summer months of most years. Since enzootic transmission of WEE and SLE viruses continues to occur annually along the Colorado River and in the Imperial and Coachella Valleys, this area of southern California offers a unique opportunity to answer some of the remaining questions about the mechanisms which allow these viruses to overwinter in temperate climates.

Second, human cases of mosquito-borne encephalitis have occurred historically in the rural and suburban areas of the inland agricultural valleys of California. Thus, the occurrence of human cases of SLE in the greater Los Angeles metropolitan area, beginning in 1983, came as a surprise and has stimulated the need for in-depth ecological and epidemiological research on both mosquito vectors and SLE virus in a large urban environment.

Third, vector competence studies done in the laboratory with field and colonized strains of *Culex peus* have indicated that this species is an efficient vector of SLE virus. Since *Cx. peus* is abundant in some areas of the Prado and Los Angeles Basins during certain times of the year, it was of interest to determine if this species is involved in enzootic or epidemic transmission of SLE virus in these areas.

Finally, various members of the southern California component of the California Mosquito and Vector Control Association have asked us to initiate collaborative research to address specific problems on mosquito vector and encephalitis control that are unique to their individual districts or to southern California.

Research Objectives and Plan.

The long-term goal of this research is to expand our knowledge of the ecology and epidemiology of arboviruses and mosquito vectors that are of public health and veterinary importance in southern California. This information will provide, in part, the basis for the development of more effective or alternative strategies for the control and surveillance of mosquito-borne viral encephalitis in large suburban and urban environments.

To accomplish this goal, we have initiated four research projects in various geographical areas of southern California, including the Mojave, Palo Verde and Bard Valleys along the Colorado River,

the Imperial and Coachella Valleys, and the Prado and Los Angeles Basins. Permanent study sites have been established in each area where mosquito vector populations and encephalitis viral activity are being monitored throughout the year (not just during the usual summer encephalitis season). We are attempting to sample, and to test for virus, all mosquito species found in these areas to determine if different mosquito species are involved in the maintenance of these viruses during different times of the year.

The first project is designed to study the ecology of mosquito-borne encephalitis viruses in southeastern California along the Colorado River and in the Imperial and Coachella Valleys. Specific questions being asked include: Do WEE and SLE viruses overwinter in this area or are they reintroduced annually? Are wintertime levels of WEE and SLE viral activity predictive of summertime levels? Is there a northward movement of WEE and SLE viruses along the Colorado River and from the Imperial Valley to the Coachella Valley? Local agencies collaborating with us on this project are the Coachella Valley Mosquito Abatement District and the Imperial County, and Riverside County Health Departments, and the San Bernardino County Vector Control Program.

The second project involves field studies on the ecology of SLE virus in the greater Los Angeles area in collaboration with the Orange County Vector Control District, Southeast Mosquito Abatement District, Los Angeles County West Mosquito Abatement District and Los Angeles County Health Department. Study sites are located in representative residential areas, the remaining marshes, city parks and golf courses. Important questions being asked include: Does SLE virus overwinter in this area, and, if so, are these enzootic transmission foci in the Los Angeles Basin from which the virus spreads into the residential areas during the summer when environmental conditions are right? Which mosquito species are involved in the endemic and epidemic transmission of virus in the urban environment? Which vertebrate species serve as amplifying hosts of the virus?

The third project is being conducted in the vicinity of Chino in the Prado Basin in collaboration with the West Valley Mosquito Abatement District and Northwest Mosquito Abatement District. In this area, large populations of *Cx. peus*, as well as *Culex tarsalis* and *Culex quinquefasciatus*, are breeding in waste water effluent from dairy farms in juxtaposition to a large, non-immune human population. Thus, a unique opportunity is provided to study the comparative bionomics of three *Culex* vector mosquito species and to determine their relative roles in the maintenance, amplification and transmission of SLE virus to humans.

The final project is an ongoing study of the vector competence of various mosquito species for WEE and SLE viruses in Southern California that was begun in the late 1970's. The primary aim of the current studies is to determine if any of the non-epidemic vector mosquito species can possibly serve as reservoir hosts for these viruses and/or are involved in their wintertime transmission. This potential is being evaluated by testing the ability of field-collected female mosquitoes to serve as vertical or horizontal transmitters of virus.

With these thoughts in mind, it is now time for the scientists who are doing the research to present their data on "Mosquito Abundance and Arbovirus Activity in Southern California".

MOSQUITO ABUNDANCE AND ARBOVIRUS ACTIVITY ALONG THE LOWER COLORADO RIVER DURING 1986-1987[1]

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ABSTRACT

Culex tarsalis was the most frequently collected mosquito at study areas in the Mohave, Palo Verde and Bard Valleys along the lower Colorado River during 1986 and 1987. Relative abundance patterns measured by CO_2 traps were bimodal with peaks during spring and fall. Western equine encephalomyelitis (WEE) virus was active at study areas each year and was isolated on 22 occasions from pools of *Cx. tarsalis* females collected after the vernal population peak. Seroconversions within flocks of sentinel chickens occurred after WEE virus was isolated from *Cx. tarsalis*. Limited WEE activity was associated with the autumnal increase in *Cx. tarsalis* abundance. A low level of St. Louis encephalitis (SLE) virus activity was detected by the seroconversion of sentinel chickens during late summer during both years and SLE virus was not isolated from mosquitoes. Initial results indicated that WEE and perhaps SLE viruses were reintroduced annually from southerly foci and were disseminated progressively northward.

Introduction.

The overwintering of encephalitis viruses in California has been the topic of continuing research since the early 1950's with few encouraging results. Hypotheses that have been proposed include 1) viruses are reintroduced periodically from southern endemic areas, or 2) viruses overwinter in California in either the mosquito or vertebrate components of the primary *Culex tarsalis* Coquillett-bird or possible secondary transmission cycles perhaps involving *Aedes* mosquitoes and mammals.

The lower Colorado River provides an excellent environment to investigate these alternate hypotheses (Fig. 1). The river provides a natural N-S corridor for dispersal by migratory birds and links a series of irrigated agricultural valleys and wildlife refuges where arboviruses historically are active during each summer transmission season. Thus, the primary objective of our research was to describe the seasonality of mosquito abundance and arbovirus activity at study sites along the lower Colorado River and, in the long range, to relate these patterns to selected abiotic parameters including river flow, temperature and rainfall. The presence of other potential vector species such as *Aedes dorsalis* (Meigen), *Ae. vexans* Meigen and *Culiseta inornata* (Williston) may allow an assessment of the importance of secondary transmission cycles in virus maintenance.

Methods and Materials.

Description of study areas.

During June 1986, study areas were established at Needles, the Havasu National Wildlife Refuge (NWR), Parker Dam and Blythe (Fig. 1). The Parker Dam site was deleted during 1987 because mosquito abundance was low and virus activity was not detected during 1986. The transect was extended to the south during 1987 to include the Palo Verde and Bard areas. All collection sites were situated within 1 km of the Colorado River, but differed considerably in their ecology. The Whetmore Ranch at Needles was a rural residential site with little irrigation. The Havasu NWR, Palo Verde and supplemental trapping sites at Blythe and Bard were situated near riparian marshes which were inundated permanently or intermittently by river water depending upon the flow rate. Blythe, Palo Verde and Bard were adjacent to flood-irrigated cotton and alfalfa fields.

Mosquito abundance and arbovirus monitoring.

A cluster of 3 CO_2 traps and a flock of 25 sentinel chickens were positioned at each study area

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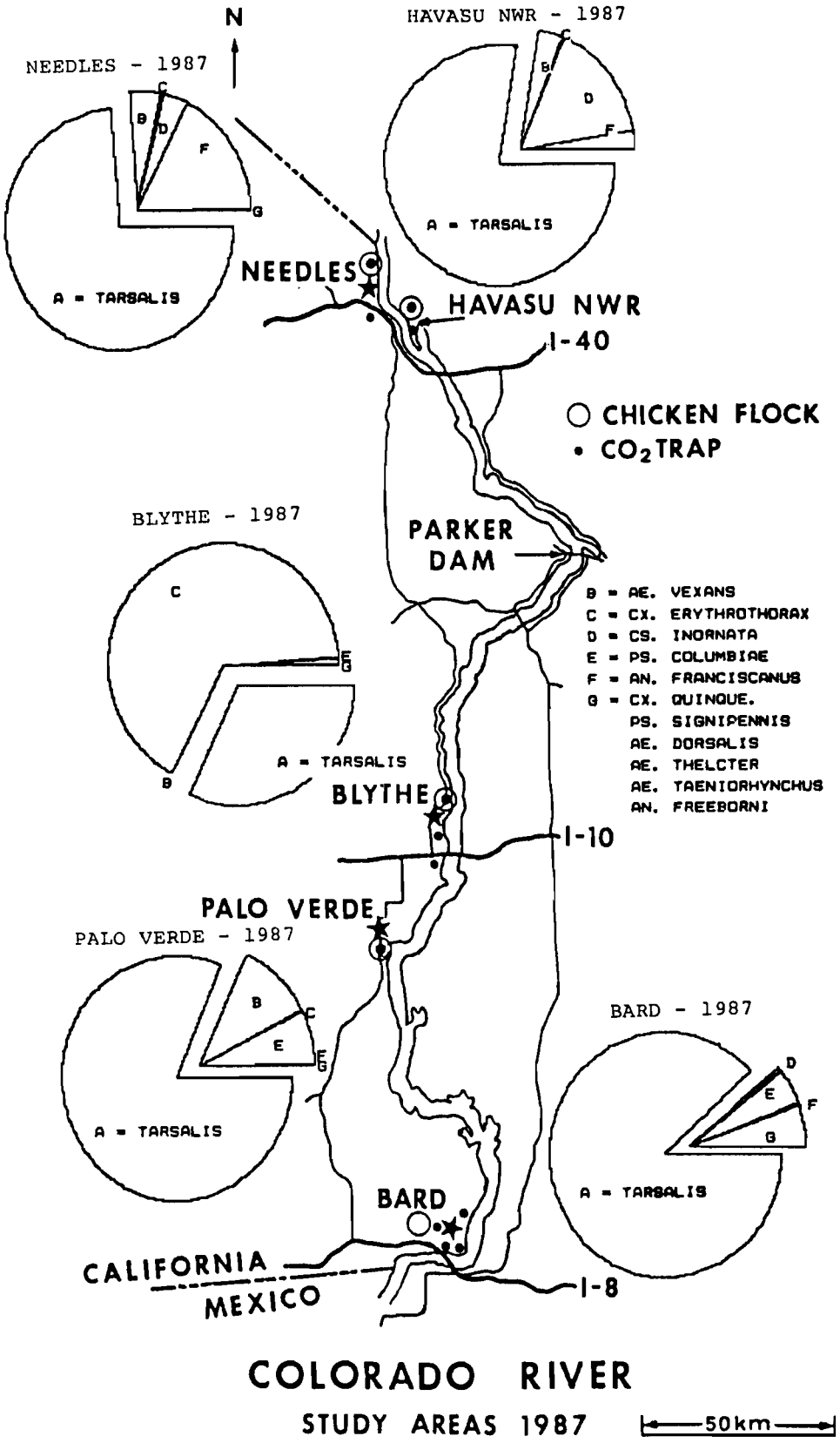

Figure 1.-Species composition of mosquitoes collected at study areas along the lower Colorado River during 1987.

(Fig. 1). An additional 6 to 9 traps were operated at supplemental locations near (1 - 10 km) each study site to enhance sampling sensitivity. CO_2 traps were run at monthly intervals during 1986, but trapping frequency was increased to weekly collections at Blythe and Palo Verde and 2-3 times per month at Needles during 1987. On the morning after collection, specimens were anaesthetized with triethylamine, sorted to species, pooled into lots of 50 females each and frozen at -70°C for later virus isolation attempts using suckling mice and/or vero cell cultures in an *in situ* enzyme-linked immunoassay (ELISA) or plaque assay system. Sentinel chickens were bled monthly and the sera tested for antibodies to WEE or SLE viruses using indirect fluorescent antibody or ELISA techniques.

Results.

Mosquito abundance.

A total of 89,667 mosquitoes comprising 14 species and 4 genera were collected during 893 trap nights from July 1986 through November 1987. *Cx. tarsalis* was the most abundant species collected during both 1986 (72%, n = 6,090) and 1987 (62%, n = 83,577) followed by *Cx. erythrothorax* Dyar (1986 = 18%; 1987 = 27%) (Fig. 1). *Cx. erythrothorax* was focally abundant at a cattail marsh near 10th Avenue in Blythe, but was relatively rare at the remaining collection sites where *Cx. tarsalis* comprised from 74 to 92% of the specimens taken. River flow remained low and channeled throughout the sampling period and thus, flood water species such as *Ae. vexans* and *Ae. dorsalis* were relatively rare in collections. Of taxonomic interest was the collection of 4 specimens of *Aedes purpureipes* Aitken from the Havasu NWR in 1986 (Meyer et al. 1987) and 15 specimens of *Ae. thelcter* Dyar from Bard Valley in 1987 (Meyer et al. 1988) which were new records for California. Prior to the recent collection of 4 *Ae. purpureipes* in the Parker and Yuma areas of Arizona by Jakob et al. (1985), the known distribution of this species in the United States was restricted to southeastern Arizona. Similarly, *Ae. thelcter* had not been recorded west of the Pecos River in New Mexico prior to our collections during October 1987 (Darsie and Ward 1981).

Mosquitoes were bimodally abundant during spring and fall at collection sites along the lower Colorado River (Fig. 2). Cold winter and hot, dry mid-summer conditions caused drastic reductions in host-seeking activity even through agricultural irrigation provided breeding sites throughout the year. Mean monthly temperatures were relatively similar at study areas and varied temporally from a low of 10°C in December to a high of 36°C in July. Extremes ranged from occasional freezes during winter to temperatures in excess of 45°C during mid-summer. Rainfall was low and ranged from 7 to 13 cm, falling mostly during late summer as part of a southerly monsoon air flow from the Gulf of Mexico.

Arbovirus activity.

A total of 41,641 females in 935 pools were tested for arboviruses during 1986 and 1987 (Emmons et al. 1987, 1988). Species were tested in approximate proportion to their abundance, and thus, *Cx. tarsalis* (31,527 females in 692 pools) followed by *Cx. erythrothorax* (7,110 females, 155 pools) were the most frequently tested species. A total of 22 western equine encephalomyelitis (WEE) and 14 Turlock (TUR) isolations were made from *Cx. tarsalis*. WEE virus was recovered from all sites except Parker Dam, while TUR was isolated only at the northern end of the Palo Verde Valley at 10th Avenue in Blythe.

Virus was not isolated from any of the remaining mosquito species tested, even though the 10th Avenue area where TUR virus was isolated supported an abundant population of *Cx. erythrothorax*. Although most frequently associated with *Cx. tarsalis* and birds, TUR virus also has been isolated from small mammals. Thus, the continued failure to isolate TUR virus from *Cx. erythrothorax* would seem to be related to poor vector competence rather than differences in host feeding patterns or low abundance at sites where TUR virus is active.

WEE virus infections in *Cx. tarsalis* were detected during early summer. In the Mohave Valley, WEE virus was isolated on 3 occasions during August 1986, but was not recovered during 1987 (Fig. 2a). The pattern of sentinel seroconversion to WEE virus by chickens at the Havasu NWR indicated continued virus transmission activity throughout the summer with conversions detected during August, September and October of 1986 (Fig. 3a). Chickens at the Havasu NWR and the Whetmore Ranch did not convert serologically to WEE positive during 1987, agreeing well with the low *Cx. tarsalis* abundance and absence of virus isolations.

A single isolation of WEE virus was made from Blythe during July 1986 (Fig. 2b) and 65% of sentinel chickens converted to WEE positive by August (Fig. 3b) indicating a brief, but intense, period of virus transmission. The seasonal pattern

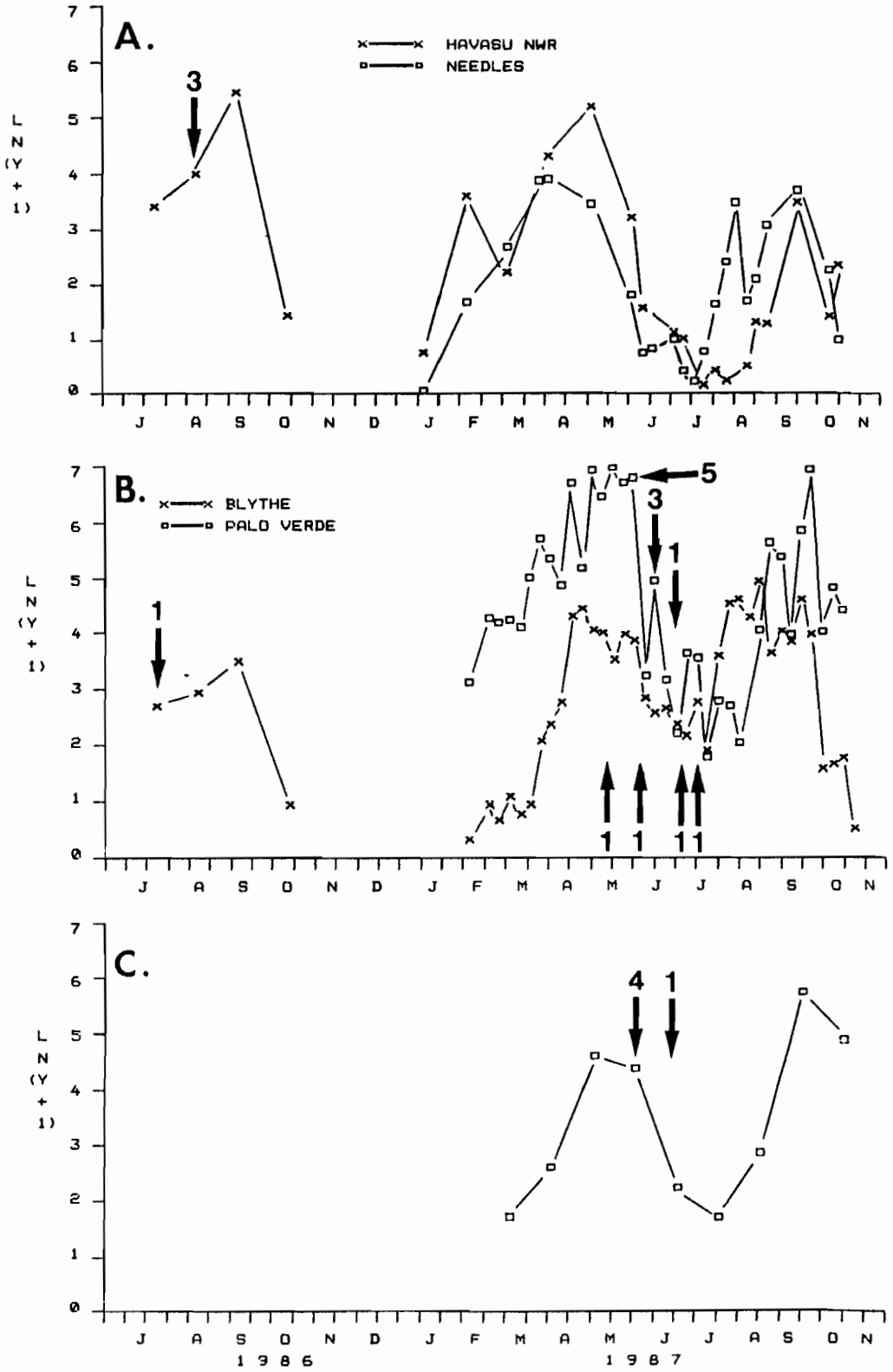

Figure 2.-Relative abundance of *Culex tarsalis* (females/$C0_2$ trap night) transformed to 1n (y + 1) at study areas in A) Mohave, B) Palo Verde and C) Bard Valleys plotted as a function of time in weeks during 1986 and 1987. Weeks and numbers of WEE virus isolations indicated by arrows.

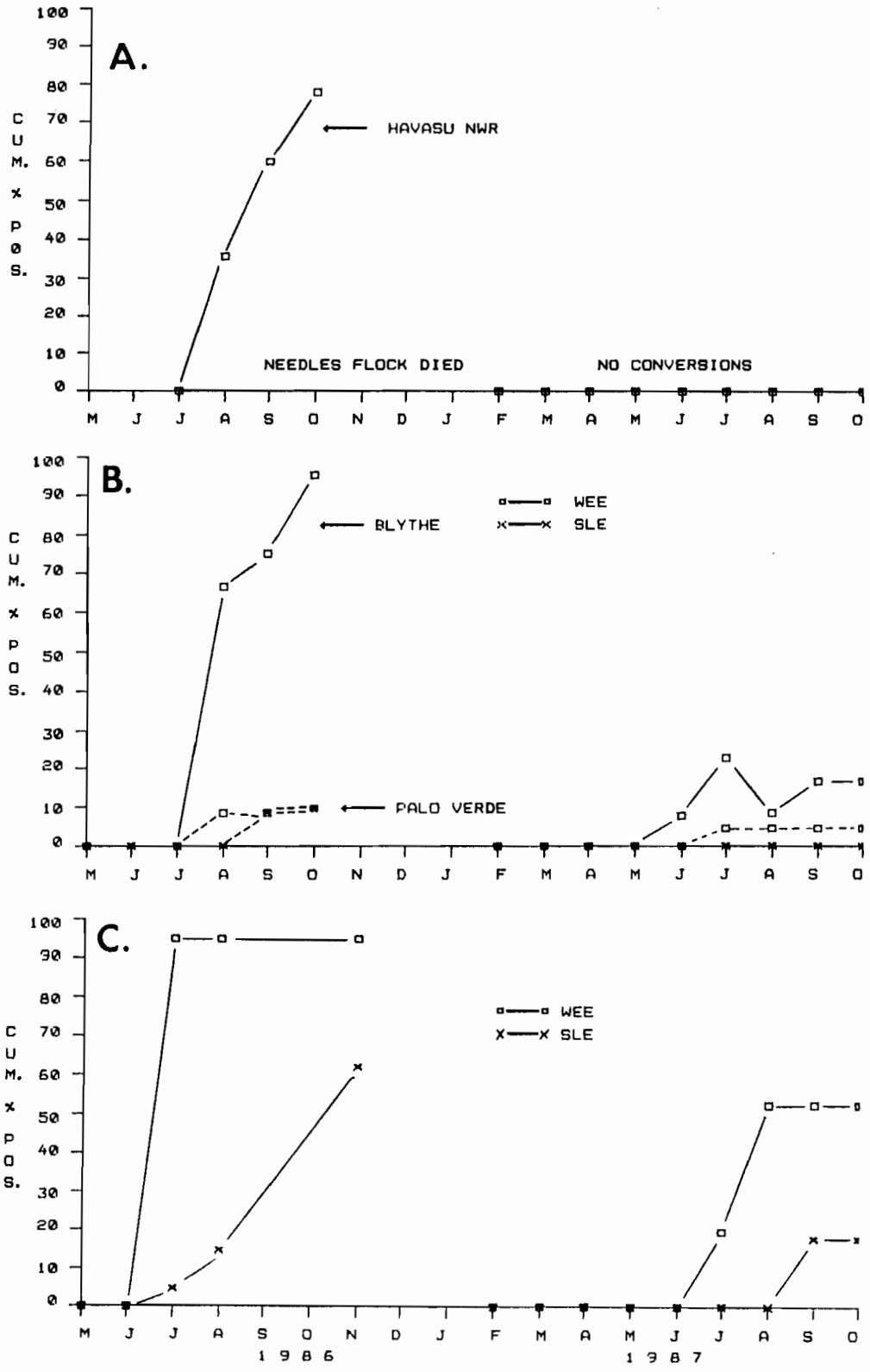

Figure 3.-Percentage of sentinel chickens seropositive for western equine encephalomyelitis (WEE) or St. Louis encephaltitis (SLE) viruses at study areas in A) Mohave, B) Palo Verde and C) Bard Valleys plotted as a function of time in months during 1986 and 1987.

was more clearly delineated during 1987 with WEE isolations made from May through July at both Blythe and Palo Verde, after the *Cx. tarsalis* population had attained maximal vernal abundance (Fig. 2b). Virus activity was not detected after July in both years, despite autumnal increases in *Cx. tarsalis* abundance. During 1987, only 2 and 1 chickens converted to WEE positive during June and July at Blythe and Palo Verde, respectively (Fig. 3b). Two chickens converted to SLE positive during August 1986, but no seroconversions occurred during 1987. SLE virus was not isolated from over 12,000 mosquitoes tested during 1987.

Mosquito sampling was done monthly at Bard Valley during 1987 (Fig. 2c). Five WEE isolates were made during late spring after the *Cx. tarsalis* population had attained maximal abundance. Similar to the pattern observed in Mohave Valley, WEE conversions occurred after the isolation of virus from *Cx. tarsalis* (Fig. 3c). Virus activity occurred slightly later during 1987 than 1986 when 95% of chickens bled in July by the Imperial County Health Department converted serologicaly to WEE positive. Similar to Palo Verde, SLE virus was detected during early autumn by seroconversion of sentinel chickens, but virus was not isolated from over 6,000 *Cx. tarsalis* or other mosquitoes.

Discussion.

Our preliminary results revealed several interesting ecological patterns:

1. Arboviruses only were isolated from *Cx. tarsalis* which was the most frequently collected mosquito at 4 of 5 study areas. Low numbers of other potential vector species during a year of low virus activity questioned the significance of alternate transmission cycles in virus maintenance. Current data indicated that virus isolated previously from other mosquitoes most likely represented a spillover from the basic *Cx. tarsalis*-bird cycle.

2. *Cx. tarsalis* abundance was markedly bimodal with peaks occurring during spring and fall. WEE isolations were made from females collected after the vernal population attained maximal abundance. Sentinel seroconversions subsequently were detected after WEE isolations were made from *Cx. tarsalis* and when virus was no longer isolated from mosquitoes. These data support the concept that virus isolation from mosquitoes provides an earlier warning of increased activity than does the seroconversion of sentinel chickens.

3. The intensity of virus transmission as indicated by sentinel seroconversion rates was difficult to predict from *Cx. tarsalis* minimum infection rates (MIR). For example, during 1987 WEE MIRs at Palo Verde and Bard were similar at 6.2 and 6.4 per 1,000 females tested during June; however, sentinel seroconversion rates were 5 and 53%, respectively.

4. Clinal S-N trends in WEE activity were detected. During 1986 when the level of virus transmission activity was high, WEE isolations from mosquitoes and sentinel seroconversions were detected earlier at Bard and Palo Verde Valleys than in the Mohave Valley. During 1987 when virus activity was low, detection was sporadic, but WEE MIRs and sentinel seroconversions appeared to decrease along a S-N cline.

5. SLE virus was detected by sentinel seroconversions in Palo Verde and Bard Valleys and also seemed to decrease in activity along a S-N cline. Neither SLE nor WEE virus was recovered during the autumnal increase in the *Cx. tarsalis* population.

In conclusion, our initial studies along the lower Colorado River indicated that WEE virus activity was associated closely with the vernal rise in the *Cx. tarsalis* population, but could not be detected prior to May. These data suggest a reintroduction and progressive northward dissemination of virus activity. However, sampling intensity was minimal in Bard and Mohave Valleys due to logistical difficulties which limited the number of specimens tested for virus. During 1988, we plan to continue our mosquito and virus monitoring activities at the same study areas and intensify sampling Bard Valley through collaboration with the Imperial County Health Department. Hopefully, expanded monitoring and careful evaluation of concurrently gathered weather and river flow data will substantiate the preliminary trends indicated by these first years of sampling.

Acknowledgments.

We especially thank V. M Martinez and B. R. Hill, Arbovirus Field Station, J. Hacker-Chavez and G. Swanson, Arbovirus Research Laboratory,

D. V. Dondero and the staff of the Arbovirus Unit, VRDL, and L. T. Hui, Vector Biology and Control Unit, for technical assistance. Mr. E. Gordon, Imperial County Health Department, kindly provided the use of chicken coops at Palo Verde and Bard.

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MOSQUITO ABUNDANCE AND ARBOVIRAL ACTIVITY IN THE COACHELLA VALLEY - 1987[1]

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ABSTRACT

Mosquito and arboviral activity were monitored at three primary and seven secondary sites positioned in a N-S transect of the Coachella Valley throughout 1987. *Culex tarsalis* was the most frequently collected species and exhibited a bimodal relative abundance pattern with peaks during the spring and fall. Western equine encephalomyelitis (WEE) virus was isolated from female mosquitoes on 44 occasions (principally from *Cx. tarsalis* after the vernal population peak). St. Louis encephalitis (SLE) virus was isolated only once (from a late winter collection of *Cx. tarsalis*) and Turlock virus was isolated three times. Sentinel chicken flocks seroconverted to WEE virus after isolates were obtained from mosquito pools. Initial results indicate that WEE (and perhaps SLE) virus may not only be re-introduced to the valley annually from southerly foci and disseminated progressively northward, but that limited overwintering foci of WEE and SLE virus may exist locally in the lower Coachella Valley.

The Coachella Valley with its seasonally large numbers of mosquitoes, abundant migrant and resident bird populations, and persistent arboviral activity provides an unique opportunity to investigate the ecology of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses. This valley not only provides a natural N-S corridor for the collection and dispersal of migratory birds but also provides a geographical link between the rural/agricultural areas of southeastern California with the urban/industrial areas of southwestern California.

Although it is possible to demonstrate the presence and transmission of both of WEE and SLE viruses within the Coachella Valley during virtually every summer, the mechanism(s) by which these viruses overwinter within and/or are periodically re-introduced to the valley remain unknown. Traditional encephalitis virus surveillance (E.V.S.) techniques (consisting of monthly mosquito collections and serological examination of a single sentinel chicken flock) provided little information other than viral presence during summer.

Thus, the primary objective of the present study was to describe the mosquito abundance and arboviral activity in the Coachella Valley on a year-round basis. Additionally, it should be possible to obtain operational information concerning the critical time period in the seasonal transmission cycle of WEE and SLE when mosquito control efforts should be maximized in order to minimize the risk of transmission to man.

Materials and Methods.

Monitoring of mosquito and arboviral activity in the Coachella Valley during 1987 was accomplished through a N-S transect of three primary and seven secondary sites (Fig. 1). Each of these sites was chosen on the basis of verified or suspected prior virus activity, proximity to avian refuges, and the abundance of vector or human populations.

Monitoring at the three primary sites consisted of: 1) weekly mosquito abundance assessment via a standard New Jersey Light Trap, 2) weekly mosquito collections for virus isolation via three CO_2-baited traps, and 3) monthly serological testing of a flock of 25 chickens for antibodies to WEE and SLE viruses. Three CO_2-baited traps were utilized at each of the seven secondary sites monthly in order to enhance overall sampling sensitivity.

Collection and processing of live mosquito samples from all CO_2-baited traps for virus isolation generally followed the guidelines established by the California Dept. of Health Services (Walsh 1987). On the morning after collection, specimens were anesthetized with triethylamine, sorted to species, pooled into lots of 50 each, and then frozen at -74°C for later virus isolation attempts.

The three primary sites utilized in this study represent typical mosquito breeding sources as found throughout the valley. Adohr Valley Farms

[1]This research is part of a collaborative effort between the Coachella Valley Mosquito Abatement District and Drs. W. K. Reisen, R. P. Meyer and J. L. Hardy of the University of California, Berkeley.

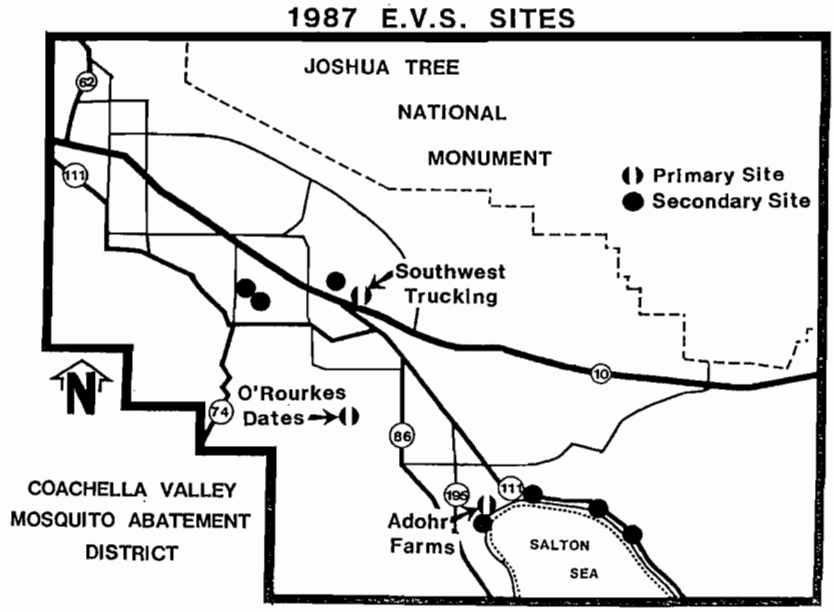

Figure 1.-Location of the three primary and seven secondary encephalitis virus surveillance (E.V.S.) monitoring sites in the Coachella Valley for 1987.

(located in Mecca) is representative of the duck hunting clubs located within 1-2 miles of the northern shore of the Salton Sea. These duck clubs each maintain hundreds of acres of standing water (and subsequent mosquito problems) from August to May in their feeding and shooting ponds. In addition, Adohr Valley Farms produces a limited number of floodwater mosquitoes in their flood irrigated date groves and permanent water mosquitoes in ornamental ponds and containers. Abundant bloodmeal opportunities exist at this site from the migratory and resident birds, a large goat herd, numerous hunting dogs, and human inhabitants.

O'Rourkes Date Grove (located in Thermal) is typical of the hundreds of date groves scattered throughout the lower Coachella Valley. The regular flood irrigation of these groves produce enormous populations of *Psorophora* and *Aedes* mosquitoes throughout the breeding season. Standing water from poor drainage, ornamental ponds, and open septic systems within O'Rourkes Date Grove also produces moderate numbers of culicine mosquitoes. Bloodmeal opportunities can be found in the cattle herd, domestic fowl and human inhabitants of this grove in addition to the numerous horses pastured on a ranch to the south.

The Southwest Trucking Company (located in Indio) periodically produces floodwater mosquitoes from the irrigation of the citrus groves within and surrounding this site. However culicine breeding is limited to the small number of sites found in the adjacent residential neighborhood and municipal golf course.

Results.

A total of 119,290 mosquitoes comprising 10 species and 5 genera were collected in CO_2-baited traps from the ten monitoring sites during 596 trap-nights in 1987. *Culex tarsalis* Coquillett was the most abundant species (83.9%) followed by *Cx. p. quinquefasciatus* Say (5.0%), *Psorophora columbiae* (Dyar and Knab) (4.8%) and *Anopheles franciscanus* McCracken (4.8%). The abundance of *Cx. tarsalis* ranged locally from a high of 95-96% at several of the secondary sites to a low of 9.2% at O'Rourkes Date Grove.

A total of 1,079 pools containing 44,596 mosquitoes (1,032 pools with 43,442 females and 47 pools with 1,154 males) were tested for arboviruses, with 70.1% of those being *Cx. tarsalis* females (699 pools containing 31,255 mosquitoes). The remaining species were submitted in relative proportion to their abundance. Thus, 142 pools (n = 5,700) of *Cx. p. quinquefasciatus*, 107 pools (N = 4,649) of *Ps. columbiae* and 57 pools (N = 909) of *Cs. inornata* were submitted for testing. A total of 43 WEE, one SLE, and three Turlock virus isolations were made from *Cx. tarsalis* pools. WEE virus appeared to be widely distributed and subsequently was isolated from over six of the 10 sites. Except for a single WEE virus isolation from *Cx. p. quinquefasciatus*

collected in a secondary site, virus was not isolated from any of the remaining mosquito species tested.

Cx. tarsalis were bimodally abundant throughout the valley during the spring and fall. This pattern was most clearly demonstrated at Adohr Valley Farms (Fig. 2) where over 3,000 females per trap-night were collected in mid-April during the vernal peak and over 2,500 females per trap-night were collected in mid-October during the autumnal peak. The relatively cool winters and very hot, dry summers in the valley were presumably detrimental to *Cx. tarsalis* reproduction and survival respectively. Consequently, this species was most abundant during the cool spring and fall periods, which coincided with the flooding of the duck clubs that provided ideal breeding conditions in the form of thousands of acres of standing water.

WEE virus was detected in *Cx. tarsalis* collected from this site during May and June on 14 occasions. This brief, but intense period of virus activity closely followed the vernal population peak. Of considerable interest here is the two single isolations of WEE virus from this site in mid-January and early March at a time when low mosquito populations were encountered. Additionally, a single SLE virus isolation was made from *Cx. tarsalis* collected at this site in mid-February; a time of year well before any previous SLE isolation in the valley.

Although mosquito collections at O'Rourkes Date Grove were primarily *Cx. p. quinquefasciatus* (47.7%) and *Ps. columbiae* (39.6%), *Cx. tarsalis* still demonstrated a similar but more widely separated bimodal abundance with an early February peak of over 45 females per trap-night and a mid-October peak of nearly 40 females per trap-night (Fig. 3). No virus isolations were made from O'Rourkes Date Grove during 1987 which is probably a reflection of the locally low vector populations.

The limited number of breeding sites and host availability at the Southwest Trucking Company proved to be much less conducive to mosquito production and, in fact, made the mosquito populations much more dependent upon water availability. Consequently, *Cx. tarsalis* collected from this site demonstrated a single population peak of over 270 females per trap-night in early August during a time when the owners were heavily watering the citrus grove (Fig. 4). WEE virus was isolated from *Cx. tarsalis* collected at this site on four separate occasions between late June and mid-August. WEE virus isolations from this site appear to be independent from the population abundance.

Collections made at each of the seven secondary sites generally supported the trends observed at the three primary sites with the previously described bimodal abundance of *Cx. tarsalis* occurring in most sites. Temporal and spatial distribution of the 24 WEE virus isolations from the secondary sites support the observation of a northward progression of viral activity as the transmission season progressed. WEE virus isolations were obtained approximately 3-4 weeks earlier in collections made near the Salton Sea than from those made further up-valley.

Sentinel chicken seroconversions to WEE virus were first noted in early June at Adohr Valley Farms and quickly reached a plateau of 58% positive (Fig. 5). These seroconversions immediately followed the recovery of virus from mosquitoes and occurred some time after the vernal mosquito population peak. Although viral isolations were not made from mosquitoes collected following the autumnal *Cx. tarsalis* population peak, further WEE viral activity was detected by additional chicken seroconversions. At the other two primary sites, seroconversions to WEE virus first occurred about one serological testing period later than it did at Adohr Valley Farms and levelled off at much lower levels. Although mosquito collections failed to detect WEE viral activity at O'Rourkes Date Grove, 8% of the sentinel chickens seroconverted to WEE virus.

Examination of climatological information for 1987 (Fig. 6) indicates that following a mild winter, the valley experienced an unseasonably warm spring, cool summer and mild fall. By providing earlier conditions suitable for breeding and extending the breeding season later into the fall, this weather may have acted to amplify the vernal and autumnal vector populations and thus enhance viral transmission possibilities.

Summary.

Although limited in scope, the first year of this study revealed some interesting observations:

1. Arboviruses were isolated almost exclusively from *Cx. tarsalis* and not from any other species, re-emphasizing the importance of this species as the principle arboviral vector in rural/agricultural areas of California such as the Coachella Valley.

2. The abundance of *Cx. tarsalis* was clearly bimodal with spring and fall population peaks. These population levels may have been fur-

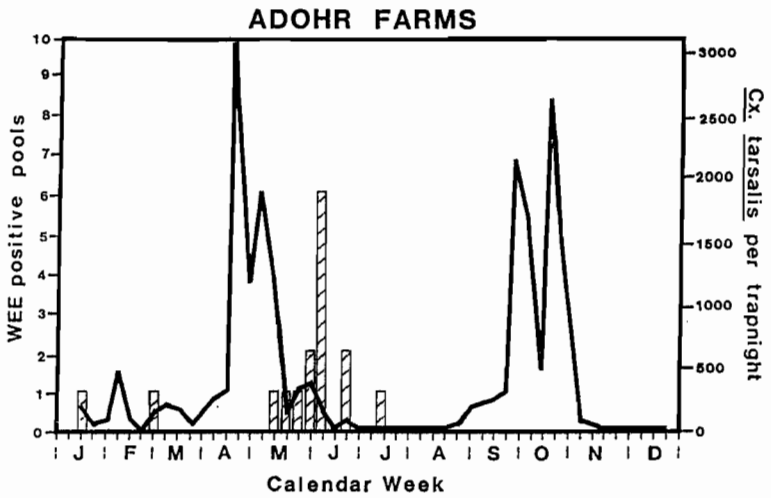

Figure 2.-Relative abundance of *Culex tarsalis* (females/$C0_2$ trap-night) and western equine encephalomyelitis (WEE) virus isolations for Adohr Valley Farms.

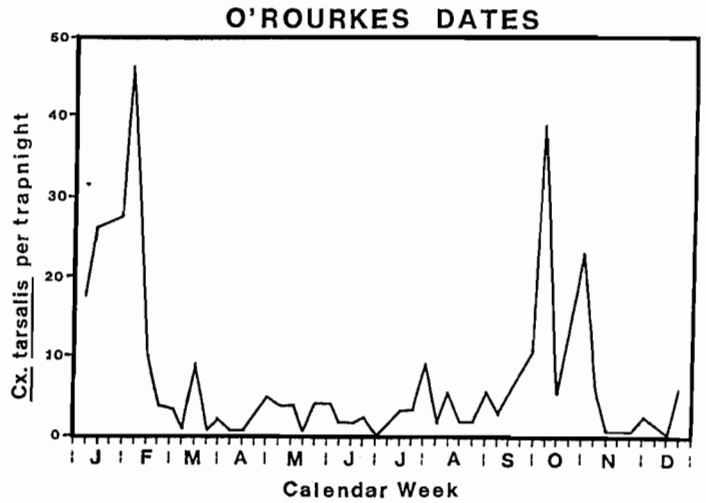

Figure 3.-Relative abundance of *Culex tarsalis* (females/$C0_2$ trap-night) for O'Rourkes Date Grove.

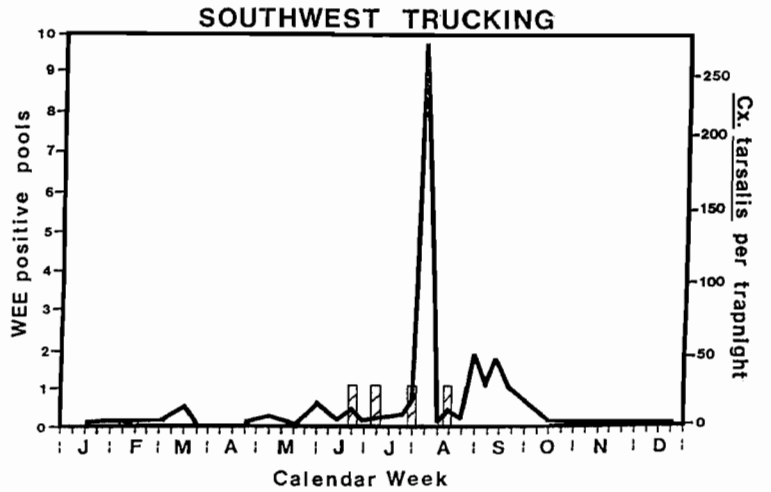

Figure 4.-Relative abundance of *Culex tarsalis* (females/$C0_2$ trap-night) and western equine encephalomyelitis (WEE) virus isolations for Southwest Trucking Company.

ther elevated by the particular weather conditions experienced in the valley during 1987.

3. Portions of the lower Coachella Valley may have served as overwintering foci of WEE and SLE viruses as indicated by the late winter isolations of both viruses from Adohr Valley Farms (in addition to an isolation of WEE virus in December 1986).

4. Whether introduced or locally overwintering, once WEE virus became active, a northward progression in activity occurred with about a one-month time lag between virus isolations from mosquitoes and seroconversions in sentinel chickens in the lower and upper Coachella Valley.

5. Future E.V.S. programs should embrace several monitoring methods to most thoroughly detect viral activity. While weekly mosquito collections provide important temporal and spatial information, sentinels may be more sensitive to virus activity (as witnessed by the serological detection of viral activity at O'Rourkes Date Grove and after the autumnal vector population peak at Adohr Valley Farms).

6. The observed seasonal sequence of a vernal vector population peak followed by virus detection and ultimately by sentinel chicken seroconversions suggests that it will be possible to develop operational strategies to maximize control efforts at the most opportune time to break the transmission cycle.

In conclusion, our initial investigations indicate that WEE virus activity occurred throughout all of 1987 in the Coachella Valley but was most closely associated with the vernal rise in *Cx. tarsalis* population. Evidence suggests that WEE virus may have overwintered locally and/or been re-introduced into the lower valley in early spring and moved progressively northward with time. In 1988, we plan to try to verify some of the spatial and temporal patterns seen during 1987 and to extend the northern limits of our monitoring site transect to the upper Coachella Valley.

Acknowledgments.

Sentinel chickens were bled by the staff of the Arbovirus Field Station, Bakersfield. Mosquito pools and chicken sera were tested by the Arbovirus Research Laboratory, University of California, Berkeley, and the California Department of Health Services Viral and Rickettsial Diseases Laboratory, Berkeley. Technical assistance and logistical support were provided by the staff of the Coachella Valley MAD. This research 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

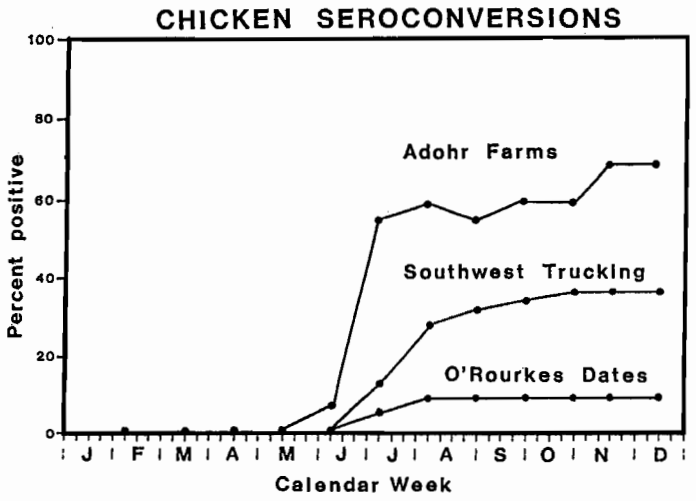

Figure 5.-Cumulative percentage of sentinel chickens seropositive for western equine encephalomyelitis (WEE) virus at the three primary sites.

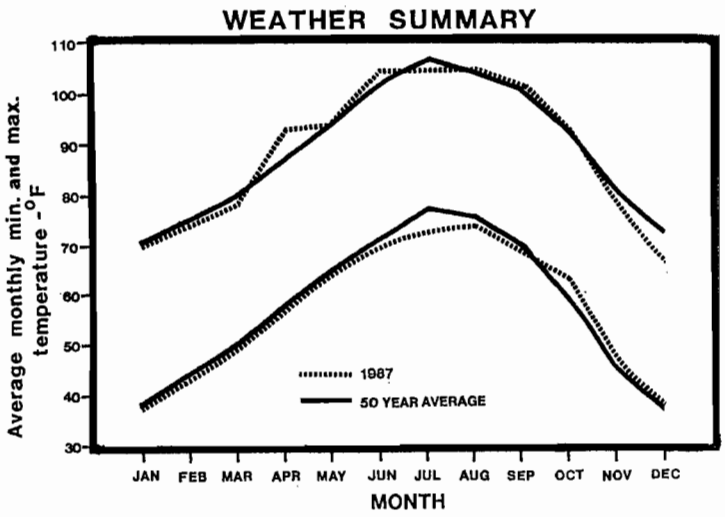

Figure 6.-Average monthly minimum and maximum temperatures (°F) for the Coachella Valley during the past 50 years and during 1987.

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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MOSQUITO ABUNDANCE AND VIRUS ACTIVITY IN THE CHINO AREA, SAN BERNARDINO COUNTY, CALIFORNIA, 1987

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ABSTRACT

Mosquito adults (*Culex tarsalis*, *Culex peus*, and *Culex quinquefasciatus*) were sampled using $C0_2$-baited traps and walk-in type red boxes. Overall, 24,395 adult mosquitoes were tested for the presence of arboviruses with negative results. Monthly blood samples taken from sentinel flocks of chickens at one urban and one rural site tested negative for WEE and SLE viruses. Lack of virus activity may be due, in part, to female mosquitoes feeding upon a readily available bovid population. Mosquito blood meal tests (in progress) may provide insight into the continued absence of arbovirus activity in the Chino Area.

Introduction.

The West Valley Vector Control District was formed in 1983 and began actual field operations in May, 1984. The District is located in the western corner of San Bernardino County in an area encompassing residential, industrial, and agricultural environments. Most mosquitoes are produced from the many impoundments used to dispose of waste water from the washing of dairy cattle. Densities of 500 to 1,000 immature mosquitoes per dip are common in highly productive sources. The most common mosquito species occurring in the District is *Culex quinquefasciatus* Say, followed by *Culex peus* Speiser and *Culex tarsalis* Coquillett.

In 1985, the District began an encephalitis virus surveillance program wherein adult female mosquitoes were trapped utilizing modified CDC-style traps baited with dry ice (Pfuntner 1979), and a flock of sentinel chickens was established. As the three predominant mosquito species inhabiting the District are known, suspected and/or laboratory competent vectors of St. Louis encephalitis virus (SLE), the outbreak of this virus in the adjoining Los Angeles and Orange Counties prompted concern that virus cases could also occur in San Bernardino County (Hardy et al. 1985, Emmons et al. 1984, 1985). Subsequently, studies were initiated to expand surveillance activities in both rural dairy and urban residential habitats during the fall, winter, and spring periods. The present paper describes mosquito abundance and virus activity patterns during 1987.

Materials and Methods.

Sentinel chicken flocks (25 each) were established at one residential site and one rural site in January of 1987, and were bled at monthly intervals. Routine mosquito control activities were maintained in and around the sampling sites. New Jersey light traps were operated seven nights per week at both locations. A walk-in red box (Meyer 1985) was placed at the rural site where resting adults were collected weekly. Adult host-seeking female mosquitoes were collected weekly by three carbon dioxide baited traps operated at each site at ground level (ca. 2 meters). Up to 10 pools of fifty males or females for each of the three *Culex* species were shipped weekly on dry ice to either the Arbovirus Research Laboratory, University of California, Berkeley, or the Viral and Rickettsial Disease Laboratory, Department of Health Services, State of California, for testing.

Results.

Of the three mosquito species, *Cx. quinquefasciatus* was the most abundant at both rural and urban sites, comprising approximately 85% of the captured adults which numbered 48,111. This species was most prevalent during August when maximum trap counts approached 1,400 females per night at the rural site and 600 females per night at the urban site (Figs. 1 and 2). *Cx. tarsalis* was the least abundant species captured (ca. 7%). Its greatest activity was in June and July (Figs. 3 and 4). *Cx. peus* comprised about 9% of the total, also with the most specimens trapped during June and July (Figs. 5 and 6).

Overall, 24,395 *Culex* adults (538 pools) were tested for virus activity, with negative results (Table 1). Monthly blood samples taken from the sentinel chickens were likewise negative.

Discussion.

The lack of viral activity in the Chino area during 1987 was consistent with the previous low levels of activity detected here and in the nearby Prado Basin (Emmons et al. 1984, 1985, 1986, 1987). The consistent absence of arboviral activity

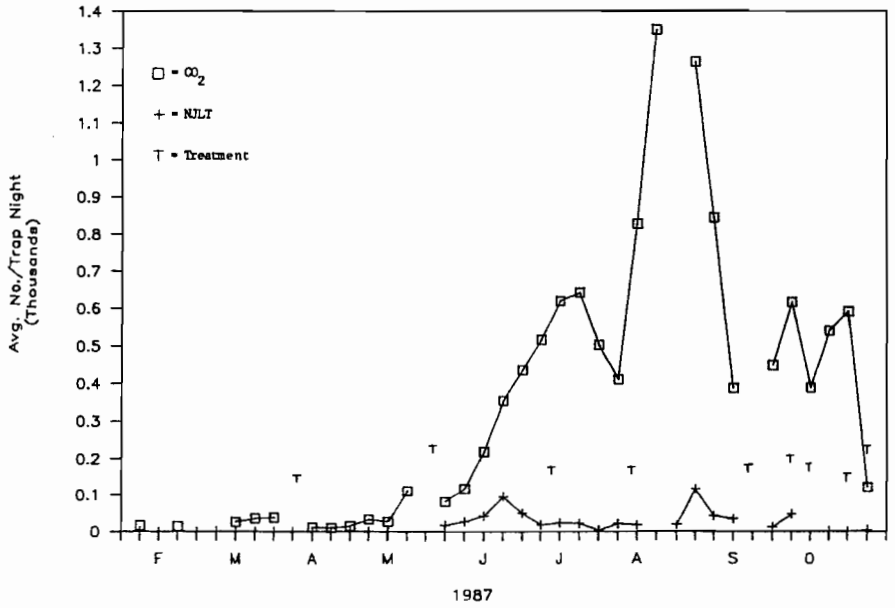

Figure 1.-Mean number of *Cx. quinquefasicatus* collected per trap night at a rural site in Chino, 1987.

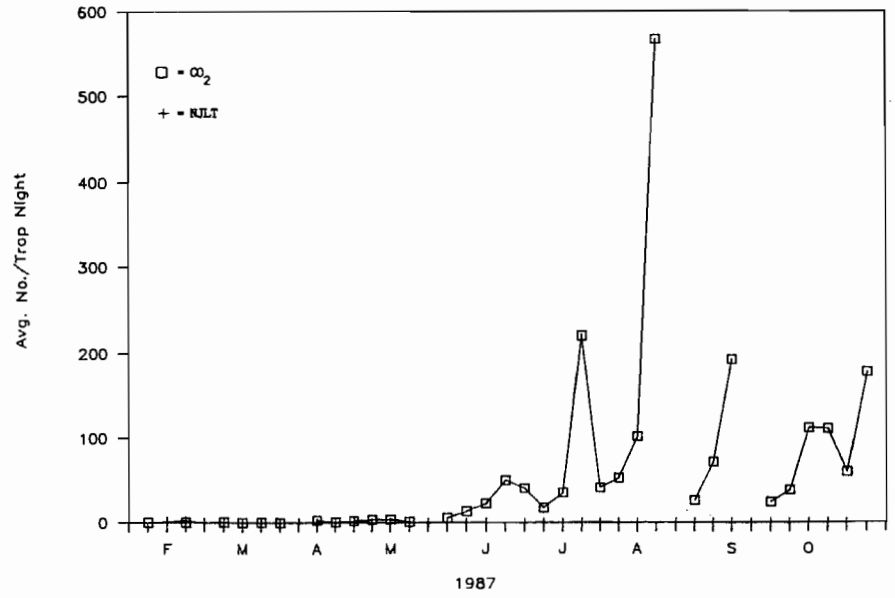

Figure 2.-Mean number of *Cx. quinquefasicatus* collected per trap night at a urban site in Chino, 1987.

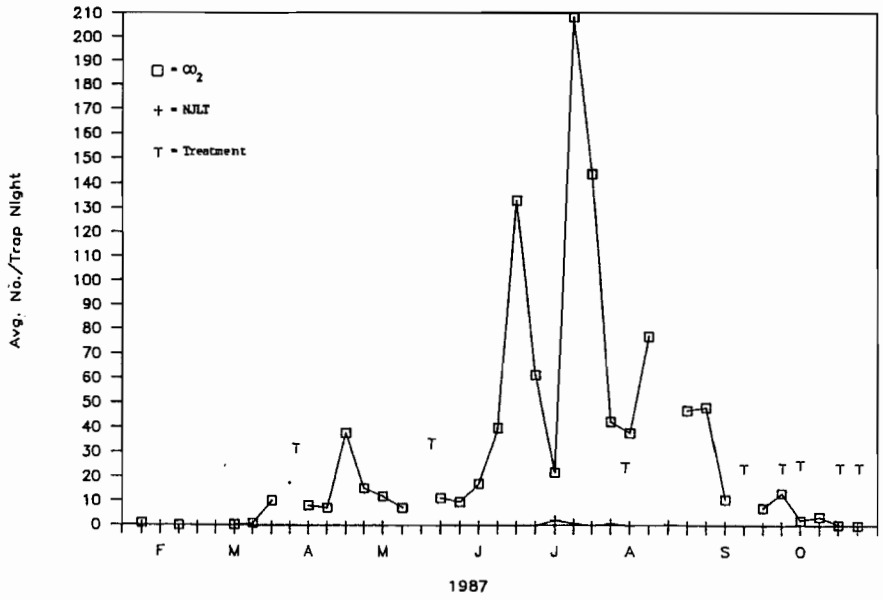

Figure 3.-Mean number of *Cx. tarsalis* collected per trap night at a rural site in Chino, 1987.

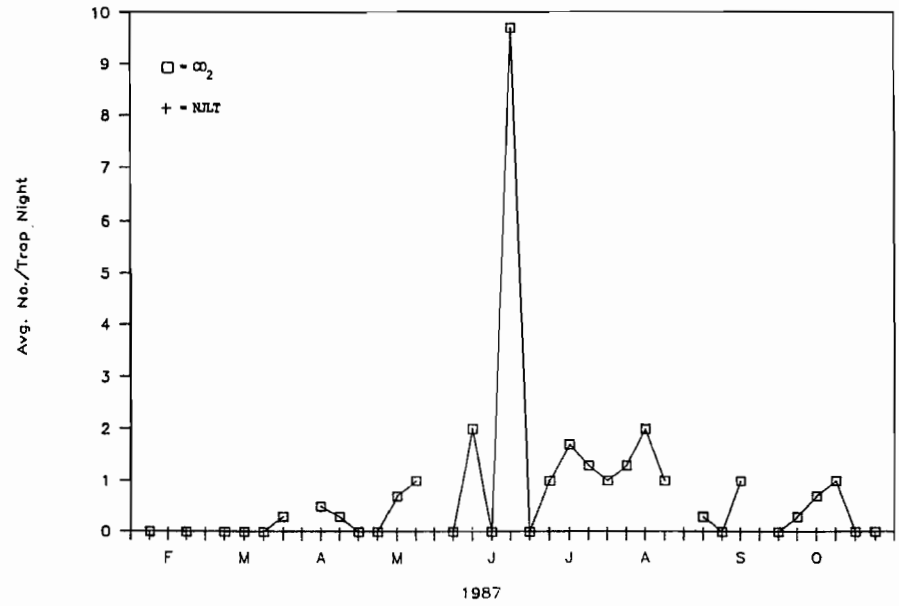

Figure 4.-Mean number of *Cx. tarsalis* collected per trap night at a urban site in Chino, 1987.

Table 1.-Mosquito collected at rural and urban sites near Chino, San Bernardino County, and tested for SLE and WEE virus infection.

Species	Sex	Numbers Tested: Pools	Numbers Tested: Total
Rural			
Cx. tarsalis	M	6	210
Cx. tarsalis	F	56	2,373
Cx. quinquefasciatus	M	48	2,064
Cx. quinquefasciatus	F	223	10,873
Cx. peus	M	21	819
Cx. peus	F	36	1,410
Urban			
Cx. tarsalis	M	0	0
Cx. tarsalis	F	11	524
Cx. quinquefasciatus	M	0	0
Cx. quinquefasciatus	F	85	3,911
Cx. peus	M	0	0
Cx. peus	F	52	2,211

was difficult to explain, since the basic ecological components for both SLE and western equine encephalomyelitis (WEE) virus transmission cycles appear to be present. As described in the present paper, mosquitoes (including *Cx. tarsalis*) were abundant in both rural and urban localities during mid-summer when SLE and WEE normally are active throughout the state. Temperature/humidity regimens seemed conducive to virus replication and mosquito survival (as indicated by parity rates). Passerine birds were abundant in both residential and dairy environs and additional bird taxa including migrants were common in the Prado Basin. Possibly, the diversion of host-seeking females to the abundant dairy cattle may have inhibited virus amplification by reducing the probability that an infective female would bite a susceptible vertebrate host. Since cows rarely produce a detectable viremia, they are considered to be a "dead-end host" in virus transmission cycles. At present, 840 *Culex* females are being tested to determine if the host selection pattern of these normally ornithophagic species has been modified in the Chino area to include a high proportion of bovid blood meals.

Acknowledgments.

Sentinel chickens were bled by the staff of the Arbovirus Field Station. Mosquitoes and sera were tested for virus by the Arbovirus Laboratory, School of Public Health, University of California, Berkeley and the Viral and Rickettsial Diseases Laboratory, California Department of Health Services. This research was funded, in part, by Research Grant 5-R22-AU-3028D 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 California Region of the California Mosquito and Vector Control Association.

The author wishes to thank Dr. W. K. Reisen for this review of the manuscript. The support and

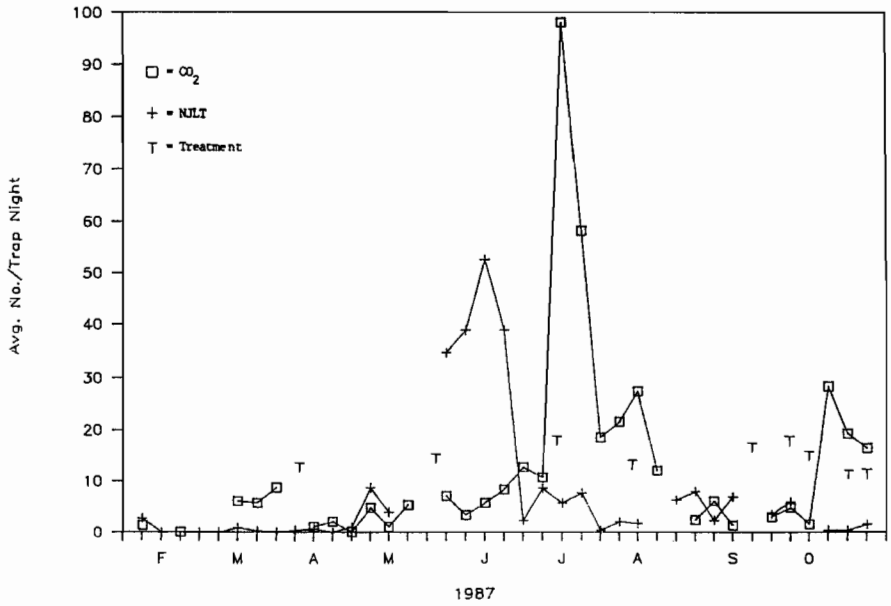

Figure 5.-Mean number of *Cx. peus* collected per trap night at a rural site in Chino, 1987.

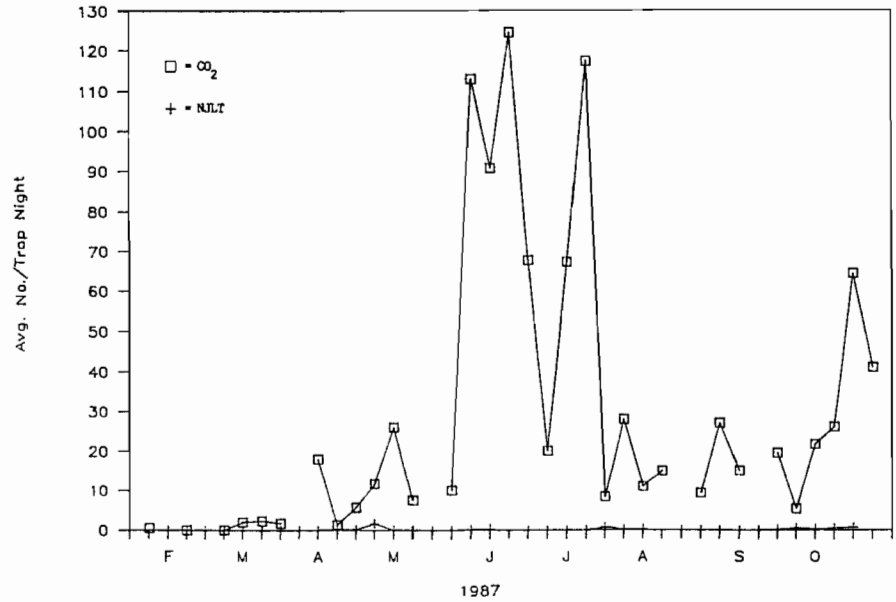

Figure 6.-Mean number of *Cx. peus* collected per trap night at a urban site in Chino, 1987.

assistance of the staff of the West Valley Vector Control District are gratefully acknowledged.

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MOSQUITO ABUNDANCE AND ARBOVIRUS ACTIVITY IN ORANGE COUNTY, 1987

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The responses by mosquito control agencies to the 1984 SLE outbreak in the Los Angles Basin (Webb and Myers 1986) have progressed from the immediate accelerated mosquito control operations to the long-term surveillance and study stages. Paramount to these stages is the determination of the mosquito-virus-bird-human interrelationships at the point or points where virus acquisition by the mosquitoes and virus transmission to humans have the highest probability of occurrence. Epidemiological data from the 1984 SLE epidemic in the Los Angeles Basin indicate that human contact with virus-infected mosquitoes may have occurred in suburban peridomestic environments (Murray et al. 1985, Webb et al. 1987).

Evaluation of each of the integral components through investigational studies should, in theory, provide insights into effective mosquito and disease control procedures. It was with this simple logical progression that the Backyard Mosquito and Small Bird Study programs were initiated in 1985 (Ritschel and Webb unpublished data, Webb et al. 1987). In addition to the usual annual Encephalitis Virus Surveillance (EVS) and Sentinel Chicken programs, in 1987, the Backyard Mosquito Program and the Small Bird Bleeding Program were greatly expanded. These programs overlapped at several sites this year and it is planned that they will be operated concurrently at the same localities next year. The EVS program yielded 713 pools of mosquitoes that were submitted to the Viral and Rickettsial Disease Laboratory for testing. None of the pools tested positive for SLE or WEE virus. The Sentinel Chicken program also produced no positive seroconversions for SLE or WEE viral antibodies. The Small Bird Bleeding program was directed by Dr. John Gruwell and produced 5,555 sera samples (Feb.-Dec.); 35 of the samples (0.63%) tested positive for SLE viral antibodies.

St. Louis encephalitis virus activity in 1987 was also low in mosquito populations in the Los Angeles Basin (1 SLE positive pool of *Culex tarsalis* from Los Angeles County) as well as in the entire state; only two SLE positive pools (both *Cx. tarsalis*) in total were reported. In contrast, numerous pools (121) in the state tested positive for WEE virus. However, most of these were from central California and the Colorado River area; none was recorded from the Los Angeles Basin.

Mosquito species composition, frequency and activity continue to be important surveillance and study criteria in Orange County in regards to arbovirus transmission. These factors have been separated ecologically into urban/suburban and rural habitats because it has been recognized from previous work (Webb unpublished data, Webb and Myers 1986, Webb et al. 1987) that both habitats demonstrate differences in mosquito and bird species occurrence and frequency. For example, in descending order of frequency, *Culex erythrothorax* Dyar, *Cx. tarsalis* Coquillett and *Culex quinquefasciatus* Say are the species commonly associated with rural marshland habitats in Orange County. In the urban/suburban habitat the most frequently collected species is *Cx. quinquefasciatus* with occasional records of *Cx. tarsalis*, *Culex peus* Speiser and *Culiseta incidens* (Thomson). Data compiled for the last five years from New Jersey light traps situated in rural habitats (Table 1) have indicated that summer populations of *Cx. tarsalis* have declined and plateaued since 1983 and 1984. New Jersey light trap data generated in urban/suburban habitats (Table 2) indicated that *Cx. tarsalis* levels were extremely low. However, *Cx. tarsalis* numbers collected at certain urban/suburban sites by modified CDC CO_2-light traps were significantly higher (Table 3). The differences here may be caused by the additional attractancy of CO_2 and the negative influence of competitive light from sources other than the mosquito traps. *Culex quinquefasciatus* levels were higher than the other species (Table 4) in the urban/suburban habitats and significantly high numbers per trap night were found throughout the entire mosquito season. The results from the Reiter/Cummings gravid female traps (Table 5) indicated that there were even higher levels of *Cx. quinquefasciatus* activity in the peridomestic surroundings than shown with the CDC traps.

Diminishment of the SLE/WEE virus and mosquito activity in Orange County since 1983 and

Table 1.-Average number of female *Culex tarsalis* per trap night - Rural Habitats* - New Jersey Light Traps.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987	0.1	0.6	0.7	3.2	4.0	2.8	14.0	11.2	6.4	6.7	1.3	- -
1986	0.4	0.5	1.6	4.2	5.7	12.5	14.3	3.6	5.8	3.3	0.3	0.1
1985	0.4	0.6	0.9	4.0	7.3	6.5	14.0	10.0	2.0	6.6	0.8	0.2
1984	0.1	0.1	0.2	0.2	0.5	1.6	18.8	8.4	5.6	3.9	0.2	0.0
1983**	0.5	0.3	0.7	1.7	9.8	19.0	37.6	9.5	12.1	3.6	1.0	0.1

* Representative Rural Habitats = Featherly Park, San Joaquin Marsh, and 20 Ranch Duck Club.
**Light trap installed 20 July at 20 Ranch Duck Club; Featherly Park light trap inoperative.

Table 2.-Average number of female *Culex tarsalis* per trap night - Suburban Habitats* - New Jersey Light Traps.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.38	0.19	0.06	0.04	- -
1986	0.00	0.00	0.13	1.84	0.79	0.64	0.06	0.00	0.00	0.00	0.08	0.00
1985	0.00	0.00	0.00	0.03	0.02	0.05	0.03	0.10	0.02	0.04	0.00	0.00
1984	0.05	0.02	0.07	0.10	0.05	0.04	0.00	0.05	0.03	0.00	0.00	0.00
1983**	0.02	0.02	0.25	1.12	2.20	0.06	0.13	0.09	0.12	0.09	0.12	0.02

* Representative Sururban Habitats = Garden Grove, Huntington Beach, Orange, and Tustin.
**No data for Garden Grove and Orange light traps.

Table 3.-Average number of female *Culex tarsalis* per trap night - Suburban Habitats CDC Light $C0_2$ Traps.

	Apr	May	Jun	Jul	Aug	Sep	Oct
Huntington Beach (Pett)	1.5 (2)*	0.2 (5)	0.4 (7)	3.1 (8)	5.0 (1)	11.5 (2)	0.0 (1)
Fullerton (Medina)	1.0 (1)	1.5 (4)	3.3 (3)	1.0 (7)	6.4 (8)	5.0 (10)	3.0 (2)
Fullerton (Fine)	1.0 (1)	ND	2.3 (3)	4.5 (10)	10.3 (9)	11.0 (12)	1.4 (7)
Orange (Elliott)	ND	0.0 (6)	0.1 (8)	0.0 (6)	0.5 (4)	0.0 (6)	0.5 (4)

*Numbers in parentheses ( ) = Trap Nights.
ND = No data.

Table 4.-Average number of female *Culex quinquefasciatus* per trap night - Suburban Habitats - CDC Light $C0_2$ Traps.

	Apr	May	Jun	Jul	Aug	Sep	Oct
Huntington Beach (Pett)	7.0 (1)*	6.6 (5)	1.8 (5)	15.6 (7)	34.0 (1)	17.0 (4)	12.0 (1)
Fullerton (Medina)	7.0 (1)	17.0 (4)	44.3 (3)	24.7 (7)	21.0 (8)	16.8 (10)	8.0 (2)
Fullerton (Fine)	1.0 (1)	ND	23.0 (3)	12.3 (7)	7.5 (12)	12.8 (15)	5.5 (8)
Orange (Elliott)	ND	2.5 (6)	13.0 (8)	15.6 (6)	15.0 (3)	12.0 (6)	6.8 (4)

*Numbers in parentheses ( ) = Trap Nights.
ND = No data.

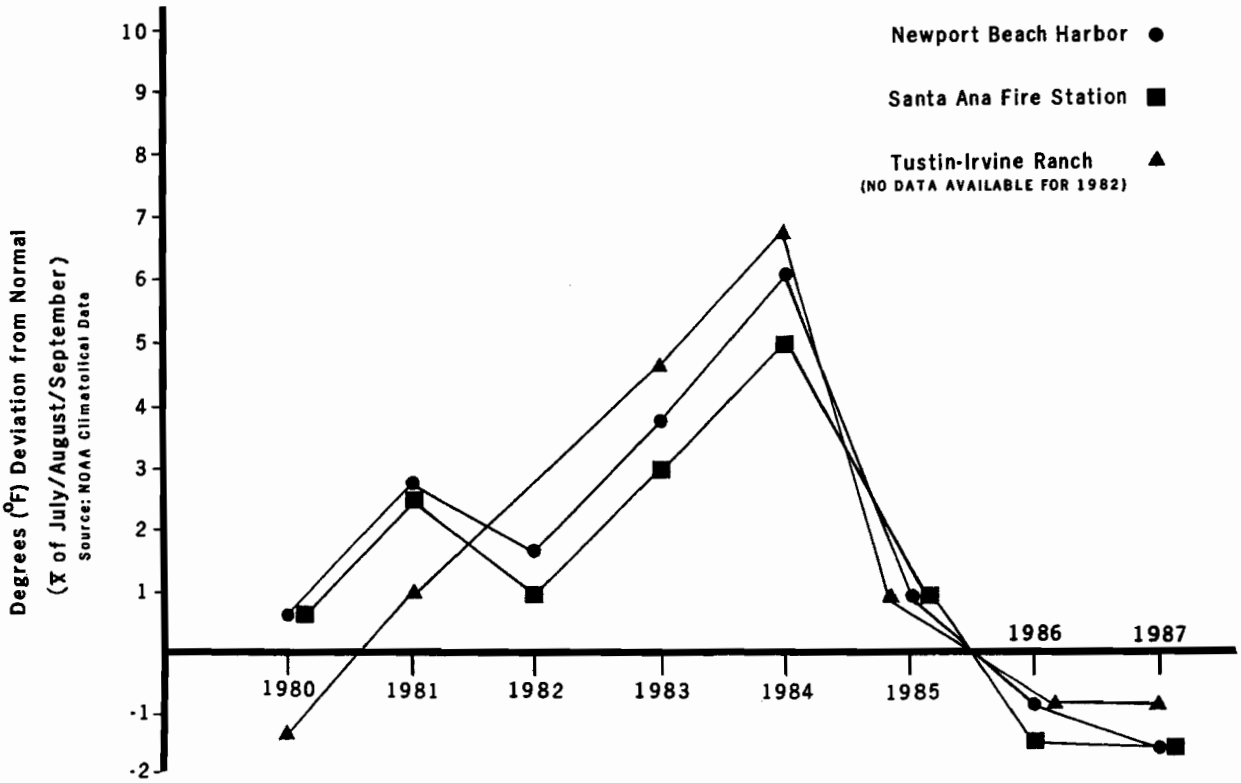

Figure 1.

Table 5.-Average number of female *Culex quinquefasciatus* per trap night - Suburban Habitats - Reiter/Cummings Gravid Female Trap.

	Jul	Aug	Sep	Oct
Fullerton (Fine)	71.8 (4)*	68.0 (4)	137.6 (5)	61.3 (3)
Whittier (Miller; Los Angeles Co.)	21.3 (3)	29.0 (4)	64.0 (4)	59.0 (1)
Huntington Beach (Pett)	65.0 (4)	95.8 (4)	63.0 (3)	51.0 (1)
El Toro (Fire Station)	2.6 (3)	23.0 (3)	43.3 (3)	16.5 (2)

*Number in parentheses ( ) = Trap Nights.

1984 may be due to the cooling trend that abruptly began in 1985 and progressed to subnormal temperatures in 1986 and 1987 (Figure 1).

Culex quinquefasciatus was the most numerous and most frequent species collected. *Culex peus* and *Cx. tarsalis* also were trapped with regularity at some sites, but were present in much lower numbers than *Cx. quinquefasciatus*. *Culiseta incidens* occasionally was taken in relatively large numbers (also see Webb et al. 1987). Any one or all of these species may be important in the transmission cycle of SLE and/or WEE virus to reservoir hosts or to humans. It is imperative that an intensified three to four year, year-round study of the peridomestic interrelationships of the mosquito-virus-bird-human factors be launched in the urban/suburban peridomestic environment of the Los Angeles Metropolitan area.

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MOSQUITO ABUNDANCE AND ARBOVIRUS ACTIVITY IN LOS ANGELES COUNTY

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St. Louis encephalitis virus has occurred in southern California, particularly Los Angeles, Riverside, and Orange Counties since 1984. In Los Angeles County, SLE human cases have been associated closely with sentinel flock seroconversions and virus isolations in abundant *Culex tarsalis* Coquillett populations occurring near the remaining riparian and marsh habitats within the densely populated urban environment. The above evidence suggests that *Culex tarsalis* may be the principle vector of SLE virus in Los Angeles County, and that SLE virus has become established within the county at the few remaining sites capable of producing abundant *Culex tarsalis* populations.

District Geography and SLE Study Sites.

The information in the present report is restricted to data collected and compiled within the Southeast Mosquito Abatement District (SEMAD). The SEMAD (within the greater Los Angeles Basin) is comprised of approximately 700 square miles of densely populated area. Geographically, the District is mostly flat terrain with poor drainage, gradually increasing in elevation in a south-north direction from sea level to several hundred feet near the inland foothills. The District is highly urbanized with typical urban mosquito sources. Several large areas of 10 or more acres of marsh and riparian habitats persist which produce abundant populations of mosquitoes, particularly *Culex tarsalis*.

Recently (1984 to 1986), several of these areas have experienced active transmission of St. Louis encephalitis (SLE) virus, and some were suspected as being foci for SLE transmission to humans. Since 1984, two of these sites have been intensively monitored for SLE activity: 1) Harbor Lake, a 50 acre marsh habitat, and 2) Sepulveda Basin, a square mile flood control basin consisting of both marsh, riparian, and agricultural habitats. In 1986, intensive SLE monitoring began in the Downey-Norwalk section of the San Gabriel River, a riparian habitat manipulated by the Los Angeles County Flood Control District for purposes of flood control and water regeneration. Monitoring for SLE activity has included one or more of the following methods: 1) capture of female mosquitoes using CO_2-baited portable light traps (CDC), 2) sentinel chicken flocks, 3) sentinel pigeon flocks, and 4) sera sampling from captured and released peridomestic birds.

Mosquito Occurrence, Abundance and Seasonal Trends.

Over 20 years of extensive larval sampling and New Jersey light trap (NJLT) collection data have shown that five species of mosquitoes commonly occur throughout SEMAD: 1) *Culex tarsalis*, 2) *Culex quinquefasciatus* Say, *Culex peus* Speiser, 4) *Culiseta incidens* (Thomson), and 5) *Culiseta inornata* (Williston). Three other species occur in restricted habitats within specific areas of the SEMAD, *Culex erythrothorax* Dyar, *Anopheles freeborni* Aitken and *Anopheles franciscanus* McCracken.

A comparison of the District's 1987 NJLT data with those averaged for the five-year period, 1982 to 1986, showed that adults of the common mosquito species occurred annually within the District in a generally repeatable and predictable trend (Fig. 1). Except for *Cx. erythrothorax* and *Anopheles* species, all adult females were captured year round in the District's NJLTs. The absence of *Cx. erythrothorax* from December to May and the anophelines from February to June is probably due to the combined effect of low seasonal abundance and limited distribution rather than seasonal inactivity. Annually, from November through May, the *Culiseta* species are most abundant (Table 1) and proportionately dominate the NJLT captures (Fig. 1). From June through October that highest percentage of NJLT captures are the *Culex* mosquitoes (Fig. 1), a six-month period when their populations are most abundant (Table 1). Among the *Culex* mosquitoes, *Cx. quinquefasciatus* and *Cx. peus* are the most abundant and require the most control effort by the District. These seasonal population trends are temperature dependent and follow a pattern well-documented by the District.

The seasonal occurrence of *Cx. tarsalis* essentially mirrors the pattern of the other *Culex*

Table 1.-Comparison of the monthly number of adult female mosquitoes collected averaged over the 5-year period 1982-1986 with monthly totals of female adult mosquitoes collected in the Southeast Mosquito Abatement District New Jersey light traps during 1987.

	Mosquitoes and Years							
	Culex tarsalis		Other *Culex* spp.		*Culiseta* spp.		Total all spp.	
	1982-86	1987	1982-86	1987	1982-86	1987	1982-86	1987
Jan	35	57	88	129	177	225	300	411
Feb	68	72	79	59	239	307	386	438
Mar	62	20	115	45	359	208	536	273
Apr	107	27	239	110	581	298	927	435
May	228	39	313	167	406	222	947	428
Jun	207	156	413	579	226	190	846	925
Jul	182	180	374	429	86	80	642	689
Aug	165	186	349	644	42	79	556	909
Sep	234	171	368	400	49	63	651	634
Oct	91	242	448	822	346	211	885	1,275
Nov	33	63	280	404	644	611	957	1,078
Dec	29	14	90	50	374	166	493	230
TOTAL:	1,441	1,227	3,136	3,838	3,529	2,710	8,126	7,725

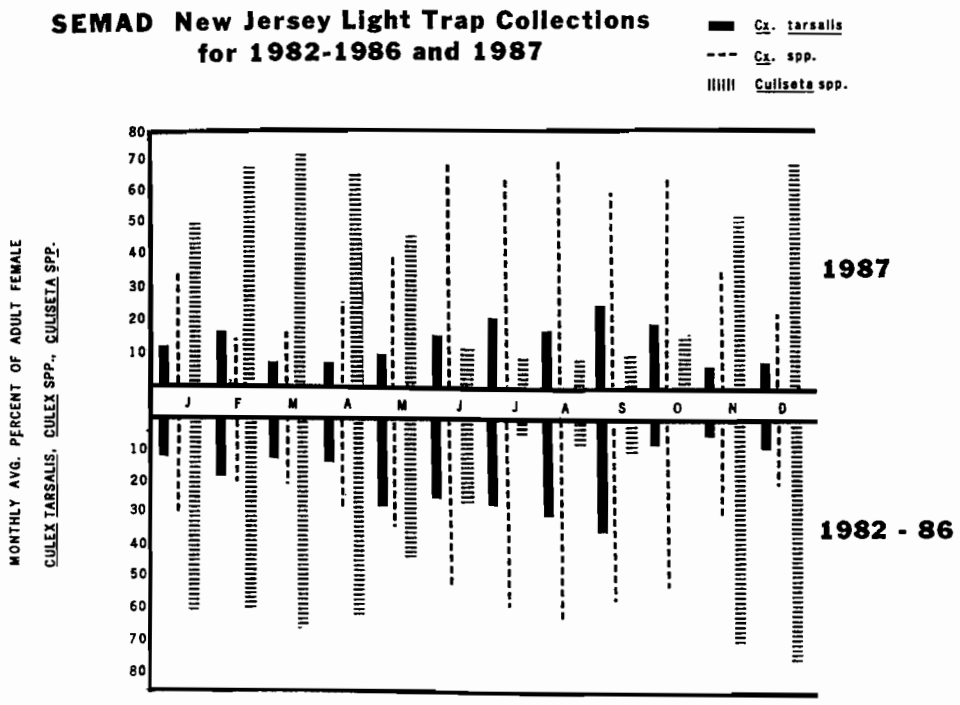

Figure 1.-Monthly average female *Culex tarsalis*, *Culex* spp., and *Culiseta incidens* New Jersey light trap collections from 1982 through 1986 and 1987, Southeast Mosquito Abatement District.

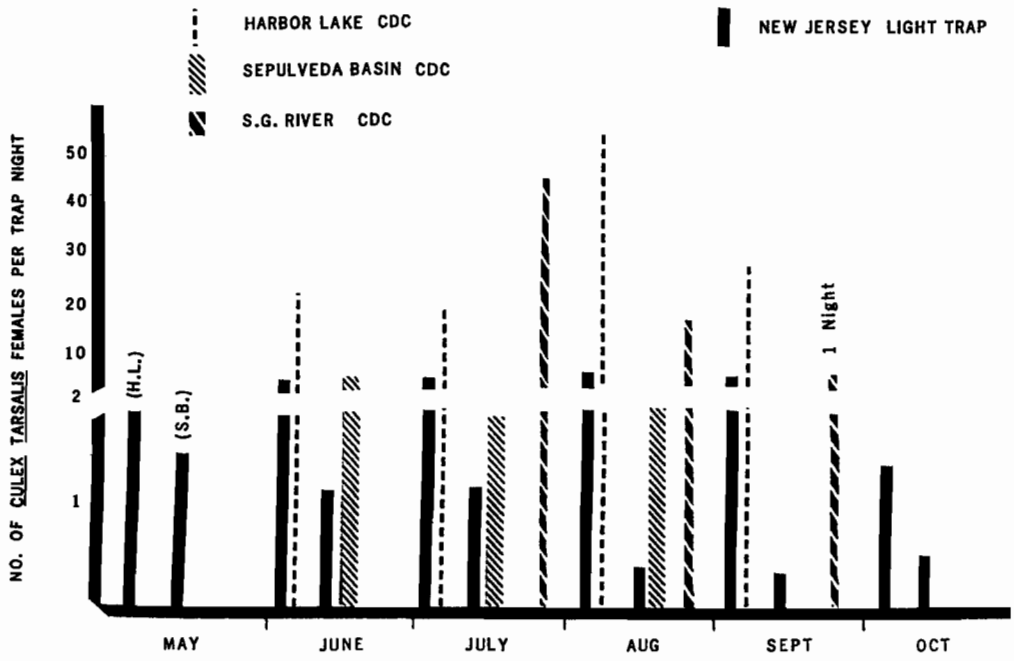

Figure 2.-The 1986 average female *Culex tarsalis* per trap night collected from New Jersey and CO_2-baited CDC light traps at Harbor Lake, Sepulveda Basin, and the San Gabriel River, Southeast Mosquito Abatement District.

mosquitoes and is slightly bimodal (Table 1). Compared to the other *Culex* species combined and captured year round, *Cx. tarsalis* populations are always proportionately lower (Fig. 1). *Cx. tarsalis* populations are least abundant from October through December (Table 1). They noticeably increase in January to moderate levels that remain steady through April. In May, another marked increase occurs and continues until October. This slight bimodality in Southern California populations of *Cx. tarsalis* is associated with the capability of this mosquito to colonize sources resulting from late winter and early spring rains. Additional staff is hired seasonally by the District to control the expected late spring and summer increases of both *Cx. tarsalis* and other *Culex* mosquitoes.

Evaluation of NJLT and CDC Trap Data from the Study Sites.

NJLT and CDC trap data for 1986 and 1987 at the three study sites were evaluated to describe *Cx. tarsalis* population trends and how they might relate to SLE activity (Figs. 2 and 3).

Harbor Lake.-Harbor Lake is a recreational lake and wildlife preserve maintained by the City of Los Angeles Recreation and Parks Department. Much of the lake is shallow, overgrown with emergent bulrush, and from early June to mid-October produces abundant populations of *Cx. tarsalis* and *Cx. erythrothorax* mosquitoes. Each year mosquito populations are not controlled at Harbor Lake because 1) chemical control is not feasible, and 2) various interest groups intervene to prevent clean-up and maintenance of the massive overgrowth of aquatic vegetation.

Except for the October 1987 data of approximately 2.6 females per trap night, the 1986 and 1987 NJLT data from Harbor Lake was similar to the usual District-wide bimodal trend for *Cx. tarsalis* (Figs. 2 and 3). Unseasonably warm temperatures and substantial rain in October 1987 promoted the unusually high early fall population. NJLT data showed that the 1986 summer population was greater and remained steadier than the 1987 population for the same period. Average monthly CDC populations for the summer did not

1987 New Jersey and CDC Light Trap Collections of Three SLE Active Study Sites

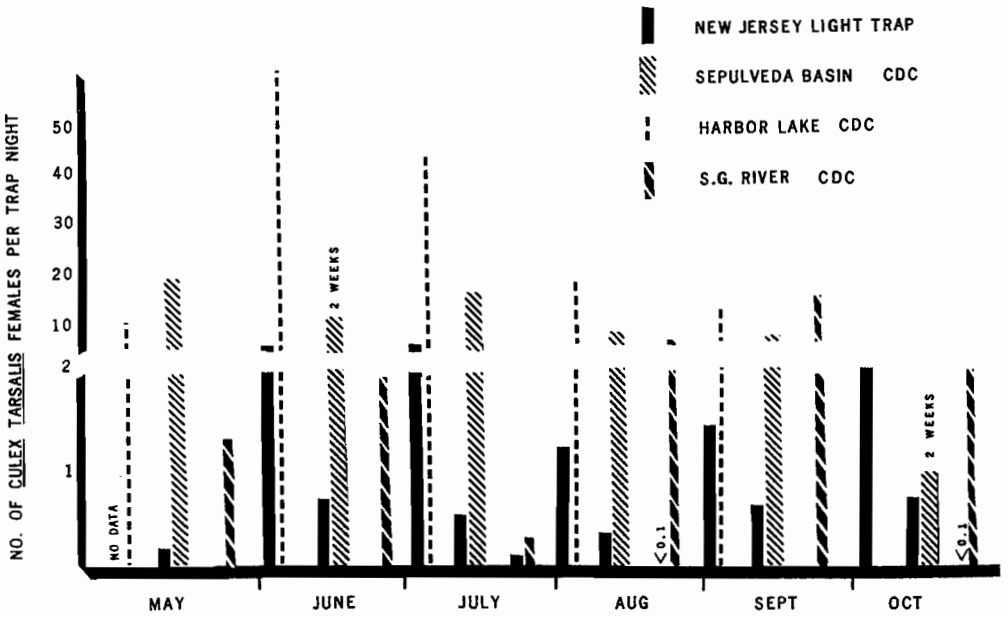

Figure 3.-The 1987 average female *Culex tarsalis* per trap night collected from New Jersey and CO_2-baited CDC light traps at Harbor Lake, Sepulveda Basin, and the San Gabriel River, Southeast Mosquito Abatement District.

fluctuate correspondingly to the NJLT data. The August-September 1986 populations were substantially higher than those of 1987 for the same period. On the other hand, the June-July CDC populations of 1987 were substantially higher than those of 1986 for the same period.

Sepulveda Basin.-The Sepulveda Basin lies almost centrally within the San Fernando Valley (Los Angeles City) and was modified by the Army Corps of Engineers as a flood control basin. This site contains marsh and riparian habitats that produce abundant populations of *Cx. tarsalis* from late spring to early fall. Unlike Harbor Lake, the District has been especially active in controlling mosquitoes in this area since 1985 because 4 human cases of SLE were contracted in 1984. The NJLT collections for *Cx. tarsalis* from both 1986 and 1987 reflect this control effort (Figs. 2 and 3). Bimodality is inapparent, and the increase in abundance expected in late spring through early fall occurred only in May, June, and July of 1986, and in June of 1987. The increased October population associated with Harbor Lake in 1987 also occurred in the Sepulveda Basin. However, the 1986 June-August and 1987 May-September CDC trap collections revealed that *Cx. tarsalis* may have been more numerous than revealed by the NJLT captures. The placement and number of traps used in each trapping method probably accounted for the observed differences. Eight to 14 fixed CDC trap sites were used to sample extensive sections of the Sepulveda Basin versus a single permanently placed NJLT. *Cx. tarsalis* control which may have been spotty throughout the Basin, may have been simply more thorough and effective near the NJLT.

In 1987, and since 1985, SLE virus isolations from mosquitoes and virus transmission confirmed from sentinel flock seroconversions have occurred in Sepulveda Basin. Despite the evidence of virus activity throughout these years, only in 1984 were human SLE cases suspected of being associated with the Basin.

Although a single virus isolate occurred in a relatively low *Cx. tarsalis* population at Sepulveda Basin on August 31, 1987 and was followed by a sentinel chicken seroconversion, a similar event

failed to occur in much greater *Cx. tarsalis* populations at Harbor Lake, where each year since 1984 mosquito isolates and sentinel seroconversions have occurred. The presumption was that the virus was not present at Harbor Lake or was present at a low and undetectable level. In general, it cannot be determined with any certainty based on the analysis of the general regional temperature data and CDC trap data from these two study sites why SLE transmission did occur at Sepulveda Basin and not at Harbor Lake.

San Gabriel River.-In July 1986, a single human case of SLE occurred in the city of Norwalk in the vicinity of the San Gabriel River, an area previously unmonitored by the District for adult mosquito activity. Intensive CDC trapping of the river revealed abundant *Cx. tarsalis* throughout most of the summer. Of 47 *Cx. tarsalis* mosquito pools collected and processed for virus presence, 17 or 36% were SLE positive, indicating a high rate of infection. Although undetermined, *Cx. tarsalis* was presumably abundant in June before monitoring began. Curiously, it could not be substantiated by larval sampling that the river was the primary mosquito breeding source. In spring, the river had been modified by the Los Angeles Flood Control District (LAFCD) to allow hundreds of acres of standing water to accumulate for ground water recharge. Early in 1987, a NJLT was established near the river and both a sentinel chicken and pigeon flock and crow trap were placed shortly afterward. In 1987, except for some intermittent upwelling and water run-off, the river remained dry. The 1987 NJLT and CDC data reflect this condition (Figs. 2 and 3). The NJLT data showed little to no *Cx. tarsalis* activity throughout the year, and CDC trapping revealed that *Cx. tarsalis* was only relatively numerous in September. No pools were positive throughout the monitoring period. No seroconversions occurred in the sentinel birds and peridomestic birds were not captured in the crow trap. Compared to 1986, water in the river in 1987 appeared insufficient to produce enough *Cx. tarsalis* to support or sustain SLE transmission.

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PRELIMINARY EVALUATION OF THE VECTOR COMPETENCE OF SOME SOUTHERN CALIFORNIA MOSQUITOES TO WESTERN EQUINE ENCEPHALOMYELITIS (WEE) AND ST. LOUIS ENCEPHALITIS (SLE) VIRUSES[1]

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Introduction.

Arbovirus surveillance in southern California between the years of 1977 and 1987 revealed that both western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses were isolated predominantly from pools of *Culex tarsalis* Coquillett (Table 1). SLE virus was isolated occasionally from pools of *Aedes dorsalis* (Meigen), *Culex erythrothorax* Dyar, *Culex peus* (Speiser) and *Culex quinquefasciatus* Say and WEE virus from pools of *Aedes vexans* (Meigen), *Cx. erythrothorax* and *Cx. quinquefasciatus*. Although *Cx. tarsalis* has been incriminated as the primary urban and rural vector of SLE and WEE viruses in California (Reeves and Hammon 1962), the fact that other species also have been found naturally infected with these viruses recently has generated considerable interest towards elucidating their possible role in the horizontal transmission cycle or the maintenance of viral endemicity via transovarial transmission.

Virus isolations from wild-caught females reveal only the presence of infected individuals and do not indicate their relative capacity to transmit virus by bite. The ability to transmit virus (i.e., vector competence) largely determines the extent to which a species can function as an effective vector in nature. Prior to this present investigation, SLE vector competence tests of southern California mosquitoes showed that *Cx. peus* transmitted SLE virus more efficiently than either *Cx. tarsalis* or *Cx. quinquefasciatus* (Hardy et al. 1985, 1986), and that *Cx. tarsalis* was overall a significantly more efficient vector of SLE than *Cx. quinquefasciatus* (Meyer et al. 1983).

In the present study, vector competence testing was expanded to include SLE and/or WEE evaluations of *Ae. vexans*, *Aedes taeniorhynchus* (Wiedemann), *Cx. erythrothorax* and *Psorophora confinnis* (Lynch-Arribalzaga). To date, SLE and WEE viruses have not been isolated from *Ae. taeniorhynchus* and there is only one recorded isolate of WEE from *Ps. confinnis* collected at Calexico, Imperial County, CA, in 1973 (Emmons et al. 1974). Therefore, it is unknown whether California populations of these species can transmit virus after feeding on infected mammals. This investigation summarizes the results of preliminary SLE and/or WEE vector competence tests of *Ae. vexans*, *Ae. taeniorhynchus*, and *Ps. confinnis*; and definitively assesses the SLE vector competence of *Cx. erythrothorax*.

Methods.

Virus Strains.-Mosquitoes were tested with the Kern County strains of SLE (BFS 1750) and WEE (BFS 1703) viruses isolated from separate pools of *Cx. tarsalis* collected in July 1953, and the Los Angeles County strain of SLE (SOUE 16-84) isolated from a pool of *Cx. tarsalis* collected at Harbor Lake in September 1984. Each strain had been passaged twice in suckling mice by intracranial inoculation. Viremogenic characterization tests have shown that the SOUE 16-84 strain of SLE is less virulent than the BFS 1750 strain in its capacity to produce viremias in chickens and mortality in suckling mice (Meyer et al. 1983, Hardy et al. 1985).

Mosquitoes.-All field strains were collected as host-seeking females with CO_2-baited traps, with the exception of *Ae. taeniorhychus* which was collected as mature larvae and pupae. The HP (high producer) laboratory strain of *Cx. tarsalis* was used as a control for each test with WEE virus and in one test with SLE virus. This strain had been se-

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Table 1.-Virus isolations from mosquitoes collected in Southern California, 1977-1987.*

Species	No. tested	No. pools	No. isolations (MIR)** WEE	SLE
		Los Angeles, Orange and San Diego Counties		
Aedes taeniorhynchus	1,586	31	0 (0.00)	0 (0.00)
Culex erythrothorax	34,689	883	0 (0.00)	0 (0.00)
Culex peus ***	19,102	573	1 (0.05)	2 (0.10)
Culex quinquefasciatus	80,895	2,002	0 (0.00)	5 (0.06)
Culex tarsalis	88,286	2,162	1 (0.01)	35 (0.40)
Culiseta incidens	3,672	106	0 (0.00)	0 (0.00)
Culiseta inornata	119	7	0 (0.00)	0 (0.00)
		Imperial, Riverside and San Bernardino Counties		
Aedes dorsalis	765	36	0 (0.00)	3 (3.92)
Aedes taeniorhynchus	84	4	0 (0.00)	0 (0.00)
Aedes vexans	2,709	70	3 (1.10)	0 (0.00)
Culex erythrothorax	6,572	179	5 (0.76)	3 (0.46)
Culex quinquefasciatus	34,498	820	8 (0.23)	0 (0.00)
Culex tarsalis	341,723	7,501	400 (1.17)	155 (0.45)
Culiseta inornata	1,841	95	0 (0.00)	0 (0.00)
Psorophora confinnis	4,754	111	0 (0.00)	0 (0.00)

* Data compiled from Emmons et al. (1978, 1979, 1980, 1981, 1982, 1983, 1984, 1985, 1986, 1987, and 1988).
** Minimum infection rate per 1,000 females tested.
***Includes pools from southwestern San Bernardino County (Chino and vicinity).

lected in the laboratory to be a highly competent vector of WEE virus (Hardy et al. 1983).

Infection of Mosquitoes.-Female mosquitoes were infected *per os* by feeding on pledgets soaked with sweetened (2.5% sucrose) defibrinated viremic chicken blood (SOUE 16-84, BFS 1750, and BFS 1703) or 10% mouse brain suspension of virus (BFS 1703) mixed in defibrinated whole rabbit blood. Females that fed to repletion were incubated at a constant 25°C for 14 days, and then tested for their ability to transmit virus using the *in vitro* capillary tube method of Aitken (1977). Females of *Cx. erythrothorax* collected at the San Joaquin Marsh and infected with the SOUE 16-84 and BFS 1750 strains of SLE virus were incubated for 18 days to assess the possibility that this species may require a longer period of extrinsic incubation to achieve infectivity. Females and saliva samples (capillary tube contents of *in vitro* transmission attempts) were tested for the presence of virus by *in situ* enzyme immunoassays (EIA) (Young-He et al. 1984, Graham et al. 1986).

Vector Competence Parameters.-Infectivity and transmission efficiency were expressed as an infection rate (% = No. infected / No. tested x 100) and a transmission index (% = No. transmitting / No. tested x 100), respectively (Meyer et al. 1986).

Results.

Virus dosages.-Female mosquitoes ingested concentrations of virus (concentration of virus present in the midgut of the mosquito is ca. Log_{10} 1.8 PFU (plaque forming unit) less than that of the pledget or host viremia) that were within the ranges of titers that would be acquired by feeding

Table 2.-Infection rates and transmission indices obtained for *Aedes taeniorhynchus*, *Aedes vexans* and *Psorophora confinnis* infected *per os* with either the SOUE 16-84 and/or BFS 1750 strain of SLE virus or BFS 1703 strain of WEE virus, 1986 - 1987.

Species	Date	Source	Virus Strain	Dosage * Log_{10} PFU	Number Tested	Infect. Rate%	Trans. Index %
Aedes taeniorhynchus			Ventura Co.				
	Sep 87	Pt. Mugu	SOUE 16-84	1.2	48	0	0
Aedes vexans		Riverside Co.					
	Sep 87	Thermal	SOUE 16-84	1.5	25	0	0
	Sep 87		BFS 1750	2.1	25	4	0
	Jul 86		BFS 1703	5.0**	48	33	10.4
Psorophora confinnis			Riverside Co.				
	Sep 87	Thermal	SOUE 16-84	1.8	45	0	0

* Concentration (0.01 ml) of virus in mosquito midgut.
**10% mouse brain suspension mixed with sweetened (2.5% sucrose) defibrinated whole rabbit blood.

on infected vertebrates in nature (Hardy 1987). SLE infections in doves and nestling house finches normally produce viremias in the range of Log_{10} 1.0-3.0 and Log_{10} 3.0-4.0 PFU, respectively. By comparison, WEE viremias in nestling house finches are much higher and are in the range of Log_{10} 6.0-9.0 PFU.

Vector competence tests with Ae. taeniorhynchus, Ae. vexans and Ps. confinnis (Table 2).-None of the females of *Ae. taeniorhynchus*, *Ae. vexans* or *Ps. confinnis* that were tested with the SOUE 16-84 strain of SLE virus became infected or transmitted virus after being incubated for 14 days at a constant temperature of 25°C. In a comparison test with the BFS 1750 strain of SLE, only 4.0% of the *Ae. vexans* tested became infected and none transmitted virus. The *Ae. vexans* population from Thermal also was tested with the BFS 1703 strain of WEE virus (10% mouse brain and rabbit blood) which produced a 33.0% infection rate and a 10.4% transmission rate of virus *in vitro*. However, in comparative test, the HP strain of *Cx. tarsalis* was more competent. More than 90.0% of the females tested became infected and 36.0% were capable of transmitting virus *in vitro* (Table 3).

Vector competence tests with Cx. erythrothorax, Cx. peus and Cx. tarsalis (Table 3).-All but 1 female (San Joaquin Marsh) of the 124 *Cx. erythrothorax* tested from 4 separate populations with the SOUE 16-84 strain of SLE virus failed to transmit virus after the standard extrinsic incubation period of 14 days at 25°C. Infection rates produced by the SOUE 16-84 strain ranged from only 5.0 to 12.0%. By comparison, the BFS 1750 strain produced a much higher infection rate (35%) in females of *Cx. erythrothorax* from the San Joaquin Marsh; however, none of those females transmitted virus *in vitro*. When the extrinsic incubation period was extended an additional 4 days to 18 days post-infection, the infection rate in the BFS 1750 group increased to 40%, and 12% of the females tested were able to transmit virus. The infection rate in the SOUE 16-84 group also was increased by 5.0%, but no females transmitted virus. In a single test with WEE virus (BFS 1703 strain), only 10% of the female *Cx. erythrothorax* collected at Blythe became infected after ingesting Log_{10} 6.9 PFU of virus in defibrinated viremic chicken blood. Of the 42 females tested, none transmitted virus *in vitro*. Conversely, 95% of the HP females tested concurrently became infected and 60.0% transmitted virus.

Table 3.-Infection rates and transmission indices obtained for *Culex erythrothorax*, *Culex peus* and *Culex tarsalis* infected *per os* with either the SOUE 16-84 and/or BFS 1750 strain of SLE virus or BFS 1703 strain of WEE virus, 1986 - 1987.

Species	Date	Source	Virus Strain	Dosage * Log_{10} PFU	Number Tested	Infect. Rate %	Trans. Index %
Culex erythrothorax		Los Angeles Co.					
	Jul 86	Harbor Lake	SOUE 16-84	1.5	25	12	0
		Orange Co.					
	May 86	San Joaq.Marsh	SOUE 16-84	2.4	20	5	5
				2.4	20	10**	0**
			BFS 1750	2.4	20	35	0
				2.4	20	40**	12**
		Riverside Co.					
	Jun 87	Blythe	SOUE 16-84	1.8	60	10	0
			BFS 1703	6.9***	42	10	0
	Jul 86	Prado Basin	SOUE 16-84	1.5	20	18	0
Culex peus		Riverside Co.					
	May 86	Prado Basin	SOUE 16-84	2.4	15	47	40
Culex tarsalis		Los Angeles Co.					
	Jul 86	Harbor Lake	SOUE 16-84	1.5	25	56	0
		Riverside Co.					
	May 86	Prado Basin	SOUE 16-84	2.4	10	100	70
	Jul 86	Prado Basin	SOUE 16-84	1.5	19	68	0
		HP laboratory					
	Sep 87	strain	SOUE 16-84	1.8	25	44	4
	Jun 87		BFS 1703	5.0+	22	91	36
	Sep 87		BFS 1703	6.9***	20	95	60

* Concentration (0.01 ml) of virus in mosquito midgut.
** Females incubated for 18 days post-infection.
***Defibrinated viremic chicken blood.
+ 10% mouse brain suspension mixed with sweetened (2.5% sucrose) defibrinated whole rabbit blood.

A population of *Cx. peus* from the Prado Basin was included as an on-line comparison in a single SLE (SOUE 16-84) test of *Cx. tarsalis* from the Prado Basin and *Cx. erythrothorax* from the San Joaquin Marsh. Results indicate that the *Cx. peus* population from the Prado Basin was less competent overall than conspecific populations tested previously from Orange and Los Angeles Counties (Hardy et al. 1985). Only 47.0 and 40.0% of the females tested became infected and transmitted virus, respectively.

Groups of female *Cx. tarsalis* tested from the Prado Basin in May and July with the SOUE 16-84 strain showed considerable variation in the infection rate and transmission index which appeared to be related to the concentration of virus ingested. Approximately 70% of the females that ingested Log_{10} 2.4 PFU of virus in the May test were able to transmit virus *in vitro*. When females representing that same field population were retested in July with a lower dosage of virus (Log_{10} 1.5 PFU), all females tested failed to transmit virus. Results were similar for the HP laboratory strain of *Cx. tarsalis* where a low transmission index (4.0%) also was obtained among females that ingested only Log_{10} 1.8 PFU of virus. The failure of HP and Prado Basin (July test) females to transmit the SOUE 16-84 strain of SLE efficiently after ingesting a concentration of virus comparable to the viremia produced in doves further demonstrated that nonvirulent strains of SLE are vectored poorly and/or that perhaps a relatively narrow threshold exists (i.e., < Log_{10} 0.50 PFU) in the concentration of virus ingested that is necessary to produce a disseminated infection and eventual infectivity.

Discussion.

Preliminary vector competence tests failed to demonstrate that *Ae. taeniorhynchus*, *Ae. vexans* and *Ps. confinnis* were experimentally capable of transmitting SLE virus. Females ingested titers of virus that would have resulted in a moderate to high transmission index (40-70%) in either *Cx. peus* or *Cx. tarsalis* (Hardy et al. 1985, 1986). Further testing of additional populations will be necessary before any definitive conclusions can be made concerning their relative status as horizontal vectors of SLE or to the possibility that these species may also be involved with maintaining viral endemicity via transovarial transmission.

During the spring of 1986, 2 isolates of SLE virus were obtained from pools of *Ae.dorsalis* collected at Bard, Imperial County, CA. Since SLE virus was not detected simultaneously in *Cx. tarsalis* tested from the same area, the early seasonal appearance of SLE in *Ae. dorsalis* suggested the possibility of transovarial transmission by that species. Therefore, we place a high priority on obtaining populations of *Ae. dorsalis* from the lower Colorado River (Bard Valley region) to evaluate the ability of this species to transmit SLE virus either horizontally by bite or vertically by transovarial transmission.

The data presented herein support our conclusion that *Cx. erythrothorax* is a poor vector of SLE virus in Southern California. Only 1 female in 124 tested was able to transmit the Harbor Lake strain (SOUE 16-84) of SLE virus. However, a slightly enhanced transmission index (12%) was achieved when females were tested with the more virulent Kern County strain (BFS 1750) of virus and incubated for 18 days. Therefore, some horizontal transmission may occur if females ingest virulent strains of virus in nature and are able to survive the duration of an apparently long period of extrinsic incubation. In the absence of ingesting virulent forms of SLE virus, it is our opinion that *Cx. erythrothorax* should pose no immediate threat to man in areas where this species is abundant and SLE virus has been isolated repetitively from *Cx. tarsalis*.

The failure of *Cx. erythrothorax* to transmit WEE virus in one test after ingesting a relatively high concentration of virus indicates that this species also is not a likely vector of WEE in Southern California, even though the virus has been isolated sporadically from field pools of females. Field isolates probably represent infected rather than infective individuals. Tests of at least 2 additional populations should provide more conclusive evidence to either substantiate or refute these initial findings.

The preliminary test of the *Ae. vexans* population from the Coachella Valley with the Kern County strain (BFS 1703) of WEE virus demonstrated that 10.4% of the females tested were capable of transmitting virus. By comparison, previous tests of field populations of *Cx. tarsalis* indicated that more than 70% of the females ingesting a comparable challenge of virus would have transmitted virus after an extrinsic incubation regimen of 14 days at 25°C. Therefore, it does not appear likely, from these initial findings, that *Ae. vexans* would function as an efficient secondary vector of WEE in the desert regions of Southern California. In addition, this species, as well as the other species of *Aedes* and *Psorophora* tested, feeds primarily on mammals which do not develop high viremia titers

in comparison to birds (i.e., Log_{10} 2.0-4.0 PFU in rabbits versus Log_{10} 6.0-9.0 in house finches) (Hardy 1987). A mammalian host-feeding pattern would make it less likely that many females would become infective after feeding on infected mammals rather than infected birds.

In summary, preliminary vector competence tests indicated that 1) *Ae. taeniorhynchus*, *Ae. vexans* and *Ps. confinnis* were unable to transmit SLE virus horizontally in the laboratory, 2) *Ae. vexans* from the Colorado Desert does not appear to be an efficient secondary vector of WEE, 3) *Cx. erythrothorax* cannot be considered a potential vector of SLE unless females ingest virulent strains of the virus and survive a long period of extrinsic incubation, and 4) *Cx. erythrothorax* failed to transmit WEE virus in a single test after ingesting a dosage of virus that produced a 70% transmission index in a highly competent laboratory strain of *Cx. tarsalis*.

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MOSQUITO ABUNDANCE AND VIRUS ACTIVITY IN SOUTHERN CALIFORNIA: SUMMARY REMARKS

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Data describing temporal and spatial trends in mosquito abundance and arbovirus activity are critical to developing effective surveillance and control programs and to incriminating vector species at which to direct these programs. For a mosquito species to be considered as an important vector of a horizontally transmitted arbovirus, it must 1) be abundant, 2) feed frequently on vertebrates which produce viremias of sufficient titer to infect most females, 3) be found infected frequently in nature, 4) be able to survive long enough to become infective, and 5) be able to transmit the acquired infection readily by bite. The collaborative research discussed today has addressed important aspects of incrimination including mosquito relative abundance, field infection rates and vector competence.

Species composition.-*Culex tarsalis* was the most abundant and widespread species collected in irrigated agricultural valleys along the Colorado River and in the Coachella Valley. *Cx. tarsalis* was focally abundant in the Los Angeles Basin where it appeared to be restricted to residual riparian and marsh habitats. Floodwater *Aedes* and *Psorophora* generally were not abundant during 1987 and were restricted almost entirely to agricultural sites which were flood irrigated, since the Colorado River flow remained low and channeled. *Culex quinquefasciatus* and, to a lesser extent *Cx. peus*, replaced *Cx. tarsalis* as the most abundant species collected at dairy and residential settings where breeding was restricted to polluted or peridomestic sources. *Culex erythrothorax* was only abundant at marshes supporting dense stands of cattails.

Seasonal abundance.-*Cx. tarsalis* abundance along the Colorado River and in the Coachella Valley was bimodal with peaks in spring (April-May) and late-summer/early-fall (August-October). Summer and winter declines in abundance were attributed to hot, desiccating and cold weather patterns. In contrast, mosquito populations in the greater Los Angeles area and the Chino area were most abundant during early summer (June) and again in fall (October). Mid-summer decreases in abundance probably reflected the impact of mosquito abatement activities which were greatest from June to September.

Virus activity.-Western equine encephalomyelitis (WEE) virus was active in the Palo Verde and Bard Valleys along the Colorado River and in the Coachella Valley with 62 isolations made between 14 January and 19 August 1987 and 37 of 182 sentinel chickens (20%) converting serologically to WEE positive. With the exception of a single isolation from *Cx. quinquefasciatus*, all WEE isolations were made from *Cx. tarsalis*, indicating that alternate transmission cycles may not have been important in the amplification of WEE virus during 1987. WEE activity detected during January and March along the north shore of the Salton Sea preceded increased activity in the Mecca (June) and Indio (August) areas. Increases in WEE activity were related directly to increases in *Cx. tarsalis* population abundance. Unfortunately, surveillance in the Imperial Valley was insufficient to determine if the increased WEE activity observed in the Coachella Valley was the result of a northward dissemination of more extensive activity in the Imperial Valley or represented enzootic amplification at an isolated focus along the north shore. We hope to investigate this situation next year by establishing early season virus monitoring at 3 sites in Imperial Valley in collaboration with the Imperial County Health Department. In 1987, WEE virus activity seemed to be restricted to these southern rural areas and did not enter the Prado or Los Angeles basins. The mechanisms which prevent the introduction and establishment of WEE virus in these basins remain cryptic, since the basic components of the transmission cycle appear to be present.

St. Louis encephalitis (SLE) virus was isolated on 3 occasions from Mecca in the Coachella Valley, Seeley in Imperial Valley and the Sepulveda Basin in Los Angeles County. The isolation of SLE virus from a pool of *Cx. tarsalis* collected at Mecca on 17 February 1987 and the seroconversion of 5 sentinel chickens at Bard were the only indication of SLE activity outside of the Los Angeles Basin. The seroconversions of 10 sentinel chickens occurred in the Los Angeles Basin from August through De-

cember at La Brea, Sepulveda Dam and El Dorado Park indicating a low, but widespread, level of SLE activity. The isolation of SLE virus from *Cx. tarsalis* in February and late season transmission activity in December detected by sentinel seroconversions speculatively suggested that SLE virus may remain active in its primary *Cx. tarsalis*-bird cycle throughout the winter in Southern California. Hopefully, continued winter sampling will support the importance of these preliminary findings and provide further indication of the endemicity of SLE virus in the Los Angeles area.

Vector competence.-Comparative vector competence studies are useful in interpreting field isolations of virus and indicating which species require further ecological study. *Cx. quinquefasciatus* previously has been shown to be an inefficient laboratory vector of both WEE and SLE viruses. The single field isolation of WEE from this species in 1987 appeared to represent a spillover from the basic *tarsalis*-bird cycle. Both WEE and Turlock (TUR) viruses were active at 10th Avenue in Blythe, a site supporting a large *Cx. erythrothorax* population. However, WEE or TUR virus infections were not detected in 6,513 *Cx. erythrothorax* females tested from this site or in 14,365 females tested from other sites in Southern California. In the laboratory, this species generally was refractory to infection and among those few females that became infected almost none (1/124) were capable of transmission.

Aedes taeniorhynchus and *Psorophora confinnis* are capable of experimentally transmitting parenterally acquired SLE virus transovarially to their progeny. However, both species were refractory to oral infection in vector competence studies, were collected infrequently in nature, and were not infected with arboviruses during 1987 with 1,431 and 5,691 specimens, respectively, tested from Southern California. *Aedes vexans* were refractory to SLE, but were moderately susceptible to infection with WEE with a small percentage capable of transmitting virus *in vitro*. However, *Ae. vexans* were not abundant during the May-July period of WEE activity and none of the 2,187 specimens tested were infected.

In conclusion, collaborative studies during 1986-1987 describing mosquito abundance, seasonality, infection rates and vector competence clearly support the concept that *Cx. tarsalis* was the primary vector of WEE and perhaps SLE viruses in southern California. Winter isolations and late season seroconversions by sentinel chickens and pigeons indicated that SLE virus may be capable of overwintering in the *Cx. tarsalis*-bird cycle. Further studies are planned to substantiate these findings and quantitate the rate of virus amplification and dissemination.

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EXPERIMENTAL INFECTION OF ROCK DOVES WITH ST. LOUIS ENCEPHALITIS VIRUS

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ABSTRACT

Viremia and antibody responses to St. Louis encephalitis (SLE) virus were measured in experimentally infected rock doves to determine their suitability to serve as sentinel hosts for the detection of SLE viral activity in an urban environment. Ten of 10 birds developed hemagglutination inhibiting antibodies to SLE virus after subcutaneous inoculation with approximately 1,000 plaque forming units of virus. Peak median antibody titers of 1:80 were attained at 14 days after infection and then median antibody titers decreased to 1:40 on days 21 and 28 and 1:10 on day 56 after infection. Three of the 10 infected birds developed low titered viremias on days 3 or 4 post-infection. These preliminary results indicate that sentinel rock doves would be sensitive indicators of SLE viral activity.

Introduction.

As part of the research to study the ecology of St. Louis encephalitis (SLE) virus in the Los Angeles Basin, rock doves (i.e., domestic pigeons) are being compared with chickens and wild birds as sentinel animals for the early detection of SLE viral activity (Gergis and Presser 1988). The rationale for this was based on knowledge that birds belonging to the family Columbiformes, which includes rock doves and mourning doves, frequently become involved in SLE viral transmission cycles in both rural and urban environments (Reeves & Hammon 1962, McLean & Bowen 1980). Further, laboratory studies have demonstrated that pigeons (Chamberlain et al. 1957) and mourning doves (J. Hardy, unpublished data) are susceptible to experimental infection with SLE virus. Finally, host preference studies carried out in the Central Valley of California indicated that *Culex* vectors of SLE virus feed frequently on columbiform birds (Reeves et al. 1963, Tempelis et al. 1965, Tempelis and Washino 1967).

After the sentinel rock doves were put out at sites in Orange County in mid-summer 1987, we began to detect what appeared to be immunological conversions to SLE virus in several birds when there was no other evidence of SLE viral activity in the area. However, some birds were seropositive by the neutralization test and negative by the hemagglutination inhibition (HI) test, and vice versa. Also, several seropositive birds became seronegative at the next monthly bleeding. This was in contrast to sentinel chickens that remain antibody positive once they seroconvert to SLE virus (LaMotte et al. 1967). Part of these problems were related to different methods being used to collect serum samples from sentinel rock doves. Nonetheless, it became apparent that we were unable to interpret the serologic results with certainty because we had no known SLE viral antibody positive rock dove serum to determine if the serologic reactions were associated with SLE virus-specific immunoglobulins. Furthermore, we had no knowledge of the magnitude and duration of SLE viral antibody responses in this avian species. Thus, it was necessary to undertake experimental infection studies with SLE virus in rock doves to sort out these ambiguities.

Materials and Methods.

Rock doves were purchased locally in Bakersfield, and as was the case with sentinel rock doves, the age of the birds was unknown. The SOUE 16-84 strain of SLE virus was used to infect birds. This SLE viral strain was isolated originally from a pool of *Culex tarsalis* Coquillett collected in September of 1984 at the Harbor Lake study site in Los Angeles County and had two intracranial passages in suckling mice.

All twelve birds were pre-bled. Each of 10 birds was then inoculated subcutaneously with approximately 1,000 PFU of virus. This is within the range of viral doses that mosquitoes would be expected to inject when feeding on a host (Hurbut 1966). The 10 inoculated and 2 uninoculated control birds were housed in the same cage and bled at selected times for viremia or antibody determinations. Blood samples for viremia tests were diluted

1:5 in viral diluent at the time of collection on post-infection days 1 thru 10, with 1 control and 5 inoculated birds each bled on alternate days starting on day 1 or day 2. Blood specimens were frozen at -70°C immediately after collection and subsequently thawed and titrated for virus by plaque assay in vero cell cultures. Bloods taken for serum samples were diluted 1:5 in physiological saline at the time of collection on post-infection days 7, 14, 21, 28 and 56. The sera were titrated for antibody by the HI test, using the BFS-1750 viral strain as the test antigen.

Results and Discussion.

All 10 of the inoculated rock doves became infected after subcutaneous inoculation with SLE virus, whereas neither of the 2 control birds housed in the same cage became infected. Thus, intracage transmission of SLE virus did not occur between rock doves, an essential prerequisite for animals being used as sentinel hosts for arbovirus surveillance.

Three of the 10 infected birds developed low titered viremias of only $10^{1.0}$ to $10^{2.0}$ plaque forming units /0.1 ml of blood on post-infection days 3 or 4. Similarly, Chamberlain et al. (1957) detected only trace amounts of SLE virus in the blood of two experimentally infected pigeons. However, even these low viremia levels are probably sufficient to infect some females of several *Culex* vector species. For example, Kramer et al. (manuscript in preparation) were able to infect 48% to 95% of the females from a highly competent laboratory strain of *Culex tarsalis* that fed on chickens circulating similar levels of SLE virus in their blood. Similar observations have been reported by Meyer et al. (1983) for *Culex quinquefasciatus* Say infected with SLE virus by feeding on viremic chickens.

HI antibody responses to SLE virus in rock doves are summarized in Table 1. Specific antibodies were first detected in all birds on day 14 post-infection when peak median titers of 1:80 occurred. Median titers then decreased to 1:40 on days 21 and 28 and to 1:10 on day 56 post-infection. This rapid decrease in SLE viral HI antibody titers also has been observed in ring-necked pheasants, tricolored blackbirds and house sparrows that were infected experimentally with another strain of SLE virus (J. L. Hardy, unpublished data). In contrast, SLE viral antibody titers remained at high levels for at least 3 months in mourning doves and house finches that were infected as part of the same experiment. Thus, the duration of the antibody responses in different species of adult wild birds appears to be quite variable following experimental infection with SLE virus. Nonetheless, results obtained in the present study indicated that serological conversions to SLE virus would be detectable in sentinel rock doves if they were bled at monthly intervals, as is the current practice in the encephalitis surveillance program in California.

We plan to rechallenge half of the infected rock doves in this study with SLE virus to determine if they develop high titered secondary antibody responses. If they do, then it may be possible to discern two sequential infections with SLE virus in a single sentinel rock dove as compared to only one in a sentinel chicken.

These preliminary studies indicated that rock doves possess some of the attributes one would desire in a sentinel animal to be used for detection of SLE viral activity in urban residential areas. These include: high susceptibility to parenteral infection, development of a detectable antibody response of suitable duration, and suitability for maintenance in

Table 1.-Antibody responses to SLE virus in ten experimentally infected rock doves.

Day after infection	Hemagglutination inhibition antibody titer	
	Median	Range
0	<1:10	-
7	<1:10	-
14	1:80	1:40 - 1:320
21	1:40	1:20 - 1:80
28	1:40	1:20 - 1:80
56	1:10	<1:10 - 1:20

backyards. Based on arboviral studies done in other areas, it is assumed that rock doves are attractive hosts of *Culex* vector species, but this remains to be demonstrated in our study areas in the Los Angeles Basin.

Acknowledgments.

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PRELIMINARY OBSERVATIONS OF THREE AVIAN SURVEILLANCE SYSTEMS FOR ST. LOUIS ENCEPHALITIS IN LOS ANGELES AND ORANGE COUNTIES, CALIFORNIA

Nasr N. Gergis[1] and Sally B. Presser[2]

Introduction.

Since St. Louis encephalitis (SLE) virus was first recognized as a disease of man in 1983, the occurrence of the disease in North America has been characterized by periodic outbreaks which punctuate years of minimal transmission (Tsai and Monath 1987, Ho and Hirsh 1985, Monath 1980). In 1984, an outbreak of SLE in Southern California resulted in sixteen serologically confirmed human cases with one fatality in Los Angeles County (LAC) and five cases in Orange County (OC) (LAC Vectorborne Disease Surveillance data, 1988). The prevention of human disease may be facilitated by the early detection of SLE virus activity; thus the development of sensitive surveillance systems is a primary concern for public health researchers.

The ability of birds to produce antibodies in response to infection with SLE virus is of interest, since an increase in antibody prevalence can be used to forecast increased activity of the virus. Serologic evidence of infection with SLE virus has been detected in species from 13 avian families (McLean and Bowen 1980).

Monitoring seroconversions in sentinel chickens previously unexposed to virus has been a useful indicator of SLE virus activity and conversion rates have been correlated with the occurrence of human cases (Bowen and Francy 1980). Sentinel chicken flocks continue to be used throughout California to detect the presence of SLE virus. New avian systems including sentinel pigeons and feral birds are being evaluated by the Los Angeles County Department of Health Services Vector Control Program, Southeast Mosquito Abatement District, Orange County Vector Control District and University of California Berkeley Arbovirus Research Unit (UCB-ARU).

The present paper summarizes SLE virus activity in sentinel chicken flocks in Los Angeles and Orange Counties for 1986 and 1987 and compares the utility and sensitivity of three avian sentinel systems that were used in 1987.

Materials and Methods.

Sentinel chicken flocks were maintained at various locations in LAC and OC (Table 1). Three avian systems were compared at four locations to detect the presence of SLE virus: sentinel chickens, sentinel pigeons, and feral birds. Flocks of 25 white leghorn chickens and 30 domestic pigeons were housed in chicken wire cages whereas feral birds were collected in Australian crow traps with the aperture of the inlet reduced to exclude large birds. Virus activity was monitored at riparian habitats: San Joaquin Marsh in Irvine, Orange County and Harbor Lakes in Wilmington, Los Angeles County, and suburban habitats: San Gabriel River in Norwalk, Los Angeles County, and Las Riendas Drive in Fullerton, Orange County.

Chicken flocks were bled once a month from 12 May to 15 December 1987, whereas pigeon flocks were deployed in June and bled monthly from August to December. During each bleeding session, 3.0-4.0 cc of blood were drawn from the jugular vein of each chicken using a vacutainer system. When this method was unsuccessful, a 3.0 cc syringe with a 22 gauge needle was used to obtain the sample from the wing vein. Blood samples from pigeons were taken from the wing vein; 0.4 cc of blood was diluted with 1.6 cc of physiological saline to increase the volume of the sample. Feral birds were trapped for one week prior to monthly sentinel flock bleeding dates in LAC and biweekly in OC. Birds were identified to species, sexed and banded prior to bleeding. When feral birds were bled, 0.2 cc of blood were drawn from the jugular vein and mixed with 0.8 cc of physiological saline.

Blood samples from pigeons and wild birds were transferred to vacutainers, held at room temperature for clot formation and then were transported to the laboratory in a cool (not frozen) ice chest. Blood samples were centrifuged at 2,000 RPM for approximately 10 minutes, and sera were collected and stored at -70°C. Chicken sera were tested by the California State Department of Health Services Viral and Rickettsial Disease Laboratory (VRDL) using an enzyme immunoassay (EIA) with positive results confirmed by an indirect

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Table 1.-Comparison of SLE virus antibodies in chicken sera in Los Angeles and Orange Counties, 1986-1987.

Locations	1986[1]	1987[2]
Los Angeles County		
Harbor Lakes, Wilmington	2/21[3]	0/25
San Gabriel River, Norwalk	No Flock	0/25
Horticulture Area, Cal Poly Univ., Pomona	2/19	0/20
Balboa Golf Company, Encino	2/18	1/16
El Dorado Park, Long Beach	0/22	2/7
La Brea Tar Pits, West Los Angeles	1/19	6/19
Total:	7/99=(7.1%)	9/112=(8.0%)
Orange County		
San Joaquin, Irvine	2/17	0/17
San Mateo Point, San Clemente	0/19	0/16
Featherly Park, Anaheim	0/17	No Flock
Fullerton	No Flock	0/24
Total:	2/53=(3.8%)	0/57=(0%)

[1]Bled through September except for flocks in Wilmington, Pomona and Encino which were bled through October.
[2]Bled through December.
[3]Number of positive birds/number of birds alive at the end of the year.

fluorescent antibody (IFA) test. The pigeon and feral bird sera were tested at the UCB-ARU by a hemagglutination inhibition (HI) test.

Results and Discussion.

A slight, but non-significant ($\chi^2=0.19$, df=1, $p \geq 0.05$), increase in SLE virus activity was observed in sentinel chickens in LAC in 1987 when compared to 1986 (Table 1). The higher activity in 1987 was attributed to the increased number of chickens that were bled during November and December, after surveillance activity normally is curtailed.

Although the prevalence of antibodies to SLE virus was greater in sentinel chickens, only one isolation of SLE virus was made from pools of *Culex tarsalis* in LAC (VRDL, 1987 unpublished). This represents the lowest number of isolations from mosquitoes in LAC and OC since 1984 (LAC Vectorborne Disease Surveillance data, 1987). Low SLE activity may have been due to drier ambient conditions that prevailed in 1987 as well as the absence of water discharged into the San Gabriel River.

No feral birds produced antibodies to SLE virus in LAC at the sites where the three different avian systems were located (Table 2). It is unclear whether this was consistent with the lower level of virus activity that was observed in the mosquito populations or that low numbers of specimens were tested. In contrast, positive serologies for SLE virus were observed for both pigeons and feral birds in OC (Table 2). An additional 26 seroconversions in feral birds were reported in OC at other locations (J. A. Gruwell, unpublished data). The detection of antibodies to SLE virus in sentinel pigeons and feral birds may indicate that these two systems are better able to detect low levels of virus activity than sentinel chickens, where no antibodies were observed.

Table 2.-A comparison of three avian systems at sites in Los Angeles and Orange Counties, 12 May-15 December 1987.

Locations	Chickens	Pigeons	Feral birds
Los Angeles County			
Harbor Lakes	0/25[1]	0/26	0/36[2]
Norwalk	0/25	0/24	0/0
Total:	0/50	0/50	0/36
Orange County			
Fullerton	0/24	3/30	2/133
San Joaquin Marsh	0/17	0/28	2/696
Total:	0/41	0/58	4/829

[1]Number of positive birds/number of birds alive at the end of the study.
[2]Number of positive birds/number of birds bled including recaptures.

Chicken flocks are the only sentinel system that have been used in LAC and OC in the past. However, because the locations where chickens may be placed are limited, their exposure to the virus has been limited to riparian/marsh habitats. This lessens their ability to serve as a sentinel animal, especially in suburban back yards where some SLE virus transmission may occur. Conversely, feral birds are dispersive and are not limited in geographical distribution. As a result, they may be a superior tool for SLE virus detection. The data presented here are preliminary, and further research will be necessary to assess the sensitivity and cost effectiveness of alternative systems to the sentinel chicken flock.

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PASSERIFORM BIRDS AS A SURVEILLANCE METHOD FOR ARBOVIRUS ACTIVITY IN RURAL AND SUBURBAN SITES IN ORANGE COUNTY, CALIFORNIA, 1987

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ABSTRACT

A year-long study to determine the prevalence of St. Louis and Western equine encephalitis arboviruses in the peridomestic and rural wild bird populations of Orange County produced 5,555 samples from 37 species for laboratory testing. Thirty-five sera samples (0.63%) were positive for SLE antibodies and 5 samples (0.09%) for WEE antibodies. Peak virus activity occurred in December, when 3.27% of all birds sampled tested positive. The data indicated that virus transmission seemed to be occurring year-round, albeit at a very low level.

Introduction.

Wild birds have been known to be hosts to St. Louis encephalitis (SLE) and Western equine encephalitis (WEE) viruses since the 1940s and human cases of WEE and SLE have been identified in California since the 1950s. Orange County remained free of recorded human cases until 1983-84, when confirmed diagnoses of SLE in humans were documented in Los Angeles, Orange, and San Diego Counties (Murray et al. 1986). Testing of wild birds for arboviral antibodies in Orange County had been sporadic from 1984 (Table 1) until 1987 when a full-time position was created by the Orange County Vector Control District (OCVCD) to monitor the prevalence of avian arboviral antibodies in the County. This study was also designed to determine which bird species would be the most suitable for future surveillance efforts (McLean et al. 1986, unpublished data).

Materials and Methods.

Birds were collected for blood sampling using modified crow traps, mist nets, and by shooting. The crow traps proved to be the most efficient method, not only in number of birds captured, but also in providing recaptured specimens for resampling.

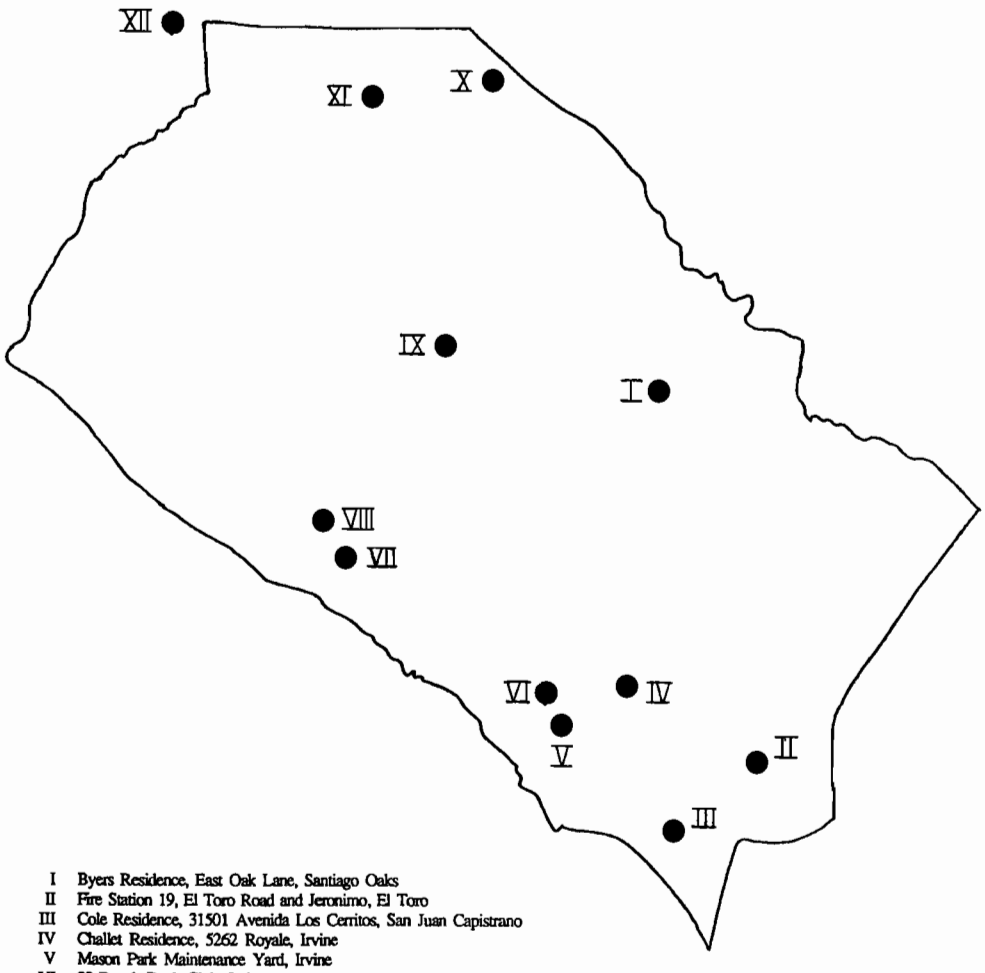
Crow Traps - 11,243 birds (6,291 recaptures)
Shooting - 568 birds
Mist Nets - 265 birds

A total of 0.1 cc of blood was removed via jugular puncture and placed in 0.9 cc of bovine diluent. Hemagglutination inhibition (HAI) tests were run by the laboratories at the University of California, Los Angeles (UCLA), Orange County Public Health Department (OCPHD), and University of California, Berkeley (UCB). The HAI positive sera will be sent to UCB for confirmation testing.

The crow traps were 6′ x 4′ x 6′ (including a variable entrance gap from 2" to 5") and constructed from wood and hardware cloth. Through trial and error, 12 traps were finally placed in relatively secure sites, which included 6 residential back

Table 1.-History of wild bird sera testing for St. Louis encephalitis viral antibodies in Orange County.

Year	Agency	No. of Months	Samples	% SLE Positive
1984	UCLA/OCVCD	Oct-Nov	100	20.00
1985	OCVCD	Aug-Sep	225	10.00
1986	CDC/OCVCD	Aug-Sep	386	2.00
1987	OCVCD	Feb-Dec	5,555	0.63



I Byers Residence, East Oak Lane, Santiago Oaks
II Fire Station 19, El Toro Road and Jeronimo, El Toro
III Cole Residence, 31501 Avenida Los Cerritos, San Juan Capistrano
IV Challet Residence, 5262 Royale, Irvine
V Mason Park Maintenance Yard, Irvine
VI 20 Ranch Duck Club, Irvine
VII Pett Residence, 608 Jana Circle, Huntington Beach
VIII Central Park Maintenance Yard, Huntington Beach
IX Modjeska Park Maintenance Yard, Anaheim
X Carbon Canyon Region Park Maintenance Yard, Brea
XI Fine Residence, 337 Las Riendas, Fullerton
XII Miller Residence, 11575 Rincon, Whitter, Los Angeles County

Figure 1.-Crow trap sites, Orange County, California, 1987.

yards, 4 parks, 1 fire station, and 1 duck club (Figure 1). Each trap was visited twice a week when birds were marked using different colored (unique to each trap site) 20-gauge wire bands, bled, and released. Food and water were replenished in the trap at the same time. During the last week of each month, all birds in all traps were bled. Major problems included vandalism and ground squirrels (*Spermophilus beecheyi*) and shrikes (*Lanius ludovicianus*) entering the traps and either carrying off all the grain or killing the captured birds.

Mist nets were set in both the park settings and at riparian and stream bed sites. Blood samples were taken using the same methodology as the crow traps. Compared to the capture rate of crow traps, mist netting was more work for the number of birds sampled and in the future will be used only in an area considered to be an active virus focus.

Shooting was conducted at county landfills (Bonita Canyon, Brea, and Santiago) for rock doves, gulls, crows, and ravens and on the Irvine Ranch (mourning doves). Samples were taken by cardiac puncture and processed as usual.

Results.

The most frequently captured bird was the house finch (*Carpodacus mexicanus*) at 40.04% of total samples, 41.36% of recaptures, 48.32% of total resamples, and 40.74% of all live captures. The house sparrow (*Passer domesticus*) was the next most frequent at 29.81% of total samples, 35.99%

Table 2.-Summary of bird blood specimen collection, February-December, 1987.

	Number Samples	Percent Total Samples	Number Recaptures	Percent Total Recaptures	Number Resamples	Percent Total Resamples	Number Live Captures	Percent Ttl Live Captures
House Finch *(Carpodacus mexicanus)*	2,224	40.04	2,602	41.36	488	48.32	4,826	40.74
House Sparrow *(Passer domesticus)*	1,656	29.81	2,264	35.99	348	34.46	3,920	33.09
Rock Dove *(Columba livia)*	383	6.89	-	-	-	-	383	3.23
Mourning Dove *(Zenaidura macroura)*	204	3.67	17	0.27	2	0.20	221	1.87
Ground Dove *(Columbigallina passerina)*	28	0.50	1	0.02	-	-	29	0.25
Spotted Dove *(Streptopelia chinensis)*	1	0.02	-	-	-	-	1	0.01
Ringed Turtle Dove *(Streptopelia risoria)*	1	0.02	-	-	-	-	1	0.01
White-Crowned Sparrow *(Zonotrichia leucophrys)*	297	5.35	674	10.71	71	7.03	971	8.20
Song Sparrow *(Melospiza melodia)*	153	2.75	191	3.04	39	3.86	344	2.90
Golden-Crowned Sparrow *(Zonotrichia atricapilla)*	6	0.10	-	-	-	-	6	0.05
Swamp Sparrow *(Melospiza georgiana)*	1	0.02	-	-	-	-	1	0.01
Lark Sparrow *(Chondestes grammacus)*	1	0.02	-	-	-	-	1	0.01
Red-Winged Blackbird *(Agelaius phoeniceus)*	261	4.70	236	3.75	21	2.08	497	4.20
Brewer's Blackbird *(Euphagus cyanocephalus)*	40	0.72	-	-	-	-	40	0.34
Brown-Headed Cowbird *(Molothrus ater)*	54	0.97	123	1.96	16	1.58	177	1.49
Common Crow *(Corvus brachyrhynchos)*	12	0.22	-	-	-	-	12	0.10
Common Raven *(Corvus corax)*	10	0.18	-	-	-	-	10	0.08
Say's Phoebe *(Sayornis saya)*	83	1.49	174	2.77	25	2.48	257	2.17
Black Phoebe *(Sayornis nigricans)*	11	0.20	-	-	-	-	11	0.09
Western Kingbird *(Tyrannus verticalis)*	1	0.02	-	-	-	-	1	0.01
California Gull *(Larus californicus)*	21	0.38	-	-	-	-	21	0.18
Starling *(Sturnus vulgaris)*	28	0.50	-	-	-	-	28	0.24
Mockingbird *(Mimus polyglottos)*	18	0.32	-	-	-	-	18	0.15
Scrub Jay *(Aphelocoma coerulescens)*	9	0.16	-	-	-	-	9	0.08
Cliff Swallow *(Hirundo pyrrhonota)*	7	0.13	-	-	-	-	7	0.06
Bullock's Oriole *(Icterus bullockii)*	7	0.13	-	-	-	-	7	0.06
Western Meadowlark *(Sturnella neglecta)*	5	0.09	-	-	-	-	5	0.04
Yellowthroat *(Geothlypis trichas)*	6	0.11	-	-	-	-	6	0.05
Audubon's Warbler *(Dendroica auduboni)*	3	0.05	-	-	-	-	3	0.03
Loggerhead Shrike *(Lanius ludovicianus)*	4	0.07	-	-	-	-	4	0.03
Yellow Warbler *(Dendroica petechia)*	3	0.05	-	-	-	-	3	0.03
Common Bushtit *(Psaltriparus minimus)*	3	0.05	-	-	-	-	3	0.03
Brown Towhee *(Pipilo fuscus)*	1	0.02	-	-	-	-	1	0.01
Anna's Hummingbird *(Calypte anna)*	1	0.02	-	-	-	-	1	0.01
Black-Headed Grosbeak *(Pheucticus melanocephalus)*	9	0.16	-	-	-	-	9	0.08
Short-Billed Marsh Wren *(Cistothorus platensis)*	1	0.02	-	-	-	-	1	0.01
California Quail *(Lophortyx californicus)*	2	0.04	-	-	-	-	-	0.02
TOTALS	5,555	100.00	6,291	100.00	1,010	100.00	11,846	100.00

of total recaptures, 34.46% of total resamples, and 33.09% of all live captures (Tables 2-6). It has been suggested (Hardy 1976, McLean et al. 1986) that these two species (Figure 2) along with rock and mourning doves are probably the best indicator of virus activity and this seemed to be the case in Orange County (Table 7). Positive birds were found throughout the County (Figure 3, Tables 8-9) with a majority, 27 (67.5%) collected from the City of Irvine and Irvine Ranch, south to San Juan Capistrano.

Peak virus activity was seen in July (0.85% of total birds positive), September (1.31%), and December (3.27%) (Figure 4). The relatively high rate in December seemed to indicate winter transmission of SLE virus because 4 birds (2 white-crowned sparrows, 1 house finch, and 1 Say's phoebe) that had been previously recaptured, resampled, and tested negative for virus yielded positive SLE results. The white-crowned sparrows are winter visitors that arrive in late September and leave around the first of May.

Discussion.

House finches and house sparrows are probably the best avian hosts upon which to concentrate for antibody sampling. These two species were the most numerous (69.85% of all samples, 77.35% of all recaptures, 82.78% of all resamples), the most easily handled, and quickly became habituated to the crow traps as a food source. The only drawback to the system of sampling was the inability to identify individual birds. All birds were banded with colored wire, a different color for each trap site. This enables the collector to catch and sample only unmarked birds during the balance of each month while releasing the others. The last week of every month, all birds, marked and unmarked, are sampled. A system of individual numbered bands would be more efficacious, but due to constraints of time and personnel and the large number of specimens involved, was virtually impossible at this point in the study.

In February, March, and the first part of April, 83 house finches were marked at the Pett

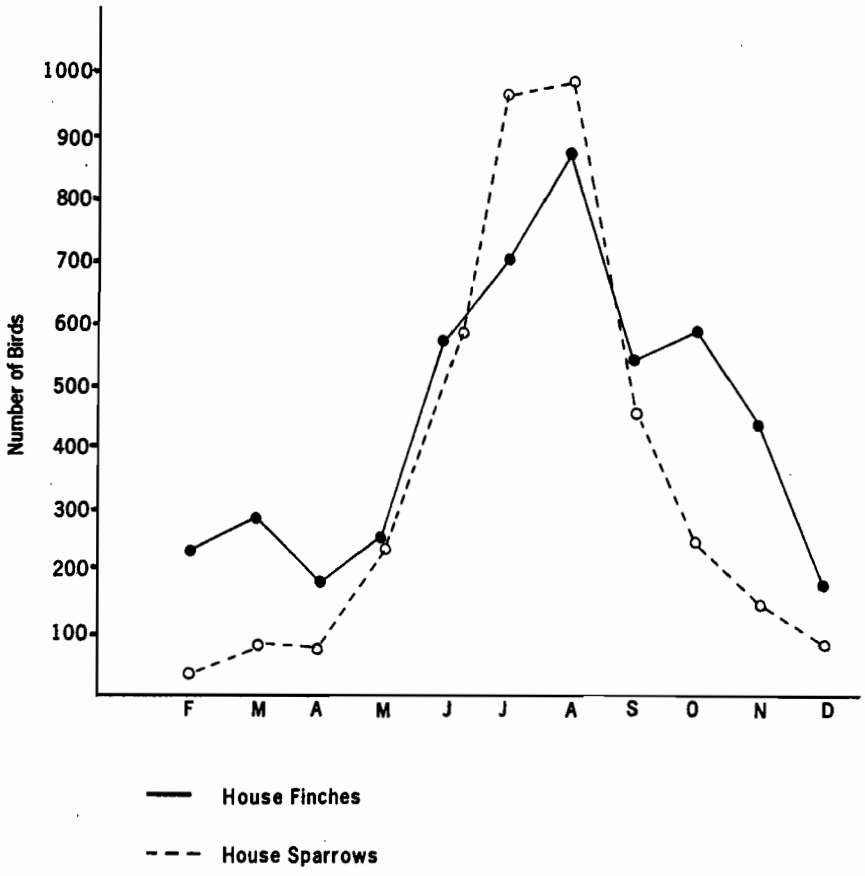


Figure 2.-Monthly live captures of house finches and house sparrows, 1987.

Table 3.-Summary of bird blood samples by month, 1987.

Common Name	Scientific Name	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% Total Samples
House Finch	*Carpodacus mexicanus*	116	133	118	144	364	440	373	149	179	131	77	2,224	40.04
House Sparrow	*Passer domesticus*	26	47	38	126	296	472	357	112	90	45	47	1,656	29.81
Rock Dove	*Columba livia*	1	-	6	-	59	107	109	101	-	-	-	383	6.89
Mourning Dove	*Zenaidura macroura*	-	-	-	-	-	33	71	58	11	16	15	204	3.67
Ground Dove	*Columbigallina passerina*	-	-	-	-	2	9	9	2	-	1	5	28	0.51
Spotted Dove	*Streptopelia chinensis*	-	-	-	-	1	-	-	-	-	-	-	1	0.02
Ringed Turtle Dove	*Streptopelia risoria*	-	-	-	-	-	1	-	-	-	-	-	1	0.02
White-Crowned Sparrow	*Zonotrichia leucophrys*	20	14	4	-	-	-	-	3	47	103	106	297	5.35
Song Sparrow	*Melospiza melodia*	1	6	14	15	15	29	32	18	11	7	5	153	2.75
Golden-Crowned Sparrow	*Zonotrichia atricapilla*	4	1	-	-	-	-	-	-	1	-	-	6	0.10
Swamp Sparrow	*Melospiza georgiana*	-	-	-	-	-	-	-	1	-	-	-	1	0.02
Lark Sparrow	*Chondestes grammacus*	-	-	-	-	-	-	-	1	-	-	-	1	0.02
Red-Winged Blackbird	*Agelaius phoeniceus*	-	-	-	-	35	3	-	-	61	31	131	261	4.70
Brewer's Blackbird	*Euphagus cyanocephalus*	-	-	1	13	20	5	-	-	1	-	-	40	0.72
Brown-Headed Cowbird	*Molothrus ater*	-	-	2	-	27	22	1	-	1	1	-	54	0.97
Common Crow	*Corvus brachyrhynchos*	-	-	-	-	5	4	3	-	-	-	-	12	0.22
Common Raven	*Corvus corax*	-	-	-	-	1	4	5	-	-	-	-	10	0.18
Say's Phoebe	*Sayornis saya*	6	3	2	5	6	22	12	9	4	3	11	83	1.49
Black Phoebe	*Sayornis nigricans*	-	-	-	-	1	6	2	2	-	-	-	11	0.20
Western Kingbird	*Tyrannus verticalis*	-	-	-	-	-	1	-	-	-	-	-	1	0.02
California Gull	*Larus californicus*	-	-	21	-	-	-	-	-	-	-	-	21	0.38
Starling	*Sturnus vulgaris*	-	-	-	5	10	11	2	-	-	-	-	28	0.50
Mockingbird	*Mimus polyglottos*	2	-	1	-	10	2	2	1	-	-	-	18	0.32
Scrub Jay	*Aphelocoma coerulescens*	2	1	-	4	-	2	-	-	-	-	-	9	0.16
Cliff Swallow	*Hirundo pyrrhonota*	-	-	-	7	-	-	-	-	-	-	-	7	0.13
Bullock's Oriole	*Icterus bullockii*	-	-	2	-	2	1	1	-	-	1	-	7	0.13
Western Meadowlark	*Sturnella neglecta*	-	-	-	-	4	-	-	1	-	-	-	5	0.09
Yellowthroat	*Geothlypis trichas*	-	-	-	4	-	1	1	-	-	-	-	6	0.11
Audubon's Warbler	*Dendroica auduboni*	3	-	-	-	-	-	-	-	-	-	-	3	0.05
Loggerhead Shrike	*Lanius ludovicianus*	-	-	-	1	2	-	1	-	-	-	-	4	0.07
Yellow Warbler	*Dendroica petechia*	-	-	-	2	-	1	-	-	-	-	-	3	0.05
Common Bushtit	*Psaltriparus minimus*	2	-	-	-	-	1	-	-	-	-	-	3	0.05
Brown Towhee	*Pipilo fuscus*	1	-	-	-	-	-	-	-	-	-	-	1	0.02
Anna's Hummingbird	*Calypte anna*	1	-	-	-	-	-	-	-	-	-	-	1	0.02
Black-Headed Grosbeak	*Pheucticus malanocephalus*	-	-	-	-	-	-	9	-	-	-	-	9	0.16
Short-Billed Wren	*Cistothorus platensis*	-	-	-	-	-	-	1	-	-	-	-	1	0.02
California Quail	*Lophortyx californicus*	-	-	-	-	-	-	2	-	-	-	-	2	0.04
	TOTAL ALL BIRDS	185	205	209	326	860	1,177	993	459	406	339	397	5,555	100.00%

residence crow trap in Huntington Beach. Thirty-six of these birds (43%) were recaptured during the year in other crow traps throughout the County (Figure 5). The potential for dispersion of virus via house finches (up to 40 miles in this case) has been dramatically demonstrated by this study and follow-up studies are strongly encouraged.

This project was intended not only to provide more data on the role of wild birds in the overall SLE virus cycle but also to serve as an early warning/surveillance system (i.e., detect the virus in avian hosts before it becomes prevalent in peridomestic mosquito populations and implement appropriate and timely control measures, thus, reducing the risk of virus transmission to humans). The Orange County Public Health Laboratory (OCPH) will be performing the serological testing in 1988 using HAI tests and results will be available 3-4 days after collection.

The sampling of peridomestic birds does not stand alone in the research into SLE and WEE cycles in southern California. Data gleaned from this work must be correlated with ongoing studies of mosquito populations, sentinel chicken and pigeon flocks, human epidemiological data, etc. In summary, it seems that SLE viral antibody exists at a very low level in the wild bird population of the County almost year-round, even though sentinel chicken and pigeon flocks showed minimal activity and no positive mosquito pools were detected. Several more years of data are needed to determine the role of wild birds in the virus cycle in Orange County.

In addition, it can be said that 1987 provided not only a wealth of data, but also a chance to learn how and where to place the crow traps in order to maximize capture and recapture levels of what appear to be "target" birds (house finches and

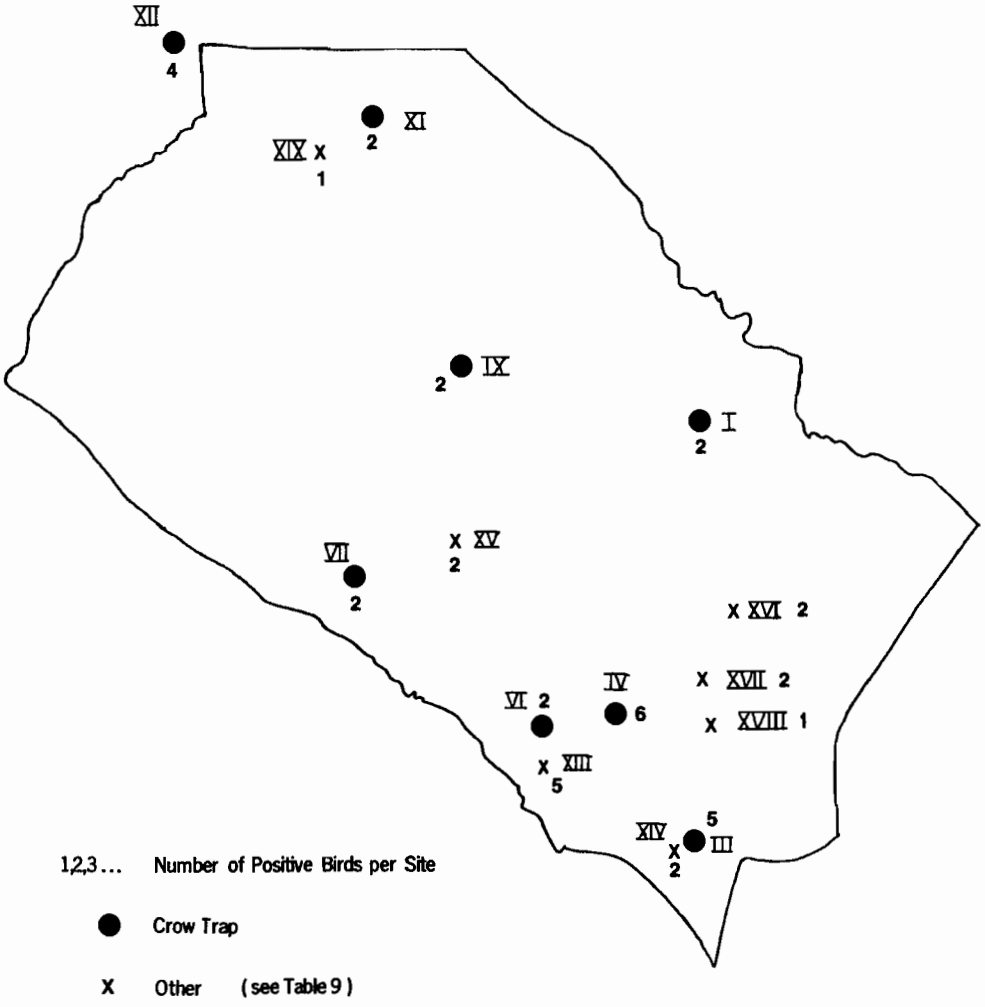


Figure 3.-Localities positive for SLE and WEE in Orange County, California, 1987 (see Table 9).

Table 4.-Summary of bird recaptures by month, 1987.

Common Name	Scientific Name	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% Total Samples
House Finch	*Carpodacus mexicanus*	109	164	72	114	207	260	500	379	399	297	101	2,602	41.36
House Sparrow	*Passer domesticus*	-	45	51	98	300	487	611	335	187	102	48	2,264	35.99
White-Crowned Sparrow	*Zonotrichia leucophrys*	24	25	2	-	-	-	-	-	81	171	371	674	10.71
Song Sparrow	*Melospiza melodia*	-	3	2	7	40	38	21	31	22	17	10	191	3.04
Say's Phoebe	*Sayornis saya*	3	3	-	11	15	26	30	23	21	15	27	174	2.77
Brown-Headed Cowbird	*Molothrus ater*	10	7	2	-	56	47	1	-	-	-	-	123	1.96
Red-Winged Blackbird	*Agelaius phoeniceus*	-	-	-	-	2	2	-	-	27	45	160	236	3.75
Mourning Dove	*Zenaidura macroura*	-	-	-	-	-	-	1	2	5	6	3	17	0.27
Ground Dove	*Columbigallina passerina*	-	-	-	-	-	-	-	-	-	-	1	1	0.02
Others													9	0.13
	TOTAL	146	247	129	230	620	860	1,164	770	742	653	721	6,291	100.00%

Table 5.-Summary of recaptured birds that were resampled each month, 1987.

Common Name	Scientific Name	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% Total Samples
House Finch	*Carpodacus mexicanus*	-	46	22	46	49	57	87	49	54	51	27	488	48.32
House Sparrow	*Passer domesticus*	-	10	6	17	58	84	75	38	37	16	7	348	34.46
White-Crowned Sparrow	*Zonotrichia leucophrys*	-	4	-	-	-	-	-	-	1	28	38	71	7.03
Song Sparrow	*Melospiza melodia*	-	-	1	3	-	16	4	7	5	1	2	39	3.86
Say's Phoebe	*Sayornis saya*	-	-	-	-	2	6	4	4	3	1	5	25	2.48
Brown-Headed Cowbird	*Molothrus ater*	-	-	-	-	14	2	-	-	-	-	-	16	1.58
Red-Winged Blackbird	*Agelaius phoeniceus*	-	-	-	-	2	-	-	-	-	2	17	21	2.08
Mourning Dove	*Zenaidura macroura*	-	-	-	-	-	-	-	-	-	2	-	2	0.20
	TOTAL	-	60	29	66	125	165	170	98	100	101	96	1,010	100.00%
	Dead in Trap	33	12	10	15	18	47	36	27	27	17	38	280	100.00%

house sparrows). Members of the family Columbidae (rock dove, mourning dove, ground dove), although difficult to collect in numbers, seem to be good virus reservoirs and will also serve as a "target" in 1988 and beyond. The significance of very low levels of both SLE and WEE viruses in the County during 1987 remains to be elucidated through continuing research during the next several years.

Acknowledgments.

The authors acknowledge the assistance given by Drs. Telford Work and Martine Jozan and Mike Medina of UCLA (currently at Coachella Valley MAD, Thermal); Dr. Rick Greenwood and Rick Alexander and Paul Stanford of OCPH; Dr. James L. Hardy, Sally B. Pressser, and Marilyn M. Milby of U. C. Berkeley; the entire staff of OCVCD, especially Gilbert Challet, Steve Bennett, Ronald Elliott, John Pett, and Steve Nippert.

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Table 6.-Summary total live captures by month (Recaptured species only).

Common Name	Scientific Name	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% Total Samples
House Finch	*Carpodacus mexicanus*	225	297	190	258	571	700	873	528	578	428	178	4,826	40.74
House Sparrow	*Passer domesticus*	26	92	89	224	596	959	968	447	277	147	95	3,920	33.09
White-Crowned Sparrow	*Zonotrichia leucophrys*	44	39	6	-	-	-	-	3	128	274	477	971	8.20
Song Sparrow	*Melospiza melodia*	1	9	16	22	55	67	53	49	33	24	15	344	2.90
Say's Phoebe	*Sayornis saya*	9	6	2	16	21	48	42	32	25	18	38	257	2.17
Brown-Headed Cowbird	*Molothrus ater*	10	7	4	-	82	69	3	-	1	1	-	177	1.49
Red-Winged Blackbird	*Agelaius phoeniceus*	-	-	-	-	37	5	-	-	88	76	291	497	4.20
Mourning Dove	*Zenaidura macroura*	-	-	-	-	-	33	72	60	16	22	18	221	1.87
Ground Dove	*Columbigallina passerina*	-	-	-	-	2	9	9	2	-	1	6	29	0.25
Others													604	5.09
	TOTALS												11,846	100.0%

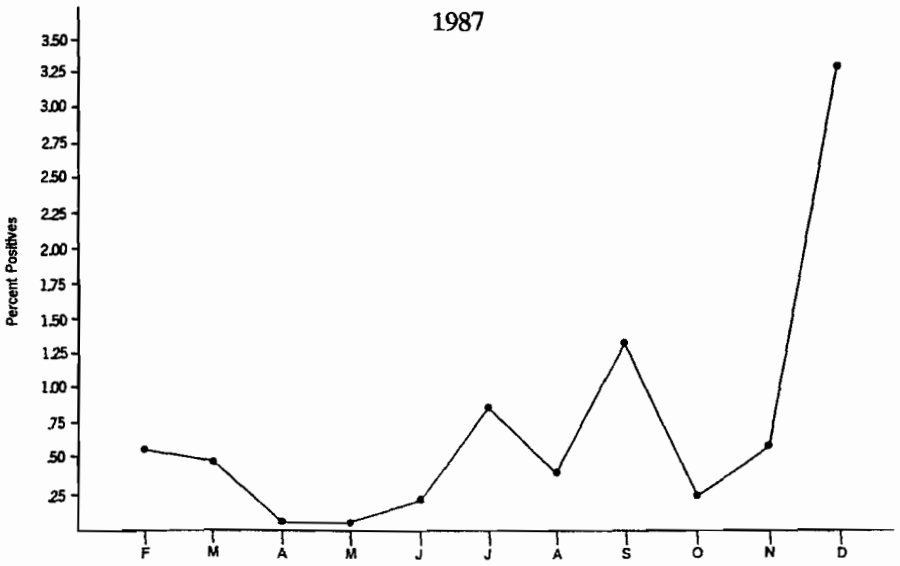

Figure 4.-Percent SLE positive small birds per month, 1987.

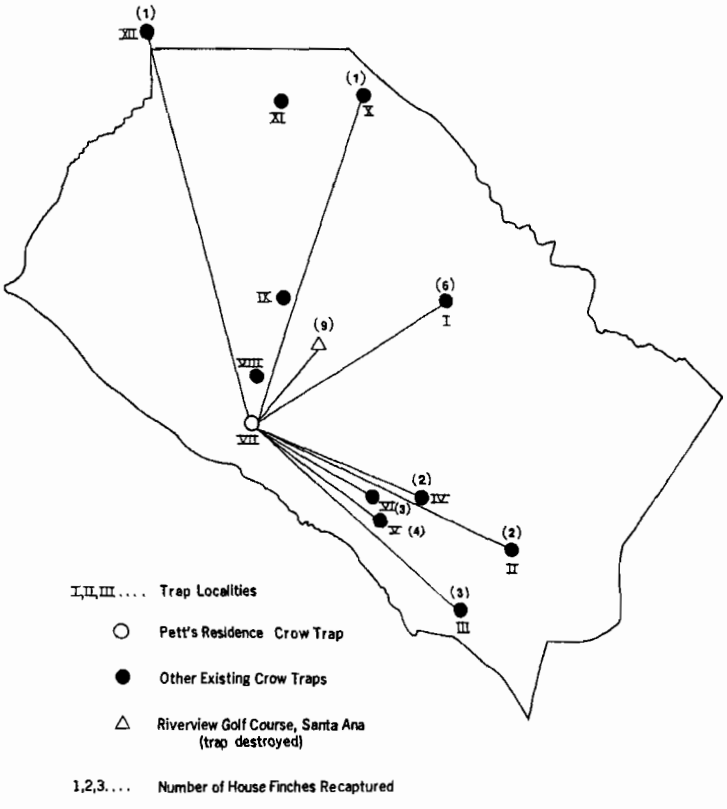

Figure 5.-Dispersal of house finches trapped and marked at Pett's Residence, Huntington Beach, California, 1987.

Table 7.-SLE and WEE positive birds by species, Orange County, 1987.

House Sparrow *(Passer domesticus)*	- 10	(1)*
House Finch *(Carpodacus mexicanus)*	- 9	
White Crowned Sparrow *(Zonotrichia leucophrys)*	- 6	
Rock Dove *(Columba livia)*	- 5	
Say's Phoebe *(Sayornis saya)*	- 2	(1)
Mourning Dove *(Zenaidura macroura)*	- 2	
Red-Winged Blackbird *(Agelaius phoeniceus)*	- 1	
California Quail *(Lophortyx californicus)*	- (1)	
Song Sparrow *(Melospiza melodia)*	- (1)	
Brown-Headed Cowbird *(Molothrus ater)*	- (1)	

SLE = 35 (0.63% total all birds)
WEE = 5 (0.09% total all birds)

*( ) = WEE

Table 8.-Localities of SLE and WEE positive birds, Orange County, 1987.

Sites I through XII same as Figure 1.

XIII	Bonita Canyon Dump, Irvine
XIV	Trabuco Creek, San Juan Capistrano
XV	Day Residence, Westminster
XVI	Rattlesnake Reservoir, Irvine Ranch
XVII	Laguna Reservoir, Irvine Ranch
XVIII	Laguna Lakes, Irvine Ranch
XIX	Hillcrest Park, Fullerton

Table 9.-SLE and WEE positive birds, Orange County, 1987.

	Catalog	A	Species	Sex	Age	Date	Locality
	JAG - 87	53	House Finch	M	Imm.	6 Feb	XIV
		216	House Finch	F	Imm.	4 Mar	VII
		1439	House Finch	F	Fledg.	22 June	IV
		1484	House Sparrow	F	Fledg.	23 June	XII
		2187	House Sparrow	F	Fledg.	10 July	XV
**		2218	Brown-Headed Cowbird	F	Ad.	13 July	III
		2221	House Sparrow	F	Imm.	13 July	III
		2394	House Finch	F	Ad.	17 July	XI
**		2514	House Finch	F	Ad.	21 July	XII
		2583	House Sparrow	F	Imm.	21 July	XV
		2613	House Sparrow	F	Imm.	21 July	XIX
		2677	House Finch	F	Ad.	24 July	XII
		2715	Rock Dove	F	Ad.	27 July	XIII
**		2892	Say's Phoebe	M	Ad.	29 July	XVIII
		3180	Rock Dove	M	Ad.	10 Aug.	XIII
**		3499	Song Sparrow	F	Ad.	19 Aug.	XIV
		3618	Rock Dove	F	Juv (Imm)	24 Aug.	XIII
**		3670	California Quail	M	Ad.	24 Aug.	XVI
		4018	Rock Dove	M	Juv (Imm)	8 Sept.	XIII
		4104	Rock Dove	M	Ad.	14 Sept.	XIII
		4115	Say's Phoebe	M	Ad.	14 Sept.	XVI
		4269	Mourning Dove	M	Ad.	23 Sept.	XVII
		4281	Mourning Dove	M	Ad.	23 Sept.	XVII
		4395	House Sparrow	M	Imm.	28 Sept.	IX
		4555	House Sparrow	F	Ad.	13 Oct.	IX
		4821	White-Crowned Sparrow	M	Imm.	2 Nov.	I
		4862	House Sparrow	F	Imm.	9 Nov.	IV
		5164	Red-Winged Blackbird	F	Ad.	1 Dec.	VI
		5173	House Sparrow	M	Imm.	3 Dec.	IV
		5174	White-Crowned Sparrow	M	Ad.	3 Dec.	IV
		5198	House Sparrow	M	Imm.	4 Dec.	XII
		5205	House Finch	F	Ad.	7 Dec.	I
		5244	White-Crowned Sparrow	M	Imm.	10 Dec.	VI
		5255	White-Crowned Sparrow	M	Imm.	11 Dec.	XI
*		5483	White-Crowned Sparrow	M	Ad.	28 Dec.	III
*		5484	White-Crowned Sparrow	M	Ad.	28 Dec.	III
*		5489	Say's Phoebe	M	Ad.	28 Dec.	III
		5497	House Finch	F	Ad.	28 Dec.	IV
		5542	House Sparrow	F	Ad.	30 Dec.	VII
*		5555	House Finch	F	Ad.	31 Dec.	IV

35 SLE (0.63%)
5 WEE (0.09%)

*Recaptures, resampled birds.
**WEE

VERTICAL DISTRIBUTION AND RESPONSE OF *CULEX* MOSQUITOES TO DIFFERING CONCENTRATIONS OF CARBON DIOXIDE

A. R. Pfuntner[1], W. K. Reisen[2] and M. S. Dhillon[3]

ABSTRACT

The effects of different carbon dioxide release rates and trap heights on the catch size of *Culex tarsalis*, *Cx. quinquefasciatus* and *Cx. peus* in CDC-style traps were evaluated in southern California. The mean catch size of *Culex tarsalis* females, but not *Cx. quinquefasciatus* or *Cx. peus*, increased significantly when CO_2 release rates were increased from 250 to 1,000 ml per min. In rural locations, trap height (2, 5 and 10 meters) did not significantly affect the number of adults captured. The parity rate of *Cx. quinquefasciatus* was greater at 10 meters than at the lower levels. The other species did not exhibit significant parity differences related to height. At urban sites, the number of *Cx. tarsalis* and *Cx. peus* adults captured tended to increase in traps placed in the tree canopy at 5 meters. Further research is indicated to determine if carbon dioxide baited traps located at ground level underestimate the abundance of host-seeking females.

Introduction.

Since the 1983 outbreak of St. Louis encephalitis virus in the Los Angeles basin, three species of mosquitoes (*Culex tarsalis* Coquillett, *Culex quinquefasciatus* Say, *Culex peus* Speiser) have been found naturally infected (Emmons et al. 1984, 1985, 1986, 1987). Vector competence studies by Hardy et al. (1985) determined that *Cx. peus* was a very efficient laboratory vector of SLE, followed by *Cx. tarsalis* and *Cx. quinquefasciatus*. All three species feed primarily on birds (Tempelis and Washino 1967) and are attracted to carbon dioxide baited traps. However, previous trap comparison studies in Kern County (Meyer 1985) and in San Bernardino County (Reisen and Pfuntner 1987) indicated that dry ice baited traps may underestimate the relative abundance of *Cx. peus* females and thereby reduce the effectiveness of arbovirus surveillance for this species.

Research was initiated to increase the catch size of *Cx. peus* captured in CO_2 traps. The present paper determined the effects of differing trap heights and carbon dioxide release rates on the abundance of *Culex* species captured in modified CDC-style traps (Pfuntner 1979).

Materials and Methods.

Study Areas.-Evaluations of trap heights were conducted at two urban and two rural locations. The urban sites were located in the suburban communities of Rossmoor (Orange County) and Norwalk (Los Angeles County). Rural sites were near the Prado Basin (Riverside County) and at a dairy adjacent to the city of Chino (San Bernardino County). The urban locations represented typical residential habitats where mosquito breeding occurred in backyard and other proximal sources such as ditches and flood control channels. The rural locations were near dairy waste water ponds or impoundments resulting from irrigation and rainwater runoff.

$C0_2$ Release Rates.-Pressurized cylinders of carbon dioxide gas coupled to regulators (Accu-Trol Model RS-4-3) and metering valves (Nupro M series were adjusted by bubble flow meter to expel 250, 500, and 1,000 ml/min. Gas was released through 3.2 mm I.D. tubing inserted into one of the four 6.4 mm discharge holes located at the bottom edge of each of the 3.8 L paint cans normally used to house dry ice. Traps were operated from dusk to dawn, at a height of two meters, at the dairy location in Chino. Each release rate was tested in three traps at three sites on three occasions.

The dry ice sublimation rates from each of three insulated 3.8 L containers were determined by periodically weighing the tared containers in the laboratory on an electronic pan balance (Ohaus E300/3000). The volume of gas produced was derived from the net weight using the following chemical equation based upon the Standard Gas Law:

$$1\ L\ CO_2/22.3\ L \times 44\ g = 1.97\ g/L\ (\text{"solid" } CO_2/L)$$

The four discharge holes on each of the three containers were approximately 6.4 mm in diameter as noted previously. The room temperature was 27 ± 2°C.

Trap Height.-Traps baited initially with approximately 1.4 kg of dry ice were operated from

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[3]Northwest Mosquito Abatement District, 6851 Granite Hill Drive, Riverside, CA 92509.

dusk to dawn at two (ground level), five and ten meters height at three fixed sites on four occasions at a dairy in Chino, and on six occasions at rural sites near Prado Basin. Traps were hung from ropes running through pulleys attached to tree limbs and thus, the traps at five and ten meters were situated adjacent to or within the tree canopy. Similarly, traps were run on four occasions at ground level and at five meters at three sites in Norwalk and at four sites in Rossmoor.

Processing.-Traps were retrieved early in the morning and were immediately transported to the laboratory where mosquitoes were anesthetized with triethylamine, sorted to species and sex and counted. During the trap height evaluation at Chino, the parity status of 40-50 females of each *Culex* species were determined by ovarian dissection using a combination of the tracheolation and dilatation methods (Detinova 1962). Since autogenous egg development can confound the interpretation of parity data, pupae were collected at the dairy site, allowed to emerge, held under insectary conditions (16:8 L:D, 25±2°C) on 10% sucrose for >6 days, and then dissected to determine ovarian development. Females with follicles matured to Stage V (Christophers 1911) were considered to be autogenous.

Statistics.-Trap catches were transformed to ln (Y+1) and then tested by analyses of variance blocked by trap sites (Sokal and Rohlf 1981). Means presented herein are back-transformed or geometric values. The variability of parity rates among heights was compared by contingency chi square.

Results.

CO_2 Release Rates.-The sublimation rate of the dry ice decreased significantly ($P<0.01$) from 1,035 ml/min. at time zero to 425 ml/min. after twelve hours exposure at 27°C (Fig. 1). The range of CO_2 released from the dry ice holder (1,000 to 300 ml/min.) was comparable to that evaluated in the CO_2 gas release experiment (1,000 to 250 ml/min.). The catch size of *Cx. tarsalis* increased as a function of the CO_2 release rate (Table 1). No significant differences were observed for the other two species. Catch sizes varied greatly both among and within trap stations making analysis, even after transformation, difficult. Since the CO_2 release rate did not influence the catch sizes for *Cx. quinquefasciatus* or *Cx. peus* and since most *Cx. tarsalis* are collected during early evening when the release rate from the dry ice was high, the range in the CO_2 release rate from dry ice baited traps probably did

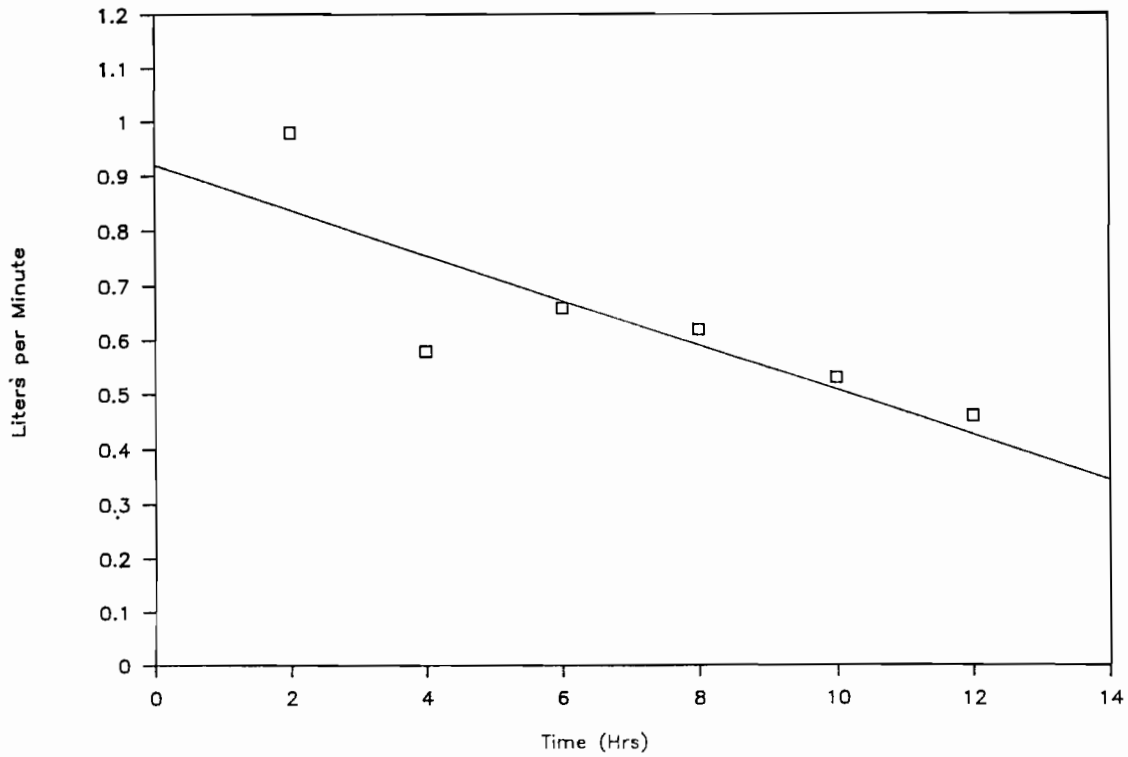

Figure 1.-Dry Ice Sublimation Rate at 27°F ($r^2 = 0.68$, $P<0.05$).

Table 1.-Geometric mean number of females collected per trap night at 3 CO_2 release rates, Chino, 1987.

	CO_2 Release Rate (ml/min.)			
Culex species	250	500	1,000	ANOVA
quinquefasciatus	378.6	518	381.4	ns
tarsalis	32.7 b	56.7 ab	104.4 a	*
peus	36.6	74.4	37.7	ns

*P<0.05, means followed by the same letter were not significantly different when tested by a multiple range test. Trap nights = 27.

Table 2.-Geometric mean number of females collected per trap night at 3 heights, Chino, 1987.

	Trap Height in Meters			
Culex species	2	5	10	ANOVA*
quinquefasciatus	374.6	287.7	423.3	ns
tarsalis	109.7	72.8	101.3	ns
peus	33.9	30.8	41.7	ns

*Trap nights = 36, NS = P>0.05.

Table 3.-Geometric mean number of females collected per trap night at sites near the Prado Basin, Corona, 1987.

	Trap Heights in Meters			
Culex species	2	5	10	ANOVA*
quinquefasciatus	15.8	61.2	59.3	ns
tarsalis	72.7	207.5	130.8	ns
peus	8.8	43.3	22.6	ns

*Trap nights = 32, NS = P>0.05.

not affect *Culex* catch sizes.

Trap Height.-The mean number of females of the three species collected at Chino did not differ significantly at two, five or ten meters (Table 2). Though not significantly different (P>0.05), the mean numbers of females of all three species captured in the Prado Basin were less at ground level than at either of the other heights (Table 3). At the urban locations, more females were collected at the traps in the tree canopy at five meters than at ground level (Table 4). However, only *Cx. tarsalis* at Rossmoor and *Cx. peus* at Norwalk showed significant differences. At the Chino site, the parity rate of *Cx. quinquefasciatus* was significantly higher at ten than at two or five meters. The percent parity for both *Cx. tarsalis* and *Cx. peus* did not differ significantly among heights (Table 5). Of the pupae collected and reared from the Chino dairy site, *Cx. tarsalis* yielded 50% autogeny (n=14), while *Cx. quinquefasciatus* showed no autogeny (n=75). No pupae of *Cx. peus* were collected.

Discussion.

In rural environments, the effectiveness of the CO_2 baited CDC-style trap in collecting female *Culex* mosquitoes was not enhanced by reducing the release rate to levels approaching one-third that routinely used. The CO_2 release rates used in the present study were all considerably higher than expected from respiring birds. For example, Reeves

Table 4.-Geometric mean number of females collected per trap night at 2 and 5 m heights at Rossmoor and Norwalk, 1987.

	Trap Height in Meters			
	Rossmoor		Norwalk	
Culex species	2	5	2	5
quinquefasciatus	53.6	65.7	0.7	4.2
tarsalis	0.8	3.1*	0.2	0.7
peus	1.9	6.2	0.3	2.0**

Means at 5 m significantly larger (*P<0.05, **P<0.01) than at 10 m, remaining means not significantly different (P>0.05). Rossmoor (Orange County) trap nights = 30, Norwalk (L.A. County) = 24.

Table 5.-Percent of parous females (N*) collected at 3 trap heights, Chino, 1987.

	Trap Heights in Meters			
Culex species	2	5	10	Chi sq.
quinquefasciatus	21 (44)	21 (47)	50 (44)	11.9**
tarsalis	18 (44)	33 (40)	34 (41)	3.27 ns
peus	29 (48)	22 (41)	20 (49)	1.15 ns

* N = number dissected.
**P<0.005, df = f; NS = P>0.05.

(1953) concluded that the CO_2 release rates of a chicken, man and a cow were about 25, 250 and 2,500 ml/min., respectively. However, he found that the catch sizes of *Cx. quinquefasciatus* did not differ markedly between release rates of 25 and 250 ml/min., although decreases occurred at release rates of 2,500 ml/min. Similar to the present study, the greatest numbers of *Cx. tarsalis* wee collected at the greatest CO_2 release rates.

Trap effectiveness at rural sites was not increased by positioning the traps below (2 meters) or within tree canopy (5 and 10 meters). Conversely, traps located in the urban environs showed a greater tendency to capture increased numbers of *Cx. tarsalis* and *Cx. peus* when placed in the tree canopy. Differences in mosquito response to CO_2 traps at rural and urban habitats were difficult to interpret, since traps were placed comparably along tree "wind breaks" at the rural sites and along trees lining streets in the urban settings. Possibly, results were influenced by increased mosquito attack rates and decreased relative host availability in rural sites where trap counts were 1 to 2 logs greater than observed at urban sites, especially for *Cx. quinquefasciatus*. Thus, elevated biting rates may have forced females to seek blood meal, in habitats less frequently searched when biting rates were low. The elevated abundance of *Cx. quinquefasciatus* also could have altered the host-seeking patterns of *Cx. tarsalis* and *Cx. peus*, since the host selection pattern are similar among all three species (Tempelis et al. 1965, 1967). These density induced changes in host-seeking patterns and possible host avoidance behavior could have contributed to the very high among-trap variability observed over time and space during the present study.

An additional confoundment was created by trap placement relative to the tree canopy. Unavoidably, some traps were positioned within the canopy and were protected by the vegetation, while other traps at the same height were relatively exposed. These differences in bait presentation also may have contributed to the variability among traps.

Additional research will be needed to evaluate further the hunting strategies of the three *Culex* species under low abundance levels to determine if ground level surveillance traps do indeed underestimate the abundance of *Cx. tarsalis* and *Cx. peus*.

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MOSQUITO ABUNDANCE IN SUBURBAN COMMUNITIES IN ORANGE AND LOS ANGELES COUNTIES, CALIFORNIA, 1987[1]

W. K. Reisen[2], R. P. Meyer[2], V. M. Martinez[2],

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ABSTRACT

During the summer of 1987, the communities of Rossmoor, Orange County, and Norwalk, Los Angeles County, were surveyed for adult and larval mosquitoes. *Culex quinquefasciatus* was the most abundant species in both communities and was sampled readily by gravid traps. Females of all culicine species were collected by CO_2 traps at ground level and in tree canopy. Adults of all species rested most frequently in shrubbery and flower beds at homes where breeding was detected and independent of home owner opinion of recent mosquito bites. The ca. 6-fold greater mosquito abundance at Rossmoor than Norwalk was attributed to differences in mosquito production from peripheral and not back yard breeding sources.

Introduction.

St. Louis encephalitis virus (SLE) recently has appeared as a public health problem in the greater Los Angeles metropolitan area, with 25 human cases confirmed since 1983. The geographical distribution of the human cases, virus isolations from mosquitoes and seroconversions among flocks of sentinel chickens has indicated widespread enzootic virus transmission with *Culex tarsalis* Coquillett as the principal vector (Emmons et al. 1984, 1985, 1986, 1987). Virus surveillance activities have emphasized residual marsh and riparian habitats and essentially have neglected the back yard environment where some of the human cases may have been contracted. Unfortunately, basic information on the bionomics of mosquitoes inhabiting the peridomestic environment essentially is lacking for the greater Los Angeles area.

The objectives of the present study were to describe quantitatively the abundance and bionomics of vector mosquitoes in representative communities in the greater Los Angeles metropolitan area. Emphasis was placed on determining 1) species composition, 2) relative abundance as estimated by different sampling methods, 3) microhabitants utilized by resting adults, 4) female *Culex* reproductive status, and 5) prevalence and relative productivity of back yard and peripheral breeding sources.

Materials and Methods.

Description of study areas.-The suburban communities of Rossmoor, Orange County, and Norwalk, Los Angeles County, were selected for study because human SLE cases previously had occurred within each community during the summers of 1985 and 1986, respectively. In addition, both communities were situated along the San Gabriel River from which 28 isolations of SLE virus were made from *Culex* mosquitoes during 1986 (Emmons et al. 1987).

The Rossmoor study site was sampled from 20-31 July 1987, encompassed an area of ca. 0.6 km^2 and contained ca. 680 homes (residence density = 1,133 homes/km^2) (Fig. 1). Homes were assessed at ca. $150,000-$250,000, and yards were well landscaped and mostly watered by automatic sprinkler systems.

The Norwalk study site was sampled during 17-27 August 1987 and was smaller than Rossmoor (0.2 km^2) with about 312 homes (density = 1,560/km^2). In comparison with Rossmoor, homes in Norwalk were less expensive ($75,000-$125,000), had smaller lots, were landscaped minimally and usually were watered by hand or with portable devices.

Mosquito sampling.-Adult mosquitoes were sampled by 6 methods at 3 to 9 fixed sites on each of 4 occasions in each community (Figs. 1 and 2).

Host-seeking females were collected by dry ice baited traps positioned in 3 habitats: 1) ground level (1.5-2.0 m) suspended from the eaves of

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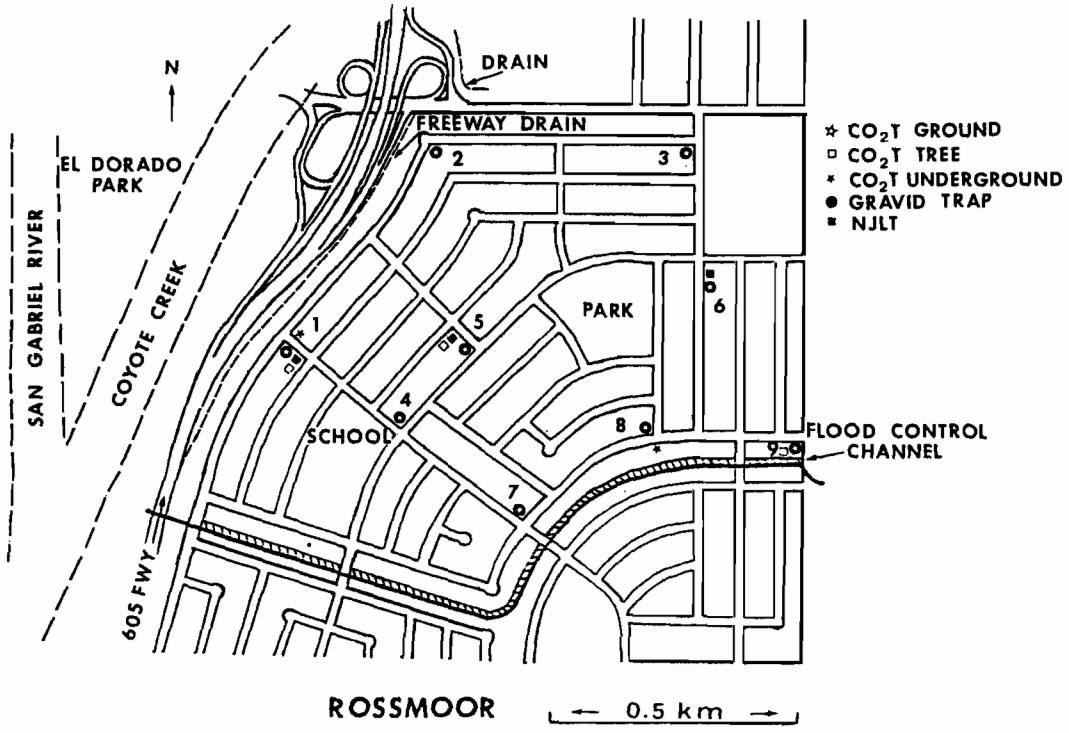

Figure 1.-Map of the Rossmoor study area, Orange County, 1987. Numbers 1 to 9 refer to fixed trapping sites and areas where 10 - 14 adjacent homes were sampled for mosquitoes.

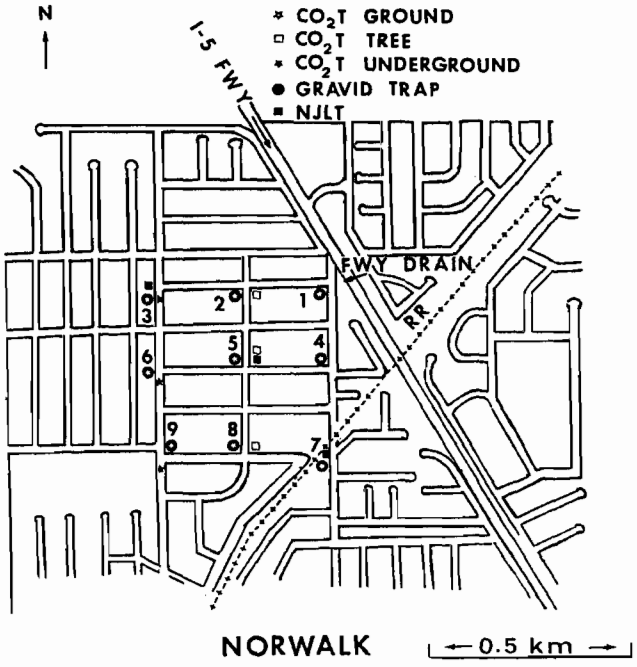

Figure 2.-Map of the Norwalk study area, Los Angeles County, 1987. Numbers 1 to 9 refer to fixed trapping sites and areas where 12 adjacent homes were surveyed for mosquitoes.

houses or adjacent vegetation, 2) 5.0 m elevation in tree canopy, and 3) underground at the entrances to catch basins (Rossmoor) or storm drain man holes (Norwalk).

Gravid females were collected by a facsimile of the Reiter (1983) trap baited with a hay infusion media.

Phototactic mosquitoes were sampled by standard NJ light traps fitted with 25 watt bulbs and operated for 7 consecutive nights at 3 locations.

From 9 to 14 residences near each of the 9 fixed trapping stations were surveyed for mosquitoes. Home owners were surveyed to determine their impression of recent mosquito contact, time spent outdoors in the evening and the numbers of pets which slept outdoors. The types of mosquito resting sites were recorded. Mosquitoes resting in vegetation in front and back yards were collected for timed intervals using a power aspirator (Meyer et al. 1983), while those resting under house eaves or in out-buildings were collected by a hand-held mechanical aspirator (Hauscherr's Machine Works, Toms River, NJ). In addition, each premise was searched for mosquito breeding. The numbers and types of habitats containing water (potential breeding sources) were recorded for each household. Immature mosquitoes were collected from each positive habitat and returned to the laboratory for identification as either L3 or L4 larvae or adults emerging from pupae.

In addition to residences, suitable breeding sites within or peripheral to the study areas were searched for mosquito breeding. Standing water in street gutters was mapped and examined for breeding. At Rossmoor, all catch basins, a flood control channel and the I-605 freeway drain were examined for breeding (Fig. 1). At Norwalk, swimming pools with a history of poor maintenance, the San Gabriel River bed (ca. 1 km W) and the I-5 freeway drain (Fig. 2) were searched for mosquito breeding.

Statistics.-Numbers of mosquitoes trapped or collected resting were transformed to 1n (y+1) to normalize the distribution and control the variance prior to performing analyses of variance (ANOVA). Means presented in tables or figures were back-transformed geometric estimates [$mean_W$ = Williams' (1951) mean]. Chi square was used to test for the goodness of fit of the dispersion pattern of mosquitoes resting at houses to the negative bionomial distribution (Poole 1974).

Results.

Species composition.-*Culex quinquefasciatus* Say was the most abundant mosquito collected, comprising 87% (n = 6,336) and 77% (n = 1,196) of the total specimens collected at Rossmoor and Norwalk, respectively. Other species collected in order of decreasing abundance were *Culex peus* Speiser (8 and 12%) *Culiseta incidens* (Thomson) (3 and 7%) and *Cx. tarsalis* (2 and 4%, respectively). At Rossmoor, 2 female *Aedes taeniorhynchus* (Wiedemann) and a single female *Culex erythrothorax* Dyar also were collected.

Sampling effectiveness.-At Rossmoor, more *Cx. quinquefasciatus* females were collected host-seeking at $C0_2$ traps than attempting to oviposit in gravid traps (Fig. 3). However, only 42.2% of host-seeking females were parous (Fig. 4) and therefore, only 23.3 and 27.4 females per trap night at ground and 5 m level, respectively, had imbibed a previous and potentially infectious blood meal which was comparable to the abundance of females collected by the gravid trap (Fig. 3). At Norwalk, where population abundance levels were considerably lower than at Rossmoor, more females were collected at gravid than at $C0_2$ traps, perhaps indicating that gravid female traps may be relatively more effective in collecting *Cx. quinquefasciatus* when population levels are low and breeding sties relatively scarce and widely dispersed.

Cx. tarsalis abundance was low throughout and females were sampled most readily at $C0_2$ traps hung in tree canopy (Fig. 5). Similar results were observed for *Cx. peus*, although specimens also were recovered from gravid and NJ light traps (Fig. 6).

Few mosquitoes of all species were collected in traps hung underground in either the entrances to catch basins (Rossmoor) or in man holes opening into storm drains (Norwalk) (Figs. 3,5,6). These data indicated that underground sites were not a major source of mosquito breeding in the current survey.

New Jersey light traps collected few mosquitoes (Figs. 3,5,6). Most houses had front and back porch lights and all streets were well lit increasing the background illumination levels and thereby reducing overall trap effectiveness.

Abundance patterns of mosquitoes at residences.-The dispersion pattern of adults collected resting at residences was decidedly clumped for the four common mosquito species (Table 1). The ratios of the variance to the arithmetic mean were greater than 1 throughout and were highest at Rossmoor where abundance levels were greater than at Norwalk. These data implied that the vari-

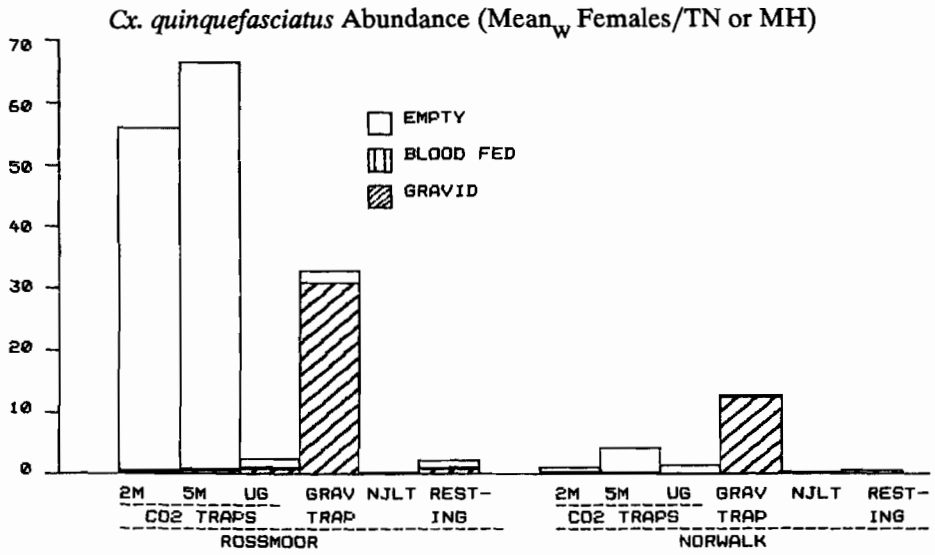

Figure 3.-Geometric mean$_w$ numbers of female *Culex quinquefasciatus* of each reproductive state (empty, blood fed or gravid) collected per trap night or man hour by dry ice-baited ($C0_2$) traps hung at ground level (2 m), in tree canopy (5 m) or underground (UG), gravid traps, NJ light traps (NJLT) or by sweeper or hand held aspirator resting at houses in Rossmoor or Norwalk, Los Angeles Basin, 1987.

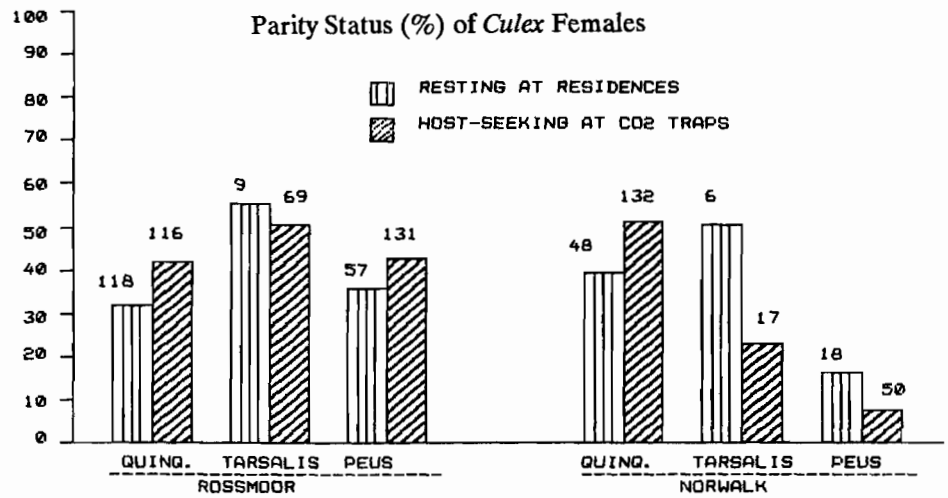

Figure 4.-Percentage of *Cx. p. quinquefasciatus*, *Cx. tarsalis* and *Cx. peus* collected resting at houses or host-seeking at ground level and tree canopy $C0_2$ baited traps that were parous, Los Angeles Basin, 1987.

Table 1.-Dispersion patterns of four species of mosquitoes resting at residences in Rossmoor and Norwalk, 1987.

Freq. class	Numbers of males and females collected per house[a]							
	Rossmoor (n = 106)				Norwalk (n = 108)			
	quinq.	*tars.*	*peus*	*incid.*	*quinq.*	*tars.*	*peus*	*incid.*
0	44	72	41	82	68	94	83	97
1	15	22	21	11	14	3	12	6
2	6	5	10	5	11	1	6	2
3	5	4	8	3	3	1	3	1
4	3	0	6	2	3	0	3	0
5	3	1	5	1	3		0	0
6	3	1	3	0	2		0	1
7	4	0	2	1	1		1	1
8	5	1	0	0	0		0	0
9	3	0	3	0	1			
10	2		1	0	0			
11	0		1	0	0			
12	0		3	0	0			
13	1		0	0	0			
14	0		0	0	0			
15	1		0	0	0			
>15	10		2	1	2			
Max.	132	8	27	18	38	4	7	7
Mean	5.85	0.59	2.59	0.64	1.49	0.20	0.48	0.24
Variance	222.5	1.58	16.82	4.23	19.55	0.37	1.27	0.98
s^2/x	38.04	2.65	6.51	6.59	13.12	1.85	2.65	4.08
k^b	0.29	0.46	0.54	0.15	0.23	0.23	0.25	0.08
Chi^2	102.9**	5.02ns	12.97ns	2.09ns	6.33ns	1.12	1.85ns	2.49ns

[a] *quinq.* = *Cx. quinquefasciatus*, *tars.* = *Cx. tarsalis* and *incid.* = *Cs. incidens*.

[b] k, an index of the degree of clumping, calculated for the negative binomial distribution using the method described in Poole (1974). Model tested by Chi square for goodness of fit by comparing expected frequencies with observed frequency distributions. **P<0.01, ns P>0.05.

Table 2.-Mean$_w$ number of adult mosquitoes collected resting at houses positive or negative for reported recent mosquito bites or detected breeding sources, Rossmoor and Norwalk, 1987[a].

	Mosquito bites[b]				Breeding sites			
	Rossmoor		Norwalk		Rossmoor		Norwalk	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Sample size	19	39	28	74	11[c]	92	7	101
quinque. males	-	-	-	-	11.30*	2.82	4.16*	1.01
females	2.29	2.60	0.76	1.29	7.17*	2.13	2.78*	1.03
tarsalis males	-	-	-	-	0.38	0.42	0.99*	0.16
females	0.93*	0.17	0.16	0.15	0.68	0.42	0.70	0.12
peus males	-	-	-	-	2.71*	0.08	0.99	0.52
females	1.83	1.25	0.19	0.32	2.90	1.48	0.00	0.35
incidens males	-	-	-	-	0.90	0.40	0.34	0.22
females	0.13	0.42	0.06	0.15	1.03*	0.20	0.00	0.13

[a]Paired means$_w$ (pos. vs neg.) followed by a * were significantly greater ($P<0.05$).

[b]Only houses surveyed or with residents responding included.

[c]Three houses with positive street gutters also included.

ance was not independent of the mean. The distribution of adult mosquitoes among houses was found to fit a negative binomial model for all species, with the exception of *Cx. quinquefasciatus* at Rossmoor where large numbers of adults were collected at few houses. The degree of clumping of males or females among houses was greater at Norwalk than at Rossmoor as indicated by the lower values of k from the negative binomial distribution. Thus, mosquitoes were aggregated more highly when population abundance was low than when abundance was high.

Resident opinion of recent mosquito attack was essentially independent of the distribution of resting female abundance at houses (Table 2). With the exception of *Cx. tarsalis* at Rossmoor, mean$_w$ female mosquito abundance was not significantly different between houses reporting or not reporting recent mosquito bites. In Norwalk, residents also were asked if they frequently were outdoors during the period of peak mosquito host-seeking activity (1900-2200 hrs). Overall, 78% of the 28 residents reporting mosquito bites also reported that they spent time outdoors in the evenings. Paradoxically, the percentage was not significantly different from 70% of the residents reporting no mosquito bites. Dogs were the most common household pet sleeping outdoors; however, mosquito relative abundance also varied independently of the number of dogs per household. The abundance of pets did not vary spatially within study areas, although the abundance of dogs was greater at Norwalk (0.7/house) than at Rossmoor (0.5/house).

In contrast, houses positive for mosquito breeding (mostly *Cx. quinquefasciatus*) had significantly more adult mosquitoes than houses where breeding sources could not be found (Table 2). Breeding sites at houses were not clumped among trap sites at either Rossmoor or Norwalk; i.e., the probability of encountering a back yard breeding source was independent of house location in the study area. Our data indicated that house inspections for breeding would be a more reliable indica-

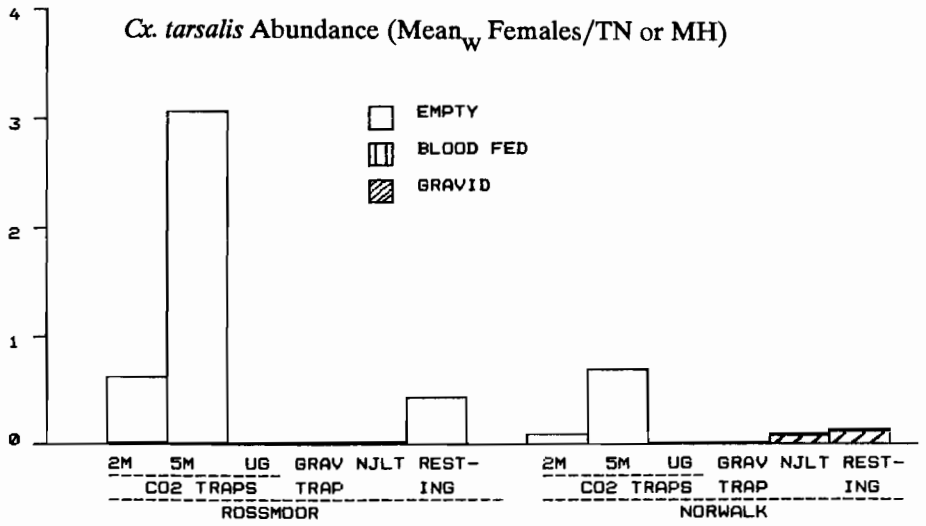

Figure 5.-Geometric mean$_w$ numbers of female *Culex tarsalis* of each reproductive state (empty, blood fed or gravid) collected per trap night or man hour by dry ice-baited ($C0_2$) traps hung at ground level (2 m), in tree canopy (5 m) or underground (UG), gravid traps, NJ light traps (NJLT) or by sweeper or hand held aspirator resting at houses in Rossmoor or Norwalk, Los Angeles Basin, 1987.

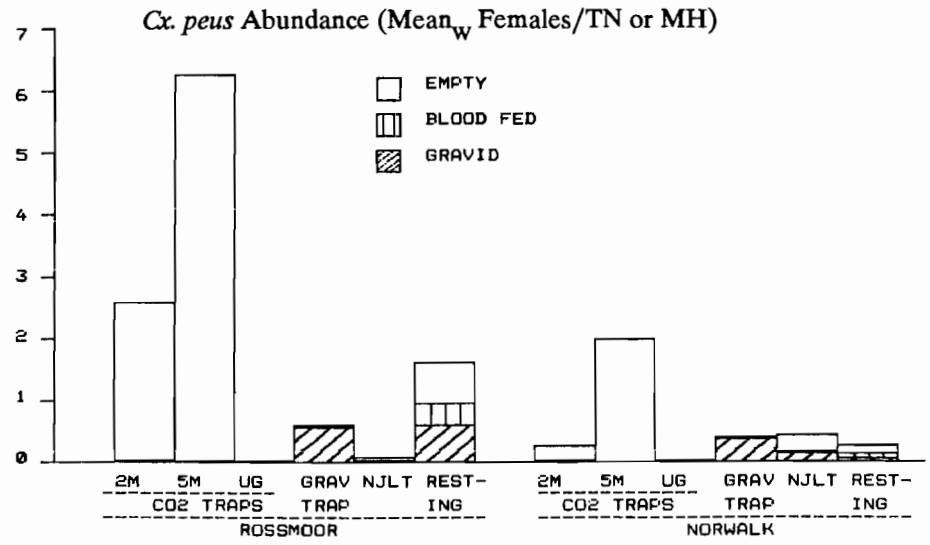

Figure 6.-Geometric mean$_w$ numbers of female *Culex peus* of each reproductive state (empty, blood fed or gravid) collected per trap night or man hour by dry ice-baited ($C0_2$) traps hung at ground level (2 m), in tree canopy (5 m) or underground (UG), gravid traps, NJ light traps (NJLT) or by sweeper or hand held aspirator resting at houses in Rossmoor or Norwalk, Los Angeles Basin, 1987.

Table 3.-Percentage of total mosquitoes collected resting in microhabitats sampled in Rossmoor and Norwalk, 1987.

Species	Sex	Total Coll.	Habitat types: Shrubs/[a] flowers	House[b] eaves	Porch/[c] patio	Misc.[d] bldgs.	Yard[e] debris	Holes/[f] drains
			Rossmoor					
Cx. quinq.	M	437	80	2	8	8	1	1
	F	188	69	9	8	10	<1	4
Cx. tarsalis	M	29	79	3	0	14	3	0
	F	32	75	6	6	6	6	0
Cx. peus	M	165	91	5	1	3	1	0
	F	108	75	7	12	6	1	0
Cs. incidens	M	38	84	5	3	0	8	0
	F	114	68	17	4	10	1	1
			Norwalk					
Cx. quinq.	M	108	82	0	1	5	7	7
	F	52	87	0	2	4	4	4
Cx. tarsalis	M	15	86	0	0	7	7	0
	F	7	86	0	0	14	0	0
Cx. peus	M	30	67	0	0	17	7	10
	F	19	74	0	0	21	0	5
Cs. incidens	M	19	53	0	0	0	47	0
	F	7	29	0	0	0	57	14

[a]Collected by sweeper from front and back yard shrubbery, flower beds and gardens.

[b]Overhanging eaves in front and back of house collected by hand aspirator.

[c]Porch/patio with associated hanging and potted plants collected by hand aspirator.

[d]Misc. bldgs. = assorted sheds, playhouses, tree houses, dog and other pet housing collected by hand aspirator and/or sweeper.

[e]Yard debris = discarded containers, tires, cars, pruning, etc.

[f]Holes/drains were excavations under house foundations, depressions in ornamental fountains, drains to swimming pools, etc.

tor of adult abundance than home owner complaints.

Collections of resting mosquitoes were kept separate by habitat and then summed over houses to provide an indication of adult resting habits (Table 3). With the exception of female *Cs. incidens* at Norwalk, more than 65% of the adults of all species were collected resting in shrubbery, flower beds or back yard gardens. Proportionately more females than males were collected resting under house eaves and from unscreened porches and patios. Most homes at Rossmoor had large shaded porches and overhanging eaves, while homes at Norwalk had smaller porches and small or no eaves. In general, mosquito resting sites were characterized by high humidity and most frequently adults were found under tree canopy or in small spaces protected by house construction. Resting site selection patterns would make control by aerial application of insecticides difficult during the day.

Female population age structure.-Female *Culex* collected resting in peridomestic habitats or host-seeking at CO_2 traps were dissected to determine parity status using a combination of tracheation and dilatation techniques (Nelson 1966). Parity rates for *Cx. tarsalis* and *Cx. quinquefasiatus* were relatively high and approached 50%, indicating parity was higher in residential communities than observed in rural agricultural habitats (e.g., Reisen et al. 1983, Reisen and Pfuntner 1987). *Cx. peus* parity rates were considerably lower than *Cx. tarsalis* or *Cx. quinquefasciatus*, agreeing with previous observations in the nearby Chino area (Reisen and Pfuntner 1987).

Breeding sites.-The prevalence of potential and positive residential breeding sites was greater at Rossmoor than at Norwalk (Table 4). The use of automatic watering devices to irrigate more extensive landscaping contributed greatly to the number of breeding sources observed at Rossmoor. Sprinkler runoff and poor street drainage provided breeding habitat for mosquitoes adjacent to residences. Frequent flooding and refugia created by leaf litter allowed breeding to persist in standing water in street gutters and catch basins despite routine treatment by the Orange County Vector Control District. Most of the positive miscellaneous small containers were potted plants which were watered too frequently to drain well. In addition to the households with potential breeding sites (i.e., sources with water), many more households had dry containers which were not recorded. During an unseasonably wet year, these habitats may become flooded and provide innumerable back yard breeding sources.

Culex quinquefasciatus was the most abundant mosquito collected from positive breeding sites at both Rossmoor and Norwalk. Habitats exploited ranged in size from bromeliad axils to a "Doughboy" swimming pool and a flood control channel. *Cs. incidens* also exploited small-sized peridomestic breeding sites such as flower pots, while *Cx. peus* was associated more frequently with larger sources such as drains, unmaintained fish ponds, etc. *Cx. tarsalis* was collected from two exposed, sunlit sources; a flood control channel at Rossmoor and a "Doughboy" pool at Norwalk.

Discussion.

Cx. quinquefasciatus was the most abundant mosquito species collected at both Rossmoor and Norwalk. Similar species composition patterns have been reported for residential communities elsewhere in Orange County (Webb et al. 1987). All four of the common species were found breeding at residences; however, only large sunlit sources were positive for *Cx. tarsalis*. Mosquito breeding at Norwalk was limited to sources at residences, whereas peripheral freeway drains, storm channels and street gutters were also positive for breeding at Rossmoor. Increased numbers of alternative breeding sources undoubtedly contributed to the considerably higher adult mosquito abundance levels (ca. 6X) estimated at Rossmoor than at Norwalk. However, increased abundance was not reflected in mosquito annoyance to residents, since the percentage of residents reporting recent mosquito bites was comparable in the 2 communities (Rossmoor = 33%, Norwalk = 27%). Collectively, these data indicated that the current abatement strategy of controlling storm drainage systems and ignoring breeding at residences probably was suited to large suburban areas where the few small back yard sources produced relatively few adult mosquitoes.

The present data were collected during a period of no rainfall or arbovirus activity. Residences at Norwalk, and to a lesser extent Rossmoor, contained numerous artificial containers which appeared capable of producing a large mosquito population if filled by rainfall. We plan to survey the same neighborhoods in late-winter/early-spring after or during the rainy season which should provide an interesting contrast to the current investigation.

Table 4.-Percentage of houses with potential breeding sources containing water (number of houses positive for source included in parenthesis), Rossmoor and Norwalk, 1987.

Source type	Percentage positive	
	Rossmoor	Norwalk
Number of houses	106	108
Man made:	36	14(1)
Plumbing	10(1)	5
Ornamental ponds	9(2)	4
Bird baths	8	5
Misc. small containers[a]	69(3)	26(5)
Pet dishes	17	25
Barrels	3(1)	0
Street gutters	?(3)	?
Natural:		
Surface pool	2(1)	0
Tree hole	3(1)[b]	0
Plant axils	0	1(1)

[a]Misc. small containers included mostly flower pots, but discarded containers such as dish pans, cans, bottles and toilets holding water were also included.

[b]Egg raft only.

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SUMMARY REMARKS AND FUTURE RESEARCH DIRECTIONS REGARDING THE PROGRAM ON MOSQUITO ABUNDANCE AND ARBOVIRUS ACTIVITY IN SOUTHERN CALIFORNIA

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A significant portion of the 1985 meeting of the CMVCA was devoted to discussion of the outbreak of St. Louis encephalitis (SLE) in southern California in 1984. I was asked at that time to provide some perspectives and predictions based on that experience. It was agreed that the occurrence of 21 cases of SLE in Los Angeles and Orange Counties was a new experience and that we must determine why it occurred if we were to prevent its recurrence. At that time, I predicted, with tongue in cheek, that if we carried out intensive and costly studies looking for answers, we could almost be certain that the virus would disappear or would be at very low levels for a few years. Prior experience had indicated this usually happened when studies were set up following major epidemics of SLE. As you know from the papers on this program, this has been the case for the past 3 years. In all of southern California, there were only 3 cases of SLE each year in 1985 and 1986 and 1 in 1987. At the same time, a major increase in surveillance allowed the detection of SLE virus at a low level in vectors and vertebrate hosts in each of those years.

I followed the above remarks by posing 7 questions that I thought deserved attention. The 15 papers in this symposium and 5 tomorrow indicate that attention has been given to those questions and considerable progress has been made.

In summary, the questions and progress are as follows:

1. How was the virus introduced and was it a new strain with unusual infectivity and pathogenic traits?

So far, there is no evidence that the virus involved in 1984 was significantly different from other strains isolated from California in previous or recent years. There is little factual evidence that the virus was introduced versus being a persistent enzootic infection in the metropolitan area as it is in nearby more rural areas.

2. How did the virus spread over such a large part of the metropolitan area so quietly and apparently rapidly?

We cannot answer that question as the surveillance system in the epidemic year was not extensive or sensitive enough to answer the question. However, virus has been active in the metropolitan area in each of the 3 succeeding years, but at too low a level to trace possible movement.

3. Were the residual marshes, reclaimed water areas and recreational waters key sites for the introduction and establishment of infection?

Such areas, as well as more residential areas, have continued to be sites where virus has been detected by the expanded surveillance program. Thus, the suspected areas still could be important and must be controlled, but residential areas should be added as a focus for control activity.

4. Which of the 4 species of *Culex* mosquitoes that are common in the area are important as vectors of SLE?

Considerable progress has been made in this area. Virus isolations indicate that *Cx. tarsalis* has higher infection rates than *Cx. quinquefasciatus*, *Cx. peus* or *Cx. erythrothorax*. Vector competence studies, with experimentally infected specimens, rank the species in their order of efficiency as *Cx. peus*, *Cx. tarsalis*, *Cx. quinquefasciatus* and *Cx. erythrothorax*. It still appears that *Cx. tarsalis* is an essential vector; however, it will require another epidemic of SLE to determine if these rankings will remain true.

5. Were the threshold levels of *Cx. tarsalis* populations that had been identified in previous studies in more rural areas, also necessary

to support endemic or epidemic spread of SLE in southern California?

It is clear that the identified threshold levels of *Cx. tarsalis* collected in New Jersey light traps are not applicable to a large metropolitan area and that this is an insensitive method to measure populations of other *Culex* species in such environments. The reasons for this will be discussed shortly.

6. What are the blood feeding habits of the *Culex* species on different hosts in a metropolitan area?

Methods to answer this question are being developed and it still must be determined if a population of over 8 million people is competitive with wild birds as a source of vector blood meals.

7. Is it possible that SLE virus has become established as a transovarial infection between female mosquitoes and their progeny which would provide an efficient reservoir of infection?

Such transmission has been shown in laboratory studies but intensive efforts to document such events in nature are still in progress and inconclusive.

Future Research Directions.

I want to turn now to discuss some research that may assist us in the prevention of future epidemics.

Surveillance.-The surveillance system to detect virus activity in southern California in 1984 was very limited but subsequently was expanded. In 1984, there were only 6 sentinel chicken flocks; there are now 28 flocks. This 4-fold increase has improved geographic representation and increased the sensitivity of the system. Additional sites have been identified where SLE and western equine encephalomyelitis (WEE) viruses are active. Current research will determine if sentinel pigeons or the frequent sampling of wild birds will be more efficient or economical than sentinel chickens.

In 1984, over 48,000 *Cx. tarsalis* were collected from 6 southern California counties and tested for virus. In 1987, this was increased more than 2-fold to over 110,000 specimens; doubling the sensitivity of virus detection. At the same time, over 97,000 specimens of other species were tested in 1987 to clarify their role as vectors. To summarize, in the past 3 years, tests on over 235,000 *Cx. tarsalis* have resulted in 111 isolations of WEE virus and 80 of SLE virus. In comparative tests of over 113,000 *Cx. quinquefasciatus*, only 1 WEE and 7 SLE virus isolations were made. Tests of 54,000 *Cx. peus* resulted in only 1 isolation of WEE and 2 of SLE virus. Tests on thousands of *Cx. erythrothorax* yielded only 1 isolate of WEE virus. While it must be granted that virus activity was low in the past 3 years, the sampling was large enough to leave no question that *Cx. tarsalis* had more contact with viremic hosts than did the other 3 *Culex* species. *Culex erythrothorax* can now be dropped from the surveillance program.

The statewide surveillance system has relied on New Jersey light traps to detect changes in the abundance of mosquito vectors in rural and urban areas of California. Threshold levels of *Cx. tarsalis* populations had been identified earlier in rural areas that were believed to sustain virus transmission or to allow its spread to humans. I believe these measures still apply to most of California. However, it is clear that the New Jersey light trap is not a sensitive instrument to measure *Cx. tarsalis* populations in metropolitan areas of southern California. It is even less satisfactory of *Cx. quinquefasciatus* measurement. I believe the inefficiency of traps reflects the influence of what I call "the urban glow". This is the curtain of light that covers large metropolitan areas at night. This degree of lighting is similar to or greater than the effect of a full moon that was shown years ago by Harry Pratt to decrease light trap efficiency. The urban glow occurs every night and not monthly as does moon glow. The use of $C0_2$ traps, oviposition traps or other collecting methods may bypass this problem. However, these alternative methods are costly and require daily attention which may make them impractical other than for research studies. I suggest that the New Jersey light trap indices be maintained and that the research on alternative methods be used to recalibrate the data from New Jersey traps from metropolitan areas. Present information from ongoing research would indicate that a *Cx. tarsalis* index of 1 or more females per trap night might be significant in a metropolitan area.

The debate over the relative importance of *Cx. tarsalis* and *Cx. quinquefasciatus* as vectors of SLE virus still must be resolved. Both of these species can transmit SLE while WEE virus has *Cx. tarsalis* as an important vector and *Cx. quinquefasciatus* cannot be infected with or transmit WEE virus. Common species of wild birds produce viremias with both viruses and they serve as the

source of vector infection. Both WEE and SLE viruses have been active in the Imperial and Coachella Valleys in most years, but for practical purposes, WEE has not been found in the metropolitan area in years when SLE has. Why hasn't WEE virus been transported into and become established in metropolitan areas, if SLE virus has been? Hypothetically, the same birds are a mode for transport of both viruses over long distances. This question is the subject of ongoing research on the population dynamics and virus infection rates in these 2 mosquito species.

The identification of 21 clinical cases of SLE in 1984 in residents of Los Angeles and Orange Counties raised the question if this epidemic had occurred in an immunologically virgin population that had little antibody from prior infections. You will recall that in earlier years, when SLE was highly endemic in areas such as Kern County, over 20% of people who had lived there for 10 years or more had been infected and were immune. The Centers of Disease Control, in collaboration with local health agencies, collected a bank of blood samples from residents of the Los Angeles metropolitan area after the 1984 epidemic. I have been informed currently that only 1.5% of the over 2,000 persons in that sample had neutralizing antibodies to SLE. Therefore, it must be assumed that almost all residents of this area still are susceptible to SLE virus. It also is obvious that it is not feasible to use serological surveys to detect inapparent infections in humans as a basic part of the surveillance system.

To enlarge on the problem of establishing an improved surveillance system: Some people assume that the purpose of a surveillance system is to detect virus activity before there is any chance of infection in any person who lives in an area or to predict each year the chances and time that virus will become active in an area. This would be a perfect system and allow immediate response of a control agency. However, I do not believe that these are realistic objectives for our surveillance program. To reach this level of sensitivity would require a greatly increased and more expensive program. At the same time, I believe the present program is realistic and it is unlikely that virus activity will reach a level that presents an epidemic threat without being detected. I also believe that the present research program will lead to improved sensitivity and effectiveness of surveillance and will identify areas for concentration of control efforts.

Vector Control.-The final area I want to consider is the problem of vector control in southern California. If the hypothesis is that virus is moved into the metropolitan or more northern areas of the state, it may become very important to reduce the levels of virus activity in the Imperial and Coachella Valleys. In those areas, *Cx. tarsalis* reaches high populations each year in the fall and spring and this is associated with activity of SLE and WEE viruses, respectively. Any reduction in these populations could decrease virus activity and decrease the chances of northern movement of viruses.

Extensive current studies on the bionomics of *Culex* mosquitoes in urban and rural areas of southern California have revealed gaps in our knowledge regarding dispersal and the life tables of the principal *Culex* species. Intensive surveys in metropolitan areas have indicated a need for costly intensified inspections and control of *Culex* in such environments if virus activity is to be minimized. Unfortunately, new methods for chemical or biological control of adult and immature mosquitoes that are acceptable and effective in urban areas are slow in development.

In looking to the future, the most encouraging aspects of the problem are the existence of a network of well staffed control agencies that are concerned with the problem and who are dedicated to its solution, an effective statewide surveillance system, and the current level of collaborative research between the University of California and control agencies.

PESTICIDE LABEL REQUIREMENTS UNDER THE ENDANGERED SPECIES ACT: A PUBLIC HEALTH EXEMPTION

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Introduction.

When the U.S. Environmental Protection Agency announced in early 1987 its timetable for implementation of its new label requirements for protection of endangered species under PL 97-304, the amended Endangered Species Act, it became immediately obvious that little allowance had been made for the protection of human health other than in the case of a full-blown insect-borne disease epidemic. To address this problem, a special committee of the CMVCA met several times to develop a workable plan for the protection of human health in areas affected by the label restrictions. Although protection of human health was of paramount importance, meaningful protection of endangered species was an important design factor. George Craig, president of the American Mosquito Control Association, gave essentially this same charge to that association's National Mosquito Control Fish and Wildlife Committee, and with a large overlap in membership with the CMVCA committee. The AMCA Board of Directors instructed the AMCA committee to use the CMVCA's public health exemption plan as a model for an AMCA plan.

A nearly finished draft plan for a public health exemption for California was produced by the CMVCA committee, but the urgency of pushing forward with a finished version was removed with EPA's announcement that the new label requirements would not be implemented in early 1988 as previously announced, but that 1988 would be used ".....to develop a solid, workable program for the long run. The goal .... remains to protect endangered species while at the same time avoid any unnecessary disruption of agriculture or other uses" (EPA 1988).

I think it is well for all concerned that we have this year or more to take a step backward and to look at the relationship between public health concerns and the protection of endangered species. That is what I plan to do now.

Protecting public health.

It seems ironic that one should have to be in a position to defend public health interests. Certainly the overwhelming passage of Proposition 65 in this state suggests that the public wants its health protected. Mostly it is a problem of education. When a public health program is carried out as successfully as is mosquito abatement in California, so that density of disease vectors rarely rises to levels sufficient to produce epidemics or large outbreaks of vector-borne disease, the public tends to take the program for granted, and to assume that vector abatement need no longer be attended to. I am going to stick my neck out. Given the size of the human population in California today, and the size of the present irrigated agricultural industry, plus the presence of mosquito and other vector breeding sources in the form of "community marshes" and other bodies of standing water, I am willing to state that if the vector populations are not maintained at levels far below their breeding potential, there will be outbreaks of human disease. This seems like a relatively straight forward thesis, one which would permit all of us to say, in the words of Thomas Jefferson: "I hold these words to be self-evident". Alas, such is not the case. The mind-set of public decision makers is invariably "Here we have human cases of human disease -- do something now! -- why weren't we warned?" Let me provide a modern day example. All of us have warned the Federal Government in general and the Centers for Disease Control in particular about the potential danger of *Aedes albopictus*. Yet almost no resources have been made available for development of new control methods for this mosquito in tires and similar habitats.

Principles of a Public Health Exemption.

It seems to me that a public health exemption plan should have two basic tenants: (1) that protection of public health is paramount and should not receive secondary consideration when planning programs to protect endangered species, and (2) effective protection of the public from vector-borne diseases depends upon management of vector populations and maintaining their densities at low levels. When cases of human disease break out, the battle has been lost, and the only recourse then is to invoke emergency methods of questionable effectiveness. At the symposium held in Berkeley last April to honor Dr. William C. Reeves, I presented a paper entitled "Strategies for Surveillance, Prevention, and Control of Arbovirus

Diseases in Western North America." In this paper (Eldridge 1987) I constructed a model based on California's Mosquitoborne Encephalitis Virus Surveillance and Control Program (Walsh 1987) and examined step by step the reliability of the various components of the system and their theoretical relationship to actual disease outbreaks. I also reviewed a number of actual disease outbreaks from the standpoint of efficacy of emergency control measures. I believe two of the conclusions of that paper bear directly on a public health exemption:

> (1) It is very difficult to lower (by spraying) the density of adult vectors to levels below that needed to stop transmission of pathogens, and such spraying should be regarded as a remedy to be used only after all preventive methods have failed.
>
> (2) Well organized mosquito abatement efforts, which include adequate sampling, vector density and virus infection determinations, and insecticide susceptibility testing, are far more effective, and in the long run, more economical.

The Public Health Exemption.

The exemption proposed by the CMVCA committee would operate on two tiers. The first would be for situations where an outbreak of human disease appears likely if there is not some kind of intervention. The second would be for situations where an outbreak of human disease is occurring. For each tier there would be two primary decision levels. The first would be a determination by the State Department of Health Services (DHS) that a probable public health emergency situation exists and that label restrictions associated with endangered species habitat would prevent adequate control. In the case of the first tier, this determination would be made after a local agency had reviewed environmental factors, vector population trends, and other relevant factors as far in advance of the probable outbreak as possible, and made a formal request to DHS for a determination. Information submitted to the DHS would include the nature of the threat to public health, vector species involved, the areas involved, the endangered species involved, the range of the endangered species, the pesticides restricted. DHS would respond within 10 days. In the case of the second tier such a determination by DHS would be more quickly rendered, probably using telephone communication.

The second level of determination would be an evaluation of the control plan in areas affected by label restrictions. The plan would include (1) the specific control actions proposed, including brand, formulation, field strength, and application method, (2) actions proposed to reduce adverse impact on endangered species, (3) approximate dates of proposed actions, (4) name of the person who will supervise the control efforts, and (5) a statement explaining why non-restricted pesticides or other control procedures cannot be substituted.

The plan would be submitted to DHS, who would send it to the U.S. Fish and Wildlife Service for review and comment. DHS would respond to the agency requesting an exemption within 30 days. In the case of a Tier 2 situation, this level of determination would also be expedited. Finally, EPA would review the entire procedure annually to determine whether or not the procedure was operating in such a way as to satisfy PL 97-304.

Final comments.

The proposed plan for a public health exemption to the label requirements for the protection of endangered species would seem to provide a level of protection to the public health of California citizens, but not to the degree they presently have in areas coinciding with the range of endangered species. The plan is tedious and except where human cases of disease are actually occurring, time-consuming. I hope it is workable, and one which can rely on state agency determinations on a case-by-case basis. It is not workable if such determinations must be made by a federal agency such as EPA, because of the time frames involved. Finally, it must be emphasized that an exemption is not *carte blanche* for routine mosquito control within the range of endangered species. Relief from label restrictions must come from more accurate range determinations and more realistic assessments of exposure factors of public health pesticides to endangered species.

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CONVENTIONAL CHEMICAL PESTICIDES FOR MOSQUITO CONTROL: PAST AND FUTURE

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Introduction.

Recently I saw an editorial in the newspaper about the financial condition of the United States economy. The editorial began with this sentence: "We have hit the iceberg, but the band plays on". As I read the article I couldn't help but be impressed by the parallels between the subject of the editorial and the long-running controversy about the use of chemical pesticides. I asked myself: "In the case of pesticide use, have we hit the iceberg, and are we ignoring the danger signals while we conduct business as usual?" More specifically, is a nearly total ban on the use of conventional chemical pesticides for mosquito control in California, if not the entire United States, relatively close at hand? Or is the truth closer to the view I have heard expressed (but really not recently) that chemical pesticides will always be with us because there are no practical alternatives available, nor will there be any for some time to come? It seems to me that there are many "radar" signals bouncing around the atmosphere which suggest that an iceberg may be closer to the ship of mosquito abatement than any of us would like to admit. In the next few minutes I would like to (1) review the pattern of use of conventional pesticides for mosquito abatement in California over the past three decades, (2) call attention to some current developments which provide clues to the future of conventional pesticide use, (3) review the status of alternatives to pesticides, and (4) provide my evaluation of the future of conventional pesticide use for mosquito control in California.

Some definitions.

Before proceeding further I must define the term "conventional chemical pesticide" and contrast these agents with other materials used in mosquito control. In the context of this address, conventional chemical pesticides are those compounds, mainly synthetic, which exert toxicity by interfering with physiological processes found in almost all biological organisms, both vertebrate and invertebrate. The margin of safety afforded non-target organisms such as human beings results from minimizing dosages and exposure. Exposure is minimized in a number of ways, including use of chemicals which break down quickly in the environment to non-toxic by-products. For some non-target organisms (especially invertebrates), however, which share the habitat of the target mosquito, and which enjoy equal exposure, there may be no margin of safety. Currently in mosquito control, conventional chemical pesticides are nearly all organophosphate, carbamate, and pyrethrum or synthetic pyrethroid compounds.

Other pesticides used for mosquito control fall under the category of insect-specific, and in some cases, a subset of that category, mosquito-specific. These compounds exert toxicity by interfering with physiological processes which insects (and perhaps other arthropods) have, but vertebrates do not. Examples would be larvicidal oils, growth regulators, and bacterial insecticides such as Bti. In some parlance, the former group would be called broad-spectrum, the latter narrow-spectrum.

There is a tendency to accept as dogma the principal that conventional chemicals are bad, insect-specific chemicals are good. That the former are dangerous, the latter are not. We should all be cautious about taking an inflexible position here, because there may be exceptions to this. It is not my intention, however, to argue the relative merits or relative environmental safety of the two groups -- rather I wish to focus only on the trends of conventional pesticide use, both past and future.

History of Pesticide Use for Mosquito Control in California.

I do not have access to records of pesticide use for mosquito control in California before 1954, the year that the California Mosquito and Vector Control Association began publishing insecticide use data for their member organizations. I assume, however, that the trend was upward between the end of World War II, when DDT first became available for civilian use, and 1954. The peak year for use of conventional pesticides was 1958, when over 400 tons of material was reported used (Fig. 1). There has been a gradual, but significant, downward trend in the use of conventional pesticides for mosquito control in California since that time. By 1980, the quantity reported was less than one-tenth that used in 1958, and reported usage has stayed at about this same level since that time.

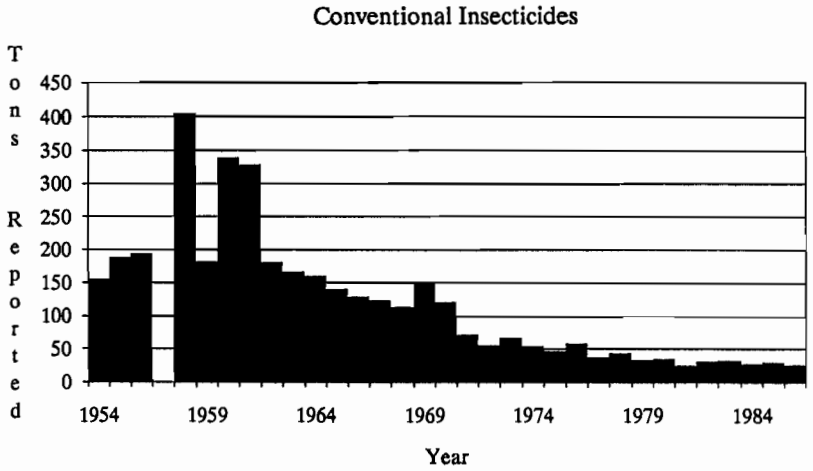

Figure 1.-Conventional insecticides for mosquito control, California, 1954-1986.

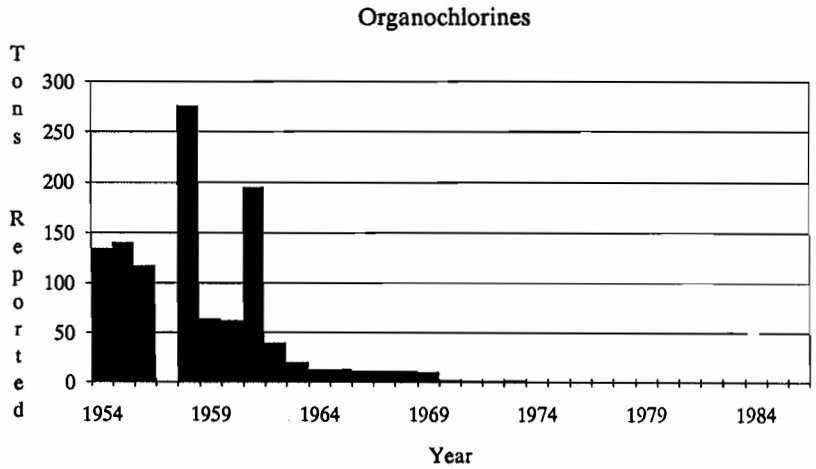

Figure 2.-Organochlorine insecticides for mosquito control, California, 1954-1986.

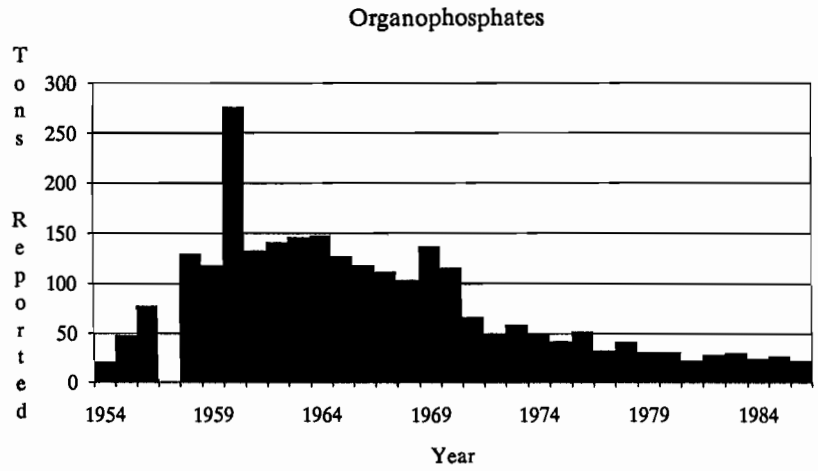

Figure 3.-Organophosphate insecticides for mosquito control, California, 1954-1986.

Reported organochlorine usage for mosquito control peaked in 1958, at about 275 tons (mostly DDT) (Fig. 2). Usage fell off drastically after 1961, DDT was suspended for all but a few uses in the United States in 1972, and no organochlorine usage was reported after 1973. It is interesting to note that organochlorine usage for mosquito control had fallen to only about one-half ton annually in California two years before the DDT ban.

When state-wide record keeping started in 1954, organophosphates made up only about 13% of reported pesticide use (Fig. 3). Usage peaked in 1960, and there has been a gradual reduction in reported use since then. In 1986, organophosphates comprised about 87% of all the conventional pesticides used. For that year, over 20 tons were applied for mosquito control, but this was still less than one-tenth of the amount applied in the peak year of 1960. The figure 20 tons may seem like an enormous quantity, so perhaps I should put this into perspective. For the year 1986, about 2,890 tons of malathion were sold in California in 326 different products (CDFA, 1987). If we assume that most of this total was actually used for insect control of one kind or another, then the use of malathion for mosquito control, which was reported by CMVCA members to be about 13 tons, comprised less than one-half of one percent of the total amount used in California for all purposes.

Other classes of conventional pesticides used are carbamates (Fig. 4), which are down to about half in reported usage from a peak of 6 tons in 1973; and pyrethrum and synthetic pyrethroids (Fig. 5), for which 1986 was a peak year (less than one ton applied).

It is impossible to make a direct comparison of the use of insect-specific materials to that of conventional chemicals during this period on a quantitative basis. This is because the units of measurement are different and there is no way to provide for a conversion. Bacterial insecticides are measured in terms of toxic units; oils in gallons. Examination of relative trends is interesting, however. The use of larvicidal oils has been remarkably stable over the years, with an average of 443,504 gallons per year used between the years 1954 and 1986 (Fig. 6). Reported usage in 1986 was only slightly below the average. There has been a striking increase in the use of growth regulators since their introduction in the early 1970's (Fig. 7), and in bacterial insecticides since their introduction in the early 1980's (Fig. 8). The year 1986 was a peak year for reported use of growth regulators, and usage of Bti was down only slightly from the year 1985.

Taken as a whole, the usage trends for conventional pesticides vs. insect-specific agents for mosquito control in California is clear, notwithstanding the impossibility of making direct quantitative comparisons. It is steadily downward for the former, steadily upward for the latter (Fig. 9).

Pressures against continued use of conventional chemicals.

So much for the past and present. Where are we today, and what does the future hold? I spoke earlier about "radar" signals warning of the iceberg of further restrictions on pesticide usage. Let me discuss briefly some of these "radar" signals. These signals mainly fall into two categories: Pesticide regulations and economics. Of course, the two are interrelated. It is an extremely difficult job to sort out the many federal and state regulations which have an impact on pesticide use in one way or another. On both levels, there are statutes and regulations which address questions of toxic materials in ground water, on food and feed, and in the air. Bill Hazeltine discussed some of these regulations at a talk he gave at the meeting of the Society of Vector Ecologists in Asilomar in November of 1987. I will restrict my comments to just a few of these pieces of legislation: The pending federal amendment of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Federal Endangered Species Act (ESA) as amended by PL 97-304, and the Safe Drinking Water and Enforcement Act of 1986.

FIFRA. There was introduced into the first session of the 100th Congress H.R. 2463 to amend the Federal Insecticide, Fungicide and Rodenticide Act. The bill, sponsored by Representative de La Garza of Texas would be the first amendment to FIFRA since 1972, and is still pending. Other sponsors include Representatives Madigan and Brown of California. The principal changes which this bill would bring about are (1) a speed-up of the re-registration process, paid for not out of the general Treasury, but through fees paid by pesticide manufacturers (2) freer public access to pesticide registration data (3) new EPA authority to protect groundwater (4) regulation of "inert" ingredients (5) stronger requirements for training and certification of pesticide applicators, and (6) greater protection of farm workers (Davis 1987).

An earlier FIFRA bill (H.R. 2482) and a companion Senate bill (S. 2792) came close to passage in the 99th Congress, but did not survive Conference Committee action. Now, both envi-

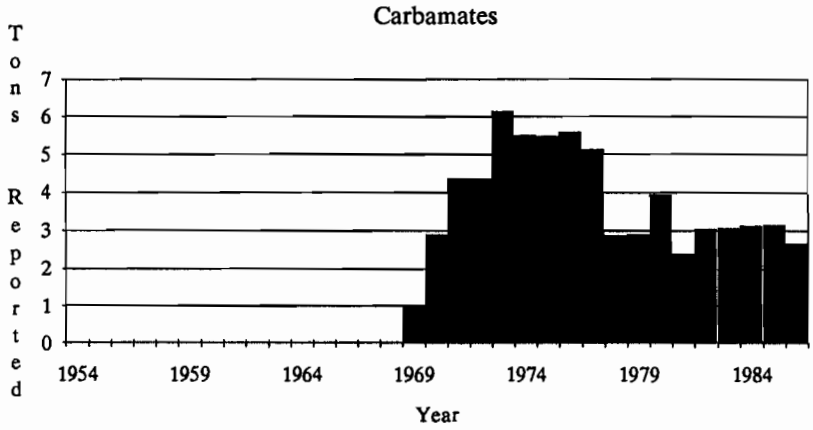

Figure 4.-Carbamate insecticides for mosquito control, California, 1954-1986.

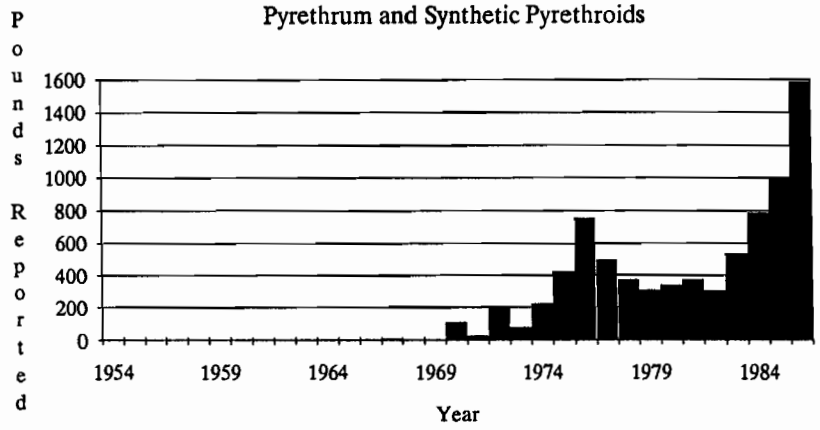

Figure 5.-Pyrethrum and pyrethroid insecticides for mosquito control, California, 1954-1986.

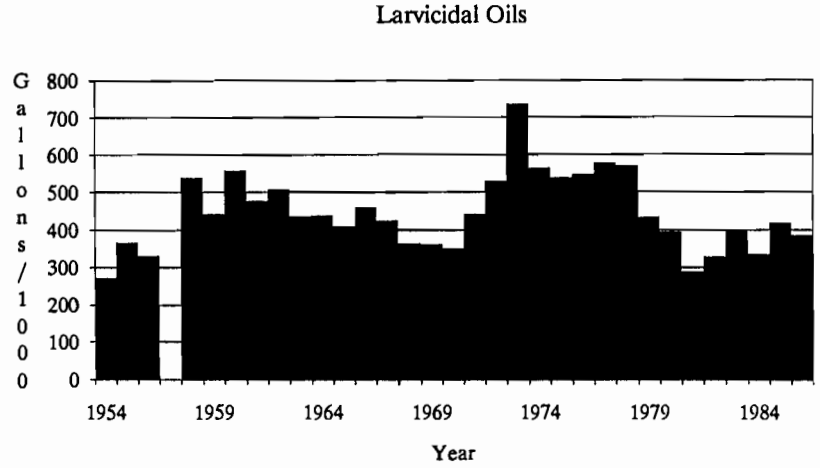

Figure 6.-Larvicidal oils for mosquito control, California, 1954-1986.

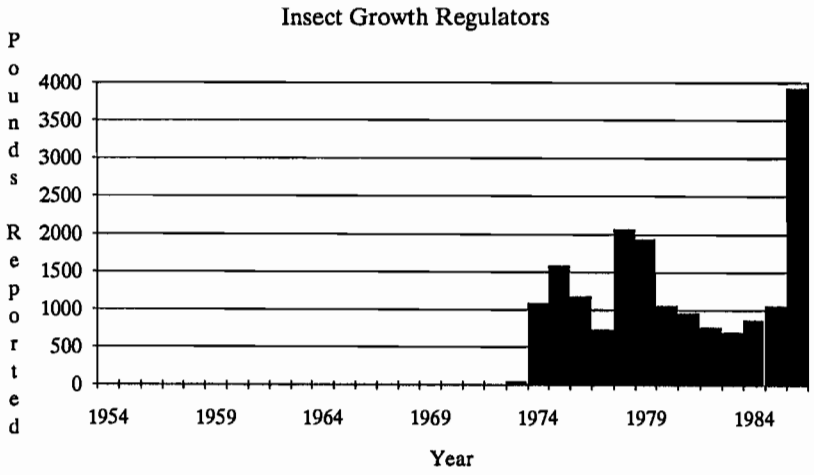

Figure 7.-Insect growth regulators for mosquito control, California, 1954-1986.

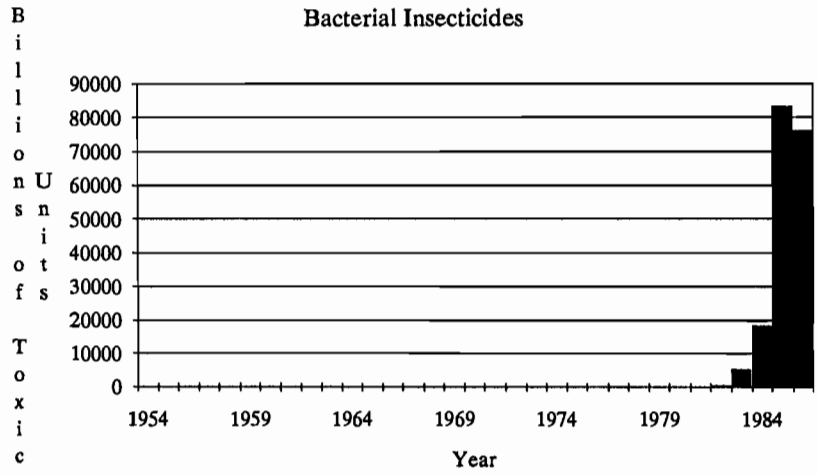

Figure 8.-Bacterial insecticides for mosquito control, California, 1954-1986.

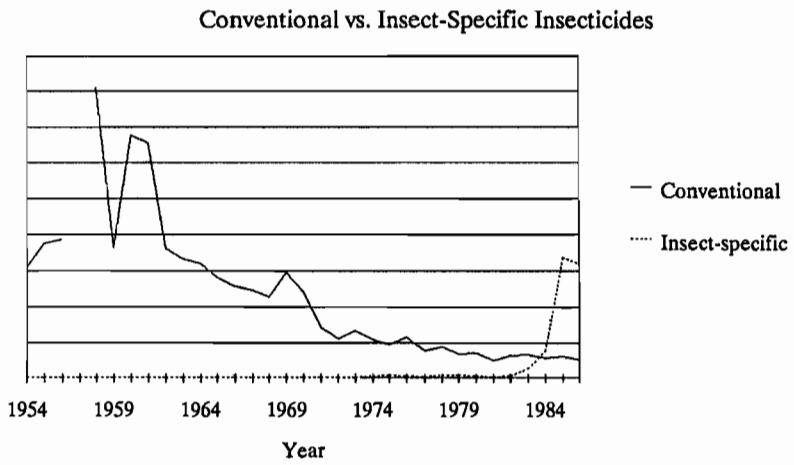

Figure 9.-Trend in insecticide use for mosquito control, California, 1954-1986.

ronmental groups and the chemical industry are insisting on changes to the bill, and the future of a FIFRA amendment is uncertain (Davis 1987a). Nevertheless, eventual passage of some kind of amendment seems inevitable, and if most of the changes I referred to above remain, I believe there are significant implications for pesticide use for mosquito control. The most direct effect would seem to be the provisions for a speed-up of the re-registration process. Presumably, large pesticide manufacturers would have little problem with the payment of fees to support re-registration of pesticides used for major agricultural crops and allowances are made for small business registrants, and for pesticides not used on major food or feed. In the case of minor uses of a pesticide, the Administrator of EPA would be able to reduce or waive the fee. Such fees would be minor, however, in comparison with costs of developing efficacy and safety data. For example, the inclusion in the bill of a requirement for data on movement of a candidate pesticide in ground water will increase both the amount of data needed and the cost of obtaining it.

The direct effects of a new FIFRA are difficult to predict. A reasonable conclusion, however, is that some pesticides used for mosquito control in aquatic environments will be in jeopardy. Development costs and data requirements for registration under the current law has already reduced new materials for mosquito control down to a trickle. Since most currently used pesticides are pending re-registration at both the state and the federal level, moreover, it is uncertain if pesticide manufacturers and formulators will be willing and able to stand the expense of providing the expanded data sets required for site-specific labels for what are very minor uses. I refer you again to the figures I quoted earlier for malathion.

Another complicating factor is pesticide resistance. In the absence of new replacement materials, resistance becomes an even more serious problem. It is interesting that H.R. 2463 calls for EPA to establish a pesticide resistance management program, including research on pesticide resistance management techniques and their demonstration. I applaud this development. I should mention further that pesticide resistance is apolitical and will not wait for legislative action.

Endangered Species Act.

An event which caused an enormous stir in the mosquito abatement community last year was EPA's announcement of its time table for implementation of PL 97-304, the 1982 amendment of the ESA under the provisions of FIFRA. After extensive discussions between EPA and affected agencies, EPA announced that the time table had been set back at least a year, if not longer. EPA's original plan would have restricted or eliminated the use of most mosquito larvicides in use in California at sites determined to be within the range of certain endangered species. The delay in implementation will provide time to gather more accurate data on ranges of endangered species, pesticide use patterns, and toxicological effects of pesticides. Unfortunately, few resources are presently available to conduct the needed studies. Although I am confident that a public health exemption procedure will be worked out, I doubt if individual abatement districts will be able to continue to use conventional mosquito larvicides in affected areas without significant changes in their operational procedures.

Safe Drinking Water and Enforcement Act of 1986.

The overwhelming passage by voter referendum of the Safe Drinking Water and Enforcement Act 1986 (otherwise known as Proposition 65) has produced a great amount of uncertainty at the planning level in a number of state agencies. Although more than a year has passed since the referendum, we have yet to see enabling legislation, although month by month the number of toxic materials added to the Governor's list of toxic substances to be included under provisions of the act grows. At this point, however, no pesticides used for mosquito control have been placed on the list, and it may be that none will be. According to Jim Seiber, Professor of Environmental Toxicology at UC Davis, current thinking is that there are plenty of other laws on the books providing regulatory control of pesticides in aquatic environments, so that there is presently no strong effort to get them added to the Proposition 65 list. For example, there is the Pesticide Contamination Prevention Act of 1985 which was intended to prevent pollution of groundwater by pesticides. Currently, however, no mosquito larvicide has been judged to have numerical values for water solubility, soil adsorption coefficient, hydrolysis, or other factors related to leaching which exceed the amount which would trigger restriction under terms of the act.

I believe that the take-home message from a consideration of the present laws regulating pesticide use, as well as those pending, is that there will be ever increasing regulatory pressures on conven-

tional pesticide use which will tend to restrict and probably eventually eliminate the use of such materials for mosquito control. There is some irony in this, because much of the pressure is coming from public concern over pesticide residues on food -- a concern which is remote from the use of pesticides for mosquito abatement. A recent survey by the Food Marketing Institute reported that 94% of consumers surveyed were concerned about pesticides in food; more than were worried about cholesterol, fats, salt, or additives of any kind (Davis 1987b).

Alternatives to conventional pesticides.

This leads me to my final topic. In an era of ever decreasing use of pesticides, where do we turn for methods to control vectors and vector-borne diseases? In the short term, we should probably look to insect-specific and mosquito-specific materials such as insect growth regulators and bacterial insecticides. Although some of these may be under the same jeopardy as conventional chemicals, the matter of human toxicity and food residues should pose fewer problems. In terms of research and development, I believe that among chemical approaches, this is where our emphasis should be. There is a vast untapped potential for customizing of present insect-specific materials, as well as the possibility of new materials.

In the case of bacterial insecticides, I believe that the coming decade will see new strains of mosquitocidal bacteria, new formulations of bacterial insecticides, and new materials based on manipulations of protein toxins using techniques of molecular biology. This entire area will present many problems for scientists, managers, and regulators. The high degree of specificity of present formulations of bacterial insecticides is probably dependent in large degree on the association of the protein toxins with bacterial crystals. These crystals, which are degraded in the gut of the mosquito at very high pH levels, serve to protect other organisms, and especially mammals, from the actual toxin. This is an oversimplification, however, and for both *Bacillus thuringiensis israelensis* and *Bacillus sphaericus*, recent studies have shown that there are a number of protein toxins associated with the crystal body. Further, the broad-spectrum beta-toxin associated with other strains of Bt are not produced by strains currently in use of either Bti or Bs. Obviously, considerable research on bacterial insecticides for mosquito control remains to be done.

In the longer run, I remain convinced of the eventual success of efforts to achieve practical application of biological control agents, both alone and in conjunction with insect-specific materials. Mosquitofish, entomopathogenic microorganisms such as bacteria and fungi, and invertebrate predator management seem the most promising at this time. We may be closest to a breakthrough here in the case of *Lagenidium giganteum*, for which an experimental use permit has been requested from EPA. Research by Kerwin and Washino at UC Davis has shown that larval populations of *Culex tarsalis* and *Anopheles freebomi* can be suppressed for an entire season by a single application of asexual stages of this fungus. It has been tested in rice fields, roadside ditches, and irrigated pastures with success. At North Carolina State University, Dick Axtell has shown season-long suppression of *Culex quinquefasciatus* in foul-water habitats by treatment with asexual stages of *Lagenidium*. Research continues on upscale production and reliable germination of sexual stages (spores) which can be stored for long periods of time.

Research is also being conducted on mosquitofish, and on other predators such as tadpole shrimp and diving beetles. Other types of fungi belonging to the genus *Coelomomyces* are under investigation. Perhaps most important of all, season-long studies are being done on combinations of methods in terms of overall mosquito population densities, both larval and adult. As we move to new approaches for mosquito abatement, we will have to move to new methods of evaluating control success. The goal, of course, is to maintain densities of adult mosquitoes at levels where they are no longer pests, and more importantly to levels where they cannot serve as vectors of human disease pathogens. The pre- and immediate post-treatment estimates of larval density are simply not suitable for testing the effectiveness of biological control agents.

Summary.

We will probably see an end to the use of conventional pesticides for mosquito control in our professional lifetimes. The time to respond to this probability is now. This is a very challenging prospect, but I believe that in California we have the spirit, energy, resources, and talents to meet the challenge. Evidence of this spirit is California's progressive action in the area of training and certification of public health pesticide applicators.

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

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PESTICIDE DISPOSAL PRACTICE OF THE 60s

HAUNTS VECTOR CONTROL DISTRICT

IN THE 80s

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On September 19, 1986, El Dorado County Vector Control received a cleanup and abatement order from the California Water Quality Control Board - Lahontan Region for an insecticide disposal pit. This pit was used by the district between 1963 and 1969 for disposal of unused or outdated pesticides. The following is a brief summary of what led to the clean-up and abatement order; the subsequent investigation and its results; and in retrospect, some suggestions on how to handle a water quality investigation.

History.

In 1982, we began a program to eliminate outdated equipment and insecticides. In 1983, we shipped five drums of outdated insecticides to a Class I disposal facility.

In 1986, we relocated our insecticide storage and tore down the old storage shed that had been in use since 1963. Because some insecticides had been spilled in the shed over the years, we tested the floor for pesticide residue. Two insecticides, heptachlor and methyl parathion were detected at levels above California action limits. Consequently, the floor was sent to a Class I disposal facility.

Further research into the pesticide use of the district resulted in the discovery of the on-site disposal pit. This pit and ten other areas on the district property were sampled for pesticide residues. Low level residues of three insecticides: the two mentioned above and beta-BHC, were detected in the pit area. All other samples were negative. The results from these tests were the basis for the cleanup order.

Prior to receiving the cleanup order, the district had tested eight nearby domestic water sources (within 1/2 mile of the property) for organochlorine and organophosphate pesticides. All of the water samples were negative.

The Investigation.

The authority and responsibilities of the Regional Water Quality Control Board (RWQCB) are outlined in the Porter Cologne Water Quality Control Act. The RWQCB is the agency responsible for protecting the quality of both the surface and ground water in California. In our case, the RWQCB was concerned that pesticides from the pit area may have migrated to the ground water below the Vector Control property.

All hazardous substance site cleanup programs are supposed to be analyzed by the RWQCBs by applying the guidelines set forth in the State Water Resources Control Board Resolution 85-26. This resolution states that all cleanup decisions are to be site specific, i.e.--based on the actual situation at the site of contamination. This requires an on-site inspection by RWQCB personnel, a characterization of the known pollutants, a determination of the probable fate of the contaminants and an analysis of the benefits of a clean-up in relation to the amount of funds that will be expended.

Our first proposal, which was developed in-house, was to dig up the pit, and test both the material in the pit and the soil underneath the pit. This proposal was rejected by the RWQCB. Although the pesticides in the pit had been there over seventeen years and the wells in the area were not affected, they requested that we start ground water monitoring immediately. They also suggested that we hire a consultant.

Our second proposal, designed by our newly hired consultant, suggested a phased approach that would first establish if any ground water contamination had occurred. The proposal called for two 30-foot deep monitoring wells, one adjacent to the pit and one approximately 30 feet downgradient from the pit in relation to the flow direction of the ground water. Two water samples would be tested. This proposal only addressed testing for organochlorine and organophosphate insecticides, the known contaminants at our site.

The RWQCB also rejected this proposal.

They now wanted:

1. Deeper, larger wells.
2. The testing expanded to include hydrocarbons, degreasers and solvents.

3. An entire hydrogeologic survey for the aquifer below the property including flow rates, specific yields, permeability, etc.
4. Soil samples from below the pit.

For the next three months we negotiated with the RWQCB. Every aspect of the investigation was questioned: the number of wells, the depth and size of the wells, the testing parameters and the number of samples. We finally agreed that in the first phase of the investigation we would:

1. Drill one monitoring well 30 feet downgradient from the pit. One water sample would be tested from this well.
2. Bore through the pit area to collect soil samples at 5-foot intervals from the bottom of the pit down to the saturated zone. The number of samples would be determined by the depth of the saturated zone.
3. Test all samples for organochlorines, organophosphates, hydrocarbons, volatile halocarbons (degreasers) and volatile aromatics (solvents).

If any contaminants were identified from this phase of the investigation, we would be required to proceed further.

Results.

The results of the samples were:

1. All samples were negative for any pesticides.
2. Low levels of hydrocarbons (30 mg/kg to 70 mg/kg) were detected in three soil samples from 6 feet to 16.5 feet below the pit. The levels were high enough to report to the RWQCB, but not high enough to require any clean-up action.

The cost of this investigation was approximately $18,000; $6,000 for the consultant, $6,500 for the drilling and $5,500 for testing.

Conclusion.

Water quality investigations should be site-specific. Every situation is different. The State Water Quality Control Board resolution 85-26 should be reviewed prior to implementing a field investigation.

Research is the key to keeping costs down. One needs to know the pesticide use history of the district, the potential of the known contaminants to affect water sources and the fate of pesticides in the soil.

Nothing is set in concrete for clean-up and abatement orders. Negotiations with the RWQCB are very important. A phased investigation such as the one we negotiated, is usually the best approach. Each phase is less costly than a complete investigation. Each phase provides important information which will be used to design the next phase.

In our investigation, only a first phase was needed. The results from the first phase investigation indicated that our pit wasn't polluting the ground water. After this phase was completed the RWQCB rescinded the clean-up and abatement order.

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MOSQUITO SOURCES CREATED BY SOIL SUBSIDENCE IN A PLANNED UNIT DEVELOPMENT: THE CASE OF HARBORTOWN

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Introduction.

Mosquito breeding sources created by soil subsidence are widespread in lagoon and bayside areas within the San Mateo County Mosquito Abatement District where there is development on bay fill and reclaimed marsh. Differential soil settlement in these areas has caused freshwater and septic lines to separate and water to impound under a large number of pile-supported structures; these include hotels, restaurants, shopping centers and planned-unit (or common interest) developments. The Harbortown planned-unit development in the City of San Mateo provides an example of the operational problems and solutions generated by these sources.

Soil Subsidence at Harbortown.

Harbortown is a 35 acre, lagoon-side townhouse and condominimum development with 312 units grouped into 31 clusters. It was constructed in stages beginning in 1979 on reclaimed marsh that had been filled during the 1960's and early '70's.

Soil subsidence was occurring on the site before, during and after construction and is continuing to subside at a rate of about one inch per year. The weight of the land fill, site grading, the filling of artificial lagoons and the weight of the structures all contribute to this soil settlement.

Two foundation types are employed at the Harbortown development: slab on pile (Fig.1) alongside an original lagoon and "floating" slab (Fig. 2) around an interior artificial lagoon. Preconstruction soils reports by Cooper, Clark & Associates indicated that utilities connected to pile-supported structures will settle 12 to 15 inches differentially with respect to the structures in the years following construction. It was expected that the soil, along with utility connections, would settle away from the slab on pile foundations which are fixed in place. Provisions made to account for this differential settlement were not adequate.

Within a few years of construction, septic and freshwater lines had separated under every pile-supported foundation in the development, creating water impoundments with little or no access. In addition soil settlement around the foundation perimeter allowed runoff from landscape watering and downspouts to impound under the slabs.

Utilities connected to the "floating" slab foundation units were not affected by differential soil subsidence. The "floating" slab settles with the soil below.

Operational Problems.

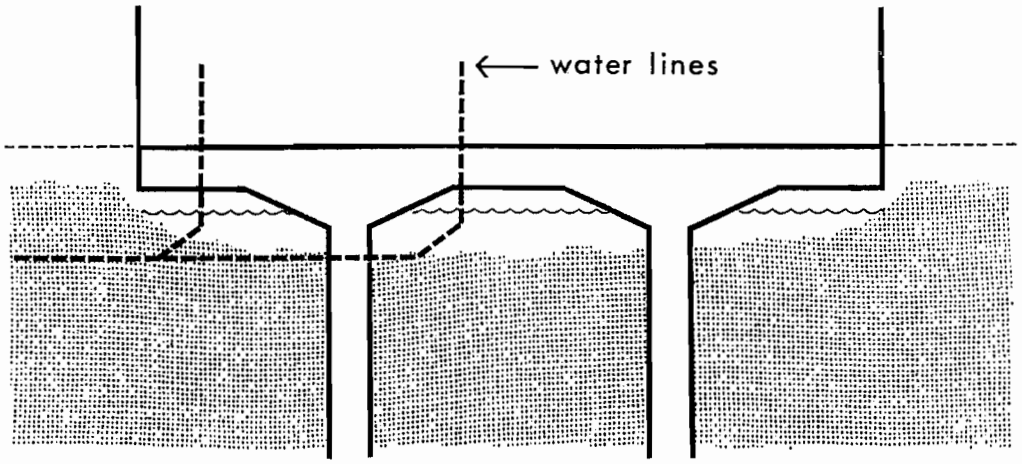
The impounded water under the slab on pile foundations provided a year-round *Culex pipiens* breeding site. In addition to our routine control program, the District received 20 - 30 requests for service each year from the residents of Harbortown. Lack of access made these breeding sources difficult to control; larval sampling was usually impossible and there was no way to knowing if treatments actually reached the target areas.

Solutions.

Problems with separated water lines and mosquito breeding continued over an eight-year period until a multi-million dollar, development-wide "retro-fitting" project was completed in 1987. These repairs were the result of a lawsuit against the developer by the Harbortown Homeowners Association. (Harbortown was the first in a series of "retro-fit" projects in the area).

Extensive underground excavation was required to gain access to repair separated lines underneath the pile-supported foundations. Tunnels were installed for access to lines and to serve as gravity drains underneath the structures. The tunnels were layered one-foot deep with gravel and perforated pipes ("French drains") were installed to drain water off to the storm drain or lagoon system. Plastic siding was attached to the foundation perimeter of all units to close the gap created by soil settlement. This also served to exclude rodent populations. Boxes were installed next to the siding for access to the water service.

To date (early 1988), all separated lines have been repaired at Harbortown. However, last winter (1987), runoff from downspouts emptied into the access boxes and impounded water behind the foundation siding, creating new breeding sources.



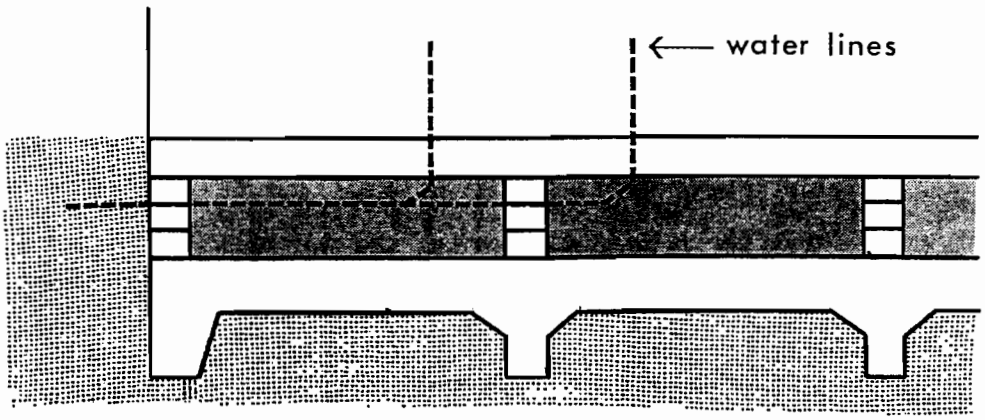
SLAB ON PILE FOUNDATION

soil level before subsidence

soil after subsidence

impounded water

Figure 1.



"FLOATING" SLAB FOUNDATION

soil level

accessible sub-area

Figure 2.

Fortunately, the solution in this case is less costly: the downspouts could be directed away from the access boxes. The District is currently working with the management company at Harbortown to eliminate this problem.

Trends in Construction.

There is a trend in the City of San Mateo towards the use of "floating" slab foundations and away from pile-supported structures. This is due to the lower cost of the "floating" slab and the failures associated with slab on pile foundations (utility connection separations and uneven settling and cracking in entrance areas).

The trend towards the use of a "floating" slab foundation in new construction is expected to reduce water line breaks. The "floating" slab settles with subsiding soil and eliminates the void underneath. This void is a feature of the slab on pile foundation and is where water impounds.

Flexible utility connections are now widely used in the City of San Mateo, but state of the art engineering is continuing to fail over time.

Recommendations.

Request soils reports on areas to be developed on landfill or reclaimed marsh; reports are available from City Building Departments. They include information on the quality of land fill, expected rates of soil settlement and its effect on utilities and recommendations concerning foundation design, drainage and runoff.

Make arrangements for your agency to receive Environmental Impact Reports from cities within your jurisdiction. Take this opportunity to address potential problems with proposed developments in advance of project approval.

As soon as you become aware of water impounding under or around foundations where there is soil subsidence, contact the property owner and the City Building Department.

Before "retro-fit", or major repair work is started, find out what kinds of changes are planned. Check to see that no new breeding sources are created as a result of corrective work.

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MOSQUITO CONTROL IN THE TWENTY FIRST CENTURY

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A year ago the Ad Hoc 21st Century Committee was formed and directed by the CMVCA president Charles Hansen to look at Vector Control Needs of the Future, to see if districts are preparing themselves for foreseeable needs. The members of the Committee were Gilbert L. Challet, OCVCD; Bruce F. Eldridge, Ph.D., UCD; Don J. Womeldorf, EMB; Steven S. Balling, Ph.D., NDVECC and myself, from CCMAD.

To look into the future isn't anything new; people have been doing it for many years, even in mosquito control. Some notable people in the CMVCA have made predictions, including Richard Peters, Art Geib, Howard Greenfield and Mel Oldham. To their credit, they foresaw many of today's issues: the growth of California (Peters 1962); the need for certification and training of technicians (Geib 1969); the use of highly sophisticated computers (Greenfield 1969); husbandry of the "super-fish" *Gambusia affinis* and the problems of waste water (Peters 1962). Art Geib (1969) gave us a list of 10 questions that are just as applicable today, including topics such as in-service training and employee qualifications. Mel Oldham (1969) even expected pasture *Aedes* to be gone due to subdivisions and the escalating costs of farming. He's probably not too far off. So it's time for another view.

Much has happened since 1970. Urbanization has spread throughout the state, technology has improved by leaps and bounds, employee-management relations have gone through different phases and politics has had many tidal shifts: from the revenue cuts of Proposition 13 to legislation on pesticide labeling for endangered species. New diseases such as Lyme disease have been identified and we have seen a resurgence of SLE in areas where it was least expected. In a nutshell, what the Committee sees is more of the same at a greater level of intensity. Yes, Future Shock has arrived. Everything is happening with a greater degree of complexity and a faster pace than in the past. We don't see things slowing down. In fact, like computer technology, we expect the pace to continue to speed up, although it may eventually plateau.

The next century, you might ask, why that's light years away. Not so. For anyone over 40, 1970 was just a few years away and we were raging over the Vietnam War and 1984 was still a piece of fiction and a computer was something only for the elite.

Mosquito control in the 21st century is such a broad subject that we broke it down into 5 main categories: Administration, Operations, Research, Training and Services. As I discuss each topic it will become apparent that our vision is for the most part restricted to the next 10-20 years due to the limited time we had to explore the subject and the difficulty of the task. Most changes will occur gradually but we wouldn't be surprised to see some big shifts, should they occur. I will cite examples from time to time, give you a report card on our level of preparation, and summarize with a glimpse of the future.

Administration.

When we talk about administration, we are talking about administration of a public agency which includes both management of the district and the legislation which enables it to function.

Let me ask you: How many Districts are using computers? As you can see, there is a mixed response. Yet this is the day and age of Management Information Systems, large data bases in small computers, word processing, photocopiers & FAX machines, and office printing that can rival professional printers. The public, the press and business see these innovations as being necessary for effective use of personnel and productivity. This technology is rapidly improving and will continue to do so. The office of today is simply a different place than that of yesterday and we should not look back.

There is a growing concern that the political organization we now see may not survive beyond another 20 years. Other agencies have picked up vector control and utilized MAD legislation while MADs have not picked up other vector services, either through surveillance or control. Ultimately, what is important to the public is that the job gets done. That a service is provided which is felt to be necessary. If vectors such as the Asian cockroach continue to be introduced, the need for broader services than commonly done by MADs will continue and someone will get the assignment. The public and their elected officials will continue to try to eliminate duplicate services whenever possible. Having both vector control and mosquito abate-

ment operating within the same political boundaries will eventually become unacceptable.

Operations.

Although the mainstay of mosquito control will continue to be field-oriented physical work, like many other elements of our society, we will see a trend toward greater sophistication, in sampling, treating of sources, data entry and analysis, and far less reliance on pesticides. For those of you who are skeptical, look at agricultural entomology. It is not what it used to be. It's much more high tech., with a more solid basis in sampling and selective treatments. It is becoming commonplace to incorporate field data onto portable datapods or field computers. You can see the trend when you to go conferences and look at the changes in equipment. Portable computers are now available for field analysis of ULV droplets and aerial spray accuracy, once the domain of research technology.

There will be much less reliance on the use of traditional pesticides as alternate materials continue to become more refined, more effective and competitively priced. Even if the price of biological insecticides doesn't drop, public pressure and increased regulations will continue to diminish the use of traditional materials such as many of the organophosphates.

Routine activities such as mapping will all be done with computer graphics programs by staff or field personnel with aptitudes and training for this. Mapping as we know it will be a thing of the past. A month's effort will be compressed into a few minutes.

For large scale sources, such as rice fields, marshes and metropolitan areas, aerial surveillance and large scale applications will become much more sophisticated through satellite technology and remote sensing. Research and limited field trials have already been conducted to demonstrate its feasibility, and several papers have been given on the subject at state and national conferences.

Population monitoring will come of age and field treatments will become based upon action thresholds as currently done with many agricultural pests. Treatment decisions will be more consistent, based upon life table information which take survival trends and dispersal habits into account. Much information is available but it is not yet standardized into operational programs. These programs will become sophisticated enough to correlate far more information than currently collected. Analysis of urban pest levels will also improve dramatically.

There will be many spinoff benefits from research, including field introductions of modified vector populations which will be resistant to infection by disease pathogens. This won't change their pest impact, just the disease level. We will see improved biological control husbandry at the operational level and far better application equipment such as miniaturized sprayers, either hand-held or power equipment, for easier use and portability. Pesticides will be much more refined, with better spreading, emulsifying or floating characteristics, and require far less material to be applied per acre. Signs of this already exist in materials such as Aerosurf, where oz/A replaces gals/A.

Research.

If you think research is high tech now, watch out for the next 20 years. The need for field analysis will not change, but the methods of doing so will. As with many other professions, equipment and capabilities are going through a transformation. The biggest issue will probably be adequate funding to support the equipment and personnel needed to conduct research on a technological level comparable to other hard sciences, and to entice graduate students into this branch of entomology.

There will be continued research on vector-borne diseases with a much better understanding of epidemiology, unravelling many of the unanswered questions of today and opening up other Pandora's boxes along the way. Methods of detection and identification of human pathogens in mosquitoes and vertebrate hosts have been improving, so agencies will be alerted much faster than at present.

The interaction between the University and MADs will improve for several reasons: computer technology will allow direct communication to UC and expand Districts' capabilities through library and database programs. As research improves, technical surveillance by Districts will probably replace the current efforts by the State Health Department.

Much more emphasis will be placed on population dynamics of both people and vectors. Districts will have modeling programs with a far greater level of credibility and accuracy. When someone asks the age old question of how the mosquitoes are this year, we'll eventually be able to answer them, and much more.

More emphasis will be placed on a system for the monitoring of mosquito populations by species, source, habitat, region and state, eventually spinning off to an even larger scale. This is an area ripe for development and it is only through recent ad-

vancements with computers that we'll be able to do this. These programs will take time and money and are just now gaining momentum through efforts such as the Ad Hoc Computer Applications Committee.

Some researchers feel new classes of pesticides will also be developed, largely due to the relatively new field of genetic engineering. However, the traditional problems of developing and registering new compounds, as recently experienced with public resistance to Ice Minus in Monterey County, coupled with the limited market of mosquito control, will probably retard their availability.

Training.

The seeds of this were sown back in the late 60's by people like Dick Peters and Art Geib, but it is just now coming of age and the time is ripe for it. Gil Challet (pers. comm.) has spoken of the need for management training and I couldn't agree more with him. There have been several influential books written in this decade that analyzed the deficiencies in American business, the need for change if the workforce is to become more productive, and hence, more competitive. The public sector is not immune to these social issues and forces. What is good for business is often good for government, and the need for technical training and employee understanding has never been greater. To its credit, the CMVCA initiated a continuing education program for certification which has been very successful. As a result, we foresee the overall level of operational training increasing somewhat, and then leveling off within 5-10 years, resulting in better trained field employees.

MADs are small agencies, but that doesn't mean they are exempt from personnel problems. Today's employees want more out of their job than a paycheck, and it is no longer enough to have an organizational chart and job descriptions. Consequently, management needs to be trained in the art of evaluation, delegation, negotiation, public affairs and other responsibilities in order to stay effective. If we expect today's employees to be productive 20 years from now, we need to start preparing our staff to deal with these problems now. The solution lies in the field of management training, as discussed by Gil.

However, there is also a great need for administrative training, which to date has been essentially overlooked. Many businesses, and some public agencies, send their staff to intensive training courses, from a few days to several weeks, and think nothing of it. A look at the comparison of the technical vs the administrative training we now conduct shows that an overwhelming majority of Districts send their staff to technical conferences, but few send them to courses on management and public administration. Both are essential to run the District. As public agencies, we should stay as current in administration as we do in our operations.

Services: Surveillance.

There are two modes of operation within the CMVCA: mosquito control and vector control. Mosquitoes are here to stay and new pests will undoubtedly continue to appear. Consider the recent introductions of the Asian Cockroach to Florida and the widespread dispersal of the so-called Asian Tiger mosquito in a very short period of time, neither of which can be ignored. Yet there is a noticeable difference between the two. MADs conduct surveillance and control of the tiger mosquito and have achieved varying degrees of success throughout the country. There is no such program for the cockroach because no one is automatically responsible for controlling it. Eventually, someone will have to take charge. There is little doubt there will be continued need for surveillance of other vectors such as these which are not currently targets of control.

We all know the public is not sympathetic to hearing "its's not in our jurisdiction", etc. We know that Districts can be very responsive, with effective programs which include surveillance and documentation of vector disease activity, if nothing else. Most of the diseases we know today will be still be with us and we can expect introductions of new ones too. In fact, many expect the distribution and number of cases to expand as people continue to migrate to the west, bringing host and vector closer together. Consequently, the burden of disease surveillance will shift to those agencies directly responsible for control programs.

Report Card: This is only a review of the above five generalized categories. This is not a report on individual districts, as some would do with safety inspections, but a report on the overall trends seen in the Association to date. Unfortunately, most Districts have spent the last ten-years recouping from the financial cuts of Proposition 13, which put everyone in a survival mode rather than a planning and growth mode. Most Districts have adapted one way or another and to everyone's credit, nobody is completely unprepared for the future.

Administration: Districts range from well equipped offices, flexible enough to deal with the challenges of today, to those that are neither prepared nor willing to adjust to changes. The facts are very apparent at this point. Where once a typewriter sufficed, now word processing is essential. Today's offices can be highly efficient hubs of activity. The cost aren't that high and training is available. The office is the first line of communication with the public and local businesses. The impact of this should not be underestimated.

We get mixed reviews for our politics. On the one hand we see resurgence of Health Department activity where once it had waned, but on the other hand we see little interest by MADs to widen their activities. The Committee isn't passing judgement one any on agency, but the implications are clear: no one agency has the legislative right to mosquito control. Where there is a need for a service, the public will respond accordingly and eventually dictate not only the structure of the agency but the services offered. The CMVCA recognized the need for vector control many years ago but there is still tremendous resistance to broadening and standardizing services.

Operations get pretty high ratings throughout the state. This is one of the Association's strengths, and it shows. We are not only staying with the times, but new equipment and techniques are quickly incorporated into programs. In fact, Districts have been responsible for many innovations and technological improvements, including power sprayers, fish transport systems and ULV sprayers. But as technology changes and becomes more miniaturized and solid state, this will be much more difficult for Districts to continue.

Research in many ways forges ahead with the best of trends and gets pretty good reviews. Their record has been good and we expect it to continue, funding provided. However, the interactions between UC and MADs has had its ups and downs. There have been shortcomings on both sides regarding research objectives and the needs of the Association.

Training gets good reviews for operations and poor reviews for administration. Recent actions still show we focus primarily on field personnel and overlook administrative needs.

Services also gets mixed reviews because there is little consensus on which services we should offer. Although there are over 1600 certified vector control technicians employed by MADs, only a few Districts actually conduct broadscale vector control. Most only focus on mosquito control. Our services aren't backing up our training programs so it seems we have the cart before the horse.

Summary.

It's difficult to forecast events, as we all know. Yet if we don't look ahead from time to time, we do ourselves a disservice. Besides, it's a lot of fun. Picture, if you will, the MAD of the future operating with remote control miniaturized fixed-wing aircraft with optical scanners that can identify the stage of mosquito larvae in the water and disperse pesticides on command. These planes would be capable of flying down creeks, flood control channels and under power lines and fly in poor weather conditions, if needed, to check levees and tidegates, etc. Picture tiny robots scurrying through storm drains, taking pictures, collecting samples and communicating back to the office. Picture U-2 aircraft at 100,000 ft. doing broad-scale or pinpoint surveillance with infrared photography, gathering and assimilating data on habitats, vector populations and their dispersal. Some of this is currently being done, and all is very possible within the next 10-20 years. Whether it will happen or not depends a lot on the rate of progress we make along the way, as summarized below.

It is the consensus of this Committee that in the next decade, as the year 2000 approaches, we will see better trained personnel at all levels, from operations to management. We will see the development of expert systems for field control programs, utilizing better equipment, particularly for surveillance and data analysis. We will see expanded research, more technological than at current levels and more interrelated with Districts through computer networks.

The overall impact of this will be greatly improved administrative and operational capabilities. In fact, we expect the employees of the future to be much more technical than at present, with greater abilities to deal with large scale data analysis and have much more sophisticated methods of surveillance and control. Their enhanced training and public education programs will undoubtedly improve their overall public image, which is essential if they are to be remembered should revenues again become scarce.

In the long run, we believe the most effective Districts will be those that are flexible and responsive to community needs and constantly interact with the local community. Those that do not will see revenues and services slip to other agencies, perhaps not as operationally oriented as MADs,

perhaps without the research capabilities of the University, but with the willingness to serve. The most effective Districts will be those that anticipate change, train their staff accordingly, and interact with the Community at a professional, technical level.

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RELATIONSHIP OF THE STATE DEPARTMENT OF HEALTH SERVICES TO MOSQUITO ABATEMENT DISTRICTS[1]

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The relationship between the Department of Health Services (DHS) and mosquito abatement districts is an evolving one. (For this discussion, mosquito abatement districts, optionally called vector control districts, and pest abatement districts will collectively be referred to simply as "districts". The term "local vector control agency" means not only districts but other governmental organizations providing services to the public.) To understand this dynamic relationship, we must identify the DHS and then look at how districts fit into the broad local-government vector control delivery system. Because this relationship is changing, we will also want to consider the past, the present, and the future.

The DHS.

To the local vector control agency, the most visible arm of the DHS is the unit originally called the Bureau of Vector Control. This became, in turn, the Bureau of Vector Control and Solid Waste Management, the Vector Control Section, the Vector and Waste Management Section, the Vector Biology and Control Section, the Vector Biology and Control Branch, the Vector Surveillance and Control Branch, and now the Environmental Management Branch--all this within the Department of Public Health, the Department of Health, and now the Department of Health Services.

Less visible, but vital to local programs, are some key support units within the DHS. The biomedical laboratories, notably the Viral and Rickettsial Disease Laboratory and the Microbial Disease Laboratory, make the analyses of specimens needed to demonstrate activity of disease pathogens in nature, which guide program actions. The Infectious Disease Branch provides epidemiological support and analysis in the event of a disease outbreak, and its Veterinary Unit is partner to the statewide mosquitoborne encephalitis surveillance system.

There is an individual within the DHS who is rarely seen by districts but whose decisions and opinions can be very important. That is the director. The director is empowered to set conditions governing cooperative agreements with districts. Cooperative agreements have been used as fiscal vehicles and to provide relief from excessive pesticide regulations and from restrictions upon land and water management for mosquito prevention. Furthermore, under both the mosquito abatement district and pest abatement district laws, the director is empowered to adjudicate disputes between the district and state and local agencies in mosquito abatement matters. As the state's top health official, the decision of the director is final and conclusive. In practice, the director delegates his authority to lower management.

Mosquito Abatement Districts and Other Entities of Local Government.

Every county is required to include a vector control element in its health program activities. In the smaller counties, this is limited to each sanitarian's answering questions from the public. A larger county may have a vector ecologist on staff who is charged with doing surveillance, conducting investigations in response to complaints, and providing information to the public on means of self-protection from vector attack.

Some communities have chosen to fund agencies to provide full vector control services to the public. There is a great deal of flexibility available to communities in choosing a system for delivering vector control services. A community which desires to provide itself with protection from mosquitoes, rats, biting gnats, or any other insect or animal of public health importance has the ability to choose the form of government it considers to be most appropriate for the job. In the great majority of cases, the district has been chosen. However, several other forms of government have been selected. Currently, there are in total, 70 units of local government involved in direct-intervention vector control. Of the total, 47 (67%) are mosquito abatement districts, some, by their option, calling themselves vector control districts; 11 (16%) are county programs, usually within the local environmental health agency; 7 (10%) are cities; 2 (3%)

[1]Presented at Trustees' Session, January 30, 1988.

are pest abatement districts, now with almost exactly the same powers as MADs under the law; 2 (3%) are county service areas; and in one instance (1%), vector control is provided by a water district.

In summary, all of California is covered by some form of governmental agency vector control, but there are two distinct levels. At the first level, vector control typically is reactive and usually is limited to a health department's providing information to an individual or a neighborhood in response to a complaint. At the second level, a governmental agency directly intervenes to prevent the public from unacceptable exposure to attack by organisms which transmit disease pathogens or are themselves injurious or annoying.

We now turn to the relationship between a district and a local health entity. A district engaged in vector control always coexists with a county (sometimes city) health or environmental health agency. The county program may be a basic, first-level one, or it may conduct an operational program dealing with vectors other than mosquitoes. Usually, the district and the county have very little to do with one another. Generally, staff of both organizations know one another. Often, the county will ask the district's entomologist to advise on various vector problems or to identify insect specimens, or the district will redirect inquiries from the public that properly should go to the county. Day-to-day operations, though, usually do not require close coordination with the county.

This all changes in the event of a disease outbreak. Then the district and many units within the county's health program suddenly begin to deal very closely with each other. The county's several health-related disciplines and their corresponding state counterparts are brought in. Authorities and responsibilities of the various entities become confused. To avoid these problems, we urge districts to prepare a plan in advance of need, bringing in county and state units as appropriate, so that events can proceed in an orderly fashion when a disease outbreak happens. Then the district can go about doing its job with a minimum of hindrance.

The variety of local vector control delivery systems is reflected in the variety of services provided by the DHS to local programs. Some of the largest local programs are budgeted and staffed greater than the Environmental Management Branch, and so the need for the state to provide hands-on service is minimal. In some of the least populous of the counties, any vector control emergency is beyond the ability of local staff to deal with, and so the Environmental Management Branch is called upon to give direct aid. An irony of 1978's Proposition 13 is that while it cut MAD funding just about in half, legislative action in the following few years culminated in passage in 1983, 1984, and 1985 of bills which recodified the mosquito abatement district and pest abatement district laws and provided districts with funding options not readily available to governmental entities receiving their revenues solely from property taxes. Districts may choose among special taxes, service charges, or benefit assessments to augment money received from property taxes. The potential outcome is that districts are less in need of direct technical support from the state than are other types of programs, although few are in a position to provide their own laboratory and epidemiological services. The differences among agencies' capabilities is currently changing their relationships with the DHS.

The Past, the Present and the Future.

The new Bureau of Vector Control was formed July 1, 1947. Its first Chief, Arve Dahl, gave its history and set forth its plans for the future in a paper published in the Proceedings of this Association (Dahl 1948). The BVC, as it is still fondly known by old-timers, was a consolidation of the Mosquito Control Section (established in 1946 and staffed by public health engineers and entomologists to administer subvention funds of $400,000 provided by the Legislature to help get new mosquito control programs started in response to fears about mosquitoborne diseases being introduced by returning World War II veterans) and the Sanitary Inspections Section (established in 1916 to direct the Department's activities in plague and rodent control). Under a fundamental plan of operation, the BVC was to be guided by an advisory committee (still in existence today) and was to carry out programs in 3 major areas. These were: (1) endemic surveys to continually evaluate vector-borne diseases of actual or potential danger to the people of the State, (2) studies and demonstrations, which were to bring into use in California those techniques and programs used elsewhere, and (3) actions to deal with control of vectors at the State and local level. The staffing was expected to be 40 full-time, permanent biologist, engineers, and others. During the height of the field season, there were expected to be 62 personnel. (In passing, I should note that the $400,000 subvention declined steadily over the years and finally was phased out completely in the 1960s. The Legislature has never seen fit to restore ongoing state assistance.)

Succeeding Arve Dahl as BVC Chief was Richard F. Peters, who held the post for nearly 30 years and who shaped the California program into a national model, emulated by those states that could do so and envied by those that could not. Soon after becoming Chief in July, 1951, Dick Peters (1952) appeared before this Association to emphasize that "The keynote of our program centers upon the word 'service' in its broadest possible sense." He offered the specialized technical services of biologists and engineers. He also pointed out the diversity of and demands upon the BVC staff: "Our direct relationship to local health departments and other local agencies and the resulting necessity for developing vector control programs pertaining to flies, gnats, ticks and other arthropods, as well as domestic and field rodent, in addition to other miscellaneous activities, all beyond mosquitoes, are such that we are frequently extended beyond our staff's numerical capability." In that same presentation, he reported upon a meeting of the Vector Control Advisory Committee, with its several recommendations, one of which led to the establishment of an investigational program which was the forerunner to the current research activities of the University of California. He advocated keeping and applying records to guide operational timing and the deploying of resources, the use of source reduction and multiagency involvement, and multidisciplinary approaches to problem solving. Years later, these concepts would be "discovered" in agriculture and called "integrated pest management."

Over a quarter-century later, Dick Peters (1978), along with a panel of staff members, again addressed this Association. The program had maintained its direction toward local-agency support: "The (state program) has a long history of emphasizing state-local program complementation as its basic policy. Certainly the state has no proper role in conducting routine vector control operations. Accordingly, our functions are largely qualitative, involving technical and administrative services which seek to enhance local vector control programs. Thus, surveys, surveillance, investigations, demonstrations, training, consultation, evaluation and emergency actions are our principal functions. We are part of a broad environmental health program which recognizes a close interface between health, safety, environmental protection, consumer protection, housing, agriculture, recreation and conservation and the assortment of federal, state and local agencies and private interests which interrelate, each seeking to serve its major objective." The topics addressed by staff who were panel participants reveal the scope of the program: mosquito and disease suppression; plague surveillance and suppression; the domestic rat problem within California; meeting the community need for the services of a vector control specialist; and the health-related aspects of solid waste management. This latter topic serves as a reminder that the state's solid waste management program had its genesis within the BVC in the 1950s and became the California Waste Management Board in 1972. The huge program of what is now Toxic Substances Control was assigned to us in that same year, soon outgrowing its parent and becoming a separate program.

Dick Peters retired at the end of 1978, and in February, 1979, I took the helm of what nearly proved to be the Titanic. Our then-Governor Jerry Brown, in response to the budget-cutting fervor following 1978's Proposition 13, called for a massive reduction in force in the Department of Health Services. Our program was selected for extensive cuts. When the smoke cleared, and following specific legislative mandates given us later that same year, we were left with a program charged with actions in five areas: providing consultation and assistance to local vector control agencies; surveillance of vectors and diseases; emergency vector control; local agency technician training and certification; and public education. A few years later we were assigned authority over importation into California of vectors which are not native to the state. To do the job, we were cut by 21 personnel and left with 25 staff, most of whom were biologists, and two of whom were engineers. There were also a few clerical and administrative support positions.

Since that time and to the present, our program has continued to provide, to the extent possible, services to local vector control agencies. We have not been able to regain staffing for our vector surveillance and control function even though we have increased considerably in size and scope to include all of the responsibilities outlined by Arve Dahl and Dick Peters, but also such activities as wastewater management, which includes some involvement with mosquito prevention, and a number of fascinating but completely non-vector responsibilities in environmental radiation management, nuclear emergency response planning, and low-level radioactive waste management. It is for those reasons that we have chosen to call ourselves by the broad title of "Environmental Management Branch."

What, then, of the future? As the century's end approaches, at least close enough so that is reasonable to think about, it is timely to cogitate upon the forthcoming years. In 1984, I appointed a group of our staff to a Committee of the Future. They were charged with examining general trends and were asked to interview managers of districts and directors of environmental health to see what the local agencies anticipated. The committee, in its final report (Hansgen et al. 1988), cited many "megatrends" identified by Naisbitt in his 1984 book by the same name. Some of particular interest include: our society is shifting from industrial to informational (need-to-know rather than need-to-do); there are demands for increased outdoor recreation; Americans are decentralizing into small towns and rural areas (yet, as we know, California continues to urbanize); as citizens, we are demanding greater participation in government and decision-making; and we are shifting, nationally, from north to south, with population growth resulting in sunny states including California. Highlights of trends more specific to vector surveillance and control included: the growing complexity of decision making, as the numbers of choices and the amount of information increase; the likelihood that increased travel and trade will create more opportunity for introduction of new vectors and diseases; anticipation that new materials and techniques would continue to be developed, but with a very limited market and so probably unprofitable for private industry; and the continuing limitations on pesticide use.

The heads of county and municipal agencies were asked which vectors and disease will be most important in the future. They ranked mosquitoes and mosquitoborne disease first, domestic rodents second, and plague third. Regarding changes in programs, they see increased emphasis on domestic flies and roof rats; changes resulting from urban and industrial development; using special districts as a means of getting a job done and as a mechanism for funding; more surveillance work; less reliance upon pesticides; and more reliance on land use controls which will lead to vector prevention. They look to the State for assistance in these areas in order of priority: (1) consultations and technical guidance, (2) surveillance, (3) training, (4) public education, (5) emergency control, and (6) enforcement and legal assistance.

As expected, the managers of districts focused upon mosquitoes. In the future, they see that *Culex tarsalis* and encephalitis will be the number one priority, with *Anopheles* spp. and malaria second, and *Cx. pipiens* complex and St. Louis encephalitis third. Program-change emphases will be in this order: more work on *Aedes sierrensis* and dog heartworm; there will be changes due to increasing urban and industrial development; geographical areas of service will increase; additional local funding through available mechanisms will be sought; surveillance will increase; chemical control will depend more heavily on bio-rational rather than conventional pesticides; land-use control and other legal enforcement mechanisms, in support of source reduction, will be relied upon more; and the use of biocontrol will increase. State assistance will be requested in this priority order: (1) surveillance, (2) consultation and technical guidance, (3) enforcement and legal assistance, (4) training, (5) emergency control, and (6) public education.

Two points in the committee's report need emphasis. One is information exchange. We have begun using computers to get surveillance and other data to you as rapidly as possible using an electronic bulletin board called "Vector Bytes", and expect that service to be expanded. A joint electronic information exchange system among your Association, the University of California and the DHS is under development. The other is the whole area of legal controls. You may ask us to increase our regulatory role, not only in support of your activities but directly over your programs. You may choose to have us as your regulator, since we, like you, are engaged in public health. Twenty years ago, you asked us to develop the cooperative agreement relationship which has allowed you relative freedom to carry out your chemical and physical control programs. Fifteen years ago, you asked us to impose a certification requirement upon your employees, and currently you are asking us to enforce mandated continuing education upon your certified technicians.

We will evaluate in detail the committee's report and its recommendations to meet the needs identified by the agencies whom we serve, and will implement program changes to accommodate as much of the demand as is possible within available resources. As we do the evaluation, we will necessarily take into account the ability of local agencies to do things for themselves.

While much of our current and planned work is very similar to what we did 40 years ago, it is and will be because you still need us to do it, not because we've always done it that way. The Vector Control Advisory Committee, which includes representation from your Association, will continue to

guide our actions. Within our legal, policy and fiscal limits, we will remain responsive to you and to the other local agencies to whom we provide services.

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COMMITTEE ACTIVITIES OF THE CALIFORNIA MOSQUITO AND VECTOR CONTROL ASSOCIATION, INC.

January 1987-January 1988

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Biological Control.-Robert L. Coykendall, Chairman.

This year's (1987) charge to the Committee was to continue to publish Bio Briefs - our Committee's newsletter. Three issues were disseminated to the membership this year. Incorporated with these newsletter issues were numerous Bio Notes - our one-sheet, informational handout representing biological control organisms. At this time, work has been completed on all originally planned Bio Notes.

The last charge to the Committee was to begin work on the revision of "Fishes in California Mosquito Control". A literature search was initiated to obtain as much new information as possible, and to that end, we completed our search and compiled a list of references which includes 349 citations. We are including it with this report to the membership and will begin to actually collect all the cited literature next year and proceed with the actual rewriting of this guide.

Chemical Control.-Michael J. Wargo, Chairman.

This Committee has advised the CMVCA membership of the "Hazardous Waste Response Plan" requirements. The California Chamber of Commerce has three handbooks available which explain various aspects of complying with hazardous materials legislation requirements. We strongly recommend the three book set for each district.

We have printed a set of notebooks dealing with calibration of spray equipment, pesticides and Material Safety Data Sheets for most MAD chemicals. These notebooks are a guide to meet requirements necessary of all abatement districts.

Malcolm A. Thompson, Environment Management Branch Specialist, has started a resistance study utilizing a new adult *Cx. tarsalis* testing program with Malathion and Chlorpyrifos. The first year's results are surprising. Eventually, it will be expanded to include other insecticides and mosquito species. During 1988, larval mosquito testing will continue on a "by request" basis only.

Dimilin has an SLN for control of aquatic midges in noncrop lakes, ponds, channels and percolation basins developed for decorative/landscaping purposes. We are looking into expanding the use of Dimilin in mosquito control for a wider range of situations such as rice, dairy lagoons and others.

We will continue to encourage the development of new compounds and to expand the range of use of present compounds such as *Bti*, *B. sphaericus*, Dimilin and other pesticides.

Finally, this Committee will continue to keep the CMVCA membership advised of how new laws/regulations will affect district control programs.

Ad Hoc Computer Applications.-Bruce F. Eldridge, Ph.D., Chairman.

The Committee met quarterly during 1987, and devoted nearly all of its energy to preliminary planning for a state-wide computerized information system for mosquito and vector abatement activities. During the year, meetings were held with representatives from three major computer equipment manufacturers; American Telephone and Telegraph Corporation (AT&T), Digital Equipment Corporation (DEC), and International Business Machines, Inc. (IBM). Also, with funds provided by the Board of Directors of CMVCA, the Committee hired Dr. John Skinner to gather information on computerized databases.

Dr. Skinner provided the Committee with a final report on December 9. This 18 page report provides complete details on about 20 different databases of potential use to mosquito control personnel. Included in the report are databases dealing with pending legislation, toxicological data, endangered species, weather data, pesticide label information, and literature citations. The report includes information on costs, equipment needed, and descriptions of the information available.

As a result of meetings with AT&T, IBM, and DEC, the Committee has made several preliminary decisions as to the configuration of a potential system. These decisions include utilizing a mini-computer as a host computer, and using UNIX as an operating system for the host computer. Basic communication with the host will be by dial-up

[1]Vice President, California Mosquito and Vector Control Association, Inc.

modem from remote terminals and microcomputers, and communication with some databases and other large computers will be by some form of high speed link, such as X.25 communication.
During the coming year, the Committee hopes to complete planning and to present to the Board of Directors a complete recommendation for implementation, including start-up and operating cost estimates, as well as recommendation for funding of the project.

Continuing Education.-B. Fred Beams, Chairman.

The Committee has continued with the development and implementation of a statewide continuing education program for certified vector control technicians. Specific projects accomplished are as follows:

1. Approval by the CMVCA Board of Directors of a Continuing Education Program.
2. Drafting of legislative language for codification of the Continuing Education Program in the California State Health and Safety Code. Language submitted to legislative counsel January 14, 1988. Bill will be introduced tomorrow by Assemblyman Norm Waters. No Bill # as yet.
3. Alignment with California Community Colleges in each region to facilitate published continuing education requirements.
4. Approval of conference and seminar offerings for continuing education credit submitted by regions.
5. Chair of the CE Committee was appointed to the Ad Hoc Advisory Committee.

Entomology.-Major S. Dhillon, Ph.D., Chairman.

During 1987, the Committee was charged with two major tasks: 1. The arbovirus workshop and 2. the revision of "Field Guide to Common Mosquitoes of California".

1. Arbovirus Workshop: The arbovirus workshop was held from May 19 - 21 in Orange County. The program was put together by Dr. Jack Hazelrigg and it primarily covered an in-depth discussion of ecological and epidemiological aspects of St. Louis encephalitis and other arboviruses. The workshop was well attended by technical personnel from various districts. The workshop was organized by Mr. Fred Beams. Both Jack and Fred did an excellent job in organizing the seminar and I would like to thank both of them.
2. Revision of the Field Guide: To get an input from various districts for the improvement of the quality of the field guide, a questionnaire was mailed in February to all the districts. Twenty responses were received with comments and suggestions. A second questionnaire was mailed on January 13, 1988 to the agencies who did not respond to the first request. As of January 28, 1988 there are still 18 districts who have not returned the questionnaire with requested information. A list of mosquito species requested in Section "B" is an important part of the revision and is needed to complete the guide. For those agencies who have not responded to the second request, please do so as soon as possible.

The Committee is in the process of revising the field guide and hopefully we will complete the revision within a year. Dr. Robert Washino has agreed to loan us some of the photographs of mosquito drawings. These photographs will be incorporated into the new "Field Guide". I would like to express my gratitude to Bob.

Last, but not least, I would like to thank all the Committee members who have put their diligent efforts in the revision of the field guide.

Environmental & Liaison.-Fred C. Roberts, Chairman.

The activities of the Committee in 1987 were directed at problems created by the Environmental Protection Agency's (EPA) plan to implement the Endangered Species Act (ESA). President Hansen assigned the matter to our committee shortly after the plan had been unveiled. The Committee reviewed the plan, determined the impact it would have on mosquito control in California, looked at the options available to resolve the conflict and recommended a course of action to the California Mosquito & Vector Control Agency (CMVCA).

The Committee found that the plan for implementation of the ESA could severely restrict larvicides and endanger the public health of the citizens of California. The Committee recommended a course of action to President Hansen and the Board of Directors of the CMVCA which would have the CMVCA make a direct request to the EPA to correct their plans and thereby avoid conflicts with vector control. A position paper was

developed by the Committee describing the problem and recommending solutions. A plan to take legal and/or political action was developed and held in abeyance in case further action was considered necessary. The Board adopted the recommended course of action authorizing President Hansen to write the EPA and include the position paper.

The EPA, because of political pressures from the agricultural industry and perhaps due to concerns expressed by the CMVCA, has delayed implementation of the Act and will allow the State of California to develop a state-specific plan under the auspices of the California Department of Food and Agriculture. The new plan to implement the ESA has incorporated procedures by which the conflicts with mosquito and vector control should be resolved. Representatives of the Committee are working with the California Department of Food and Agriculture on the plan.

Legislative.-Douglas C. White, Chairman.

During 1987, the Committee in concert with our legislative advocate, Mr. Ralph Heim, reviewed and made recommendations on the following bills which were of special concern to the Association members.

1. AB 946, Sher; Dealing with the importation of tires and their inspection. The bill was chaptered, however a Federal program will make implementation redundant.
2. AB 761, Waters; Adds language to Health and Safety Code, Section 2200, C IV "as determined by the board" with regards to detrimental effects resulting for fly larval development.
Bill was chaptered.
3. AB 1308, Wright; Exempts local vector control agencies from certain hazardous waste fees.
Bill was chaptered.
4. SB 1018, Farr; MAD in Monterey, California which annexes property adjoining that county is not a multi-county district.
Bill was chaptered.
5. SB 269, Kopp; Would make districts subject to Proposition 65. Was amended to exempt vector control activities.
Bill died because of lack of financial support.
6. SB 1491, Marks; Concerned pesticide application by governmental agencies.
Defeated in committee.
7. SB 1630, Presley; Provides funds for Department of Fish and Game to enter into contracts with landowners to restrict land use for the conservation of waterfowl.
Bill was chaptered.
8. ACA 27, Johnson; Fees and assessments would require voter approval.
Bill not moved by author.

In January of 1988, final language for legislation mandating a continuing education program was submitted to the legislative counsel for review prior to becoming a bill.

Physical Control.-John R. Stroh, Chairman.

The Committee compiled a list of legal abatement procedures utilized by many districts. This information was solicited by a questionnaire sent out to all districts in June, 1987. The Committee is now prepared to organize this information and make it available to districts looking for information on legal abatement, along with making available alternative solutions to various legal problems regarding abatement procedures.

Videotape.-B. Fred Beams, Chairman.

The Committee accomplished its charge with the completion of the "Videotape Guidelines" consisting of the following:

1. Guidelines for Assuring Videotape Coverage.
    a. Videotape Coverage Responsibility
    b. Type of Videotape Equipment
    c. Subject Material
    d. Events
    e. Disposition of Completed Videotapes
2. Videotape and Equipment Recommendations.
    a. Video Camera
    b. Videotape Recorders
    c. Remote Power Packs
    d. Videotape Recommendations
    e. Videotape Care and Storage
3. Master Copy of Videotape Production Log.

The 56th Annual Conference was videotaped and the master copies will be turned over to the Executive Director.

Future Trustees' Sessions should be taped.

BACTERIAL MOSQUITO LARVICIDES: PRESENT STATUS OF KNOWLEDGE AND FUTURE DIRECTIONS FOR RESEARCH

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University of California, Davis and Riverside

Introduction.

Mosquito larvicides derived from spore-forming bacteria became a practical reality in 1982 with the registration and commercial availability of formulations of *Bacillus thuringiensis israelensis*, otherwise known as *Bti* or *Bacillus thuringiensis* serotype H-14. Various formulations are now widely used by mosquito abatement agencies across the United States. Presently, there is considerable research being conducted in California and elsewhere on *Bti*, as well as on other species and strains of bacteria which are toxic to mosquito larvae. This research runs the gamut from the very basic to the very applied. The purpose of this paper is to: (1) review the present status of knowledge of the bacterial species and strains toxic to mosquito larvae, (2) summarize UC research on mosquitocidal bacteria, and (3) relate current research directions to goals for better mosquito larvicides.

Background.

The initial isolation of a member of the group now recognized as *Bacillus thuringiensis* (*Bt*) was made in 1902 from diseased silkworms by Ishiwata (Lüthy et al. 1982). This isolate is known today as *B. t. sotto*, or serotype 4a, 4b[1]. In 1915, Berliner isolated a bacterium from larvae of the Mediterranean flour moth from a mill in what is now the Thuringia District of the German Democratic Republic (East Germany). Berliner named the species *Bacillus thuringiensis*, and the strain is currently recognized as *B. t. thuringiensis*, or serotype 1. Berliner noted the presence of a parasporal body, and suggested the potential of the bacterium as an insecticide (Lüthy et al. 1982). During the following decades, additional strains of *Bt* were isolated, but none were toxic to insects other than lepidopterous larvae. One of the most potent of these was *B. t. kurstaki*, isolated in 1962 (Lüthy et al. 1982).

In 1976, a new strain of *Bt* was isolated from mosquito larvae collected in a small pond in Negev, Israel. This strain became known as *Bti*, or serotype 14. It was toxic to larvae of mosquitoes and black flies, but harmless to most other organisms. Following intense research in various parts of the world, materials based on this strain were registered by EPA for use in the United States against mosquitoes and black flies. Padua et al. (1980, 1984) reported the isolation of mosquitocidal strains of *B. t. darmstadiensis* (serotype 10) and *B. t. morrisoni* (serotype 8a, 8b). An isolate (PG-14) of the latter serotype is currently used for mosquito control in the Philippines (Lacey and Undeen 1986). Ohba and Aizawa (1979) described a new subspecies, *B. t. kyushuensis*, isolated from a sericulture farm in Japan, and reported that the strain was preferentially toxic to larvae of *Culex tritaeniorhynchus*.

The name *Bacillus sphaericus* (*Bs*) was applied by Neide in 1904 to a common aerobic bacterium that formed spherical spores (Yousten 1984). This species is usually a saprophyte, living in many soil and aquatic habitats (Davidson 1982). Many strains of this species have since been isolated and studied, and although a number of varietal names have been used in the past, none are now generally accepted.

The first strain of *Bs* found to be toxic to mosquitoes was the Kellen K strain, isolated in 1965 in California from *Culiseta incidens* (Kellen et al. 1965). Since then, other strains having much higher toxicity to mosquitoes (but not to black flies) have been isolated. In spite of a high level of research activity into the possible use of this bacterial species as a mosquito larvicide, no strains are currently registered by EPA for use, and generally, research on *Bti* has overshadowed development efforts for *Bs*. The strains of *Bs* toxic for mosquitoes seem quite different from non-toxic strains, but other clear-cut differences have not been discovered sufficient to justify a separate specific name (Davidson 1982, Yousten 1984).

A complex and sometimes confusing terminology has developed in relation to the bacterial insecticides, with some of the terms being rendered obsolete as the status of our knowledge of this group of organisms improves. The bacteria of interest to this discussion are all aerobic spore-forming bacilli classified in the genus *Bacillus*, and further included in two species: *B. thuringiensis* and

[1]Serotypes, or serovars as they are sometimes called, are based on a comparison of antibodies to flagellar, or "H", antigens of these bacteria.

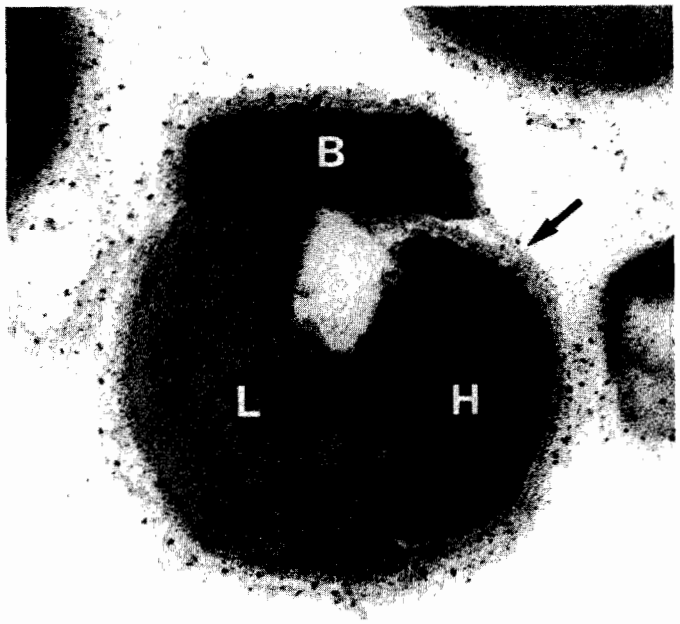

Figure 1.-Electron micrograph of a parasporal body of *Bacillus thuringiensis* subspecies *israelensis*. Note the three types of inclusions, (L) low and (H) electron density, and (B) the bar-shaped inclusion. The arrow points to the fibrous envelope that surrounds the inclusions. 60,000X.

B. sphaericus, with the former species being separated into numerous subgroups. Spores found in these bacteria are spherical bodies produced during a process in the life history of the bacteria called *sporulation* (Fig. 1). They are resistant to a wide variety of adverse environmental conditions, including relatively high temperature, desiccation, and some chemical agents. All of the strains of *Bt* and *Bs* so far studied which have mosquitocidal activity also produce, during sporulation, a structure variously called the crystal, the proteinaceous crystal, the delta endotoxin, the crystalline parasporal body, the parasporal body, the parasporal inclusion, or simply the paraspore (Heimpel 1967, Yousten 1984) (Table 1). Within this crystal resides a protein or proteins responsible for the toxic activity against mosquito larvae. In addition to several minor toxins, some strains of *Bt* also produce a beta-exotoxin having a wider range of toxic activity than the delta endotoxin (Dulmage and Aizawa 1982). This exotoxin, which has also been called the fly toxin, the thermostable toxin, and the thermostable fly factor (Heimpel 1967), is an atypical nucleotide. It is quite toxic to mammals by injection (but not by ingestion) and strains of *Bt* which produce this toxin can not be used as insecticides in the United States (Dulmage and Aizawa 1982).

Structure and mode of action of bacterial insecticides.

For both *Bti* and *Bs*, the proteins toxic to mosquitoes are contained in the parasporal bodies. Proteins associated with the parasporal bodies in both species are complex, and only recently have researchers been able to produce a fairly complete picture of the characteristics of the proteins involved in terms of relative toxicities, size, and interrelationships. The protein composition has been studied by a number of investigators; their work is summarized by Federici et al. (in press). For *Bti* (Table 2), there are probably four separate proteins associated with the parasporal body, distributed among three discrete types of parasporal body inclusions. The masses of these proteins in the intact parasporal body of *Bti* are 27, 65, 128, and 135 kDa[2], respectively. Early studies using gel chromatography detected only a single protein of 130 kDa in the high density inclusion of the parasporal body of *Bti*, but higher resolution methods detected two proteins: those having a mass of 128 and 135 kDa respectively (Federici et al. in press).

[2]The dalton is a unit of mass equal to 1.65979×10^{-24} grams. Thus a kilodalton(kDa) is 1.65979×10^{-21} grams.

Table 1.-General characteristics of mosquitocidal bacteria.

Species	Parasporal body	
	Number of inclusions	Shape
darmstadiensis	2	spherical
israelensis	3	"
kyushuensis	2	"
morrisoni (PG-14)	4	"
sphaericus 2362	1	polyhedral

Table 2.-Characteristics of parasporal body inclusions of *Bacillus thuringiensis israelensis*.

Inclusion	Characteristic	Proteins*
Large	Least electron dense, round to polyhedral, 4.3 nm lattice-spacing 40-50% of PB.	27 kDa
Bar	Moderately electron dense, rectangular in transverse section 7.8 nm lattice-spacing, 15-20% of PB.	65 kDa
High density	Highly electron dense, hemispherical to spherical, 20-25% of PB. May be two separate inclusions. Lamellae often present.	128 kDa 135 kDa

* Tentative assignments.

Table 3.-Characteristics of parasporal body inclusions of *Bacillus sphaericus*.

Inclusion	Characteristic	Proteins*
Single	Polyhedral, 6.3 nm lattice-spacing	125 kDa 110 kDa 63 kDa 43 kDa

* Tentative assignments.

In contrast to the intact parasporal body, additional proteins may appear when the parasporal body is dissolved in an appropriate solvent at high pH levels (>9.0). Because the major proteins present in the intact parasporal body show a corresponding decrease when the parasporal body is solubilized, it is thought that these new proteins are breakdown products resulting from the action of specific enzymes which are active only at high pH levels. The most significant of these proteins discovered so far is a 25 kDa protein which is cleaved from the 27 kDa protein. It is further believed that this solubilization is equivalent to what occurs naturally in the highly alkaline (pH 8-10) midgut of mosquito larvae.

The parasporal body of *Bti* is toxic to mosquitoes and certain other insects in either its intact form, or when dissolved in alkaline solvents. In the latter form, it is also toxic to mice when injected, and to a number of types of cells when introduced into various types of cell cultures (Thomas and Ellar 1983, Cheung et al. 1985). Although there is still considerable disagreement as to the specific toxicity of the various proteins associated with *Bti*, most now agree that the 25 kDa protein (the cleavage product of the 27 kDa protein) is responsible for this toxicity seen for mammalian cells. There is some dispute as to whether this protein is also toxic to mosquitoes. Current evidence also indicates that the 65, 128, and 135 kDa proteins are toxic to mosquitoes, and although detailed studies are beginning to shed light on these proteins, their specific role as mosquito toxins has not been determined. However, at this point, it appears that none of these proteins alone is as toxic per unit weight as the mixture of them found in the parasporal body.

In the case of *Bs*, the single parasporal inclusion consists of proteins having masses of 43, 63, 110, and 125 kDa (Baumann et al. 1985) (Table 3). Sporulating cells initially form the 125 kDa protein which is followed by the appearance of proteins of 110, 63, and 43 kDa (Broadwell and Baumann 1986). These 3 proteins have been purified and it has been shown that the 110 and 43 kDa proteins are toxic to mosquito larvae while the 63 kDa protein is not. Upon ingestion of the parasporal inclusions, mosquito larvae rapidly degrade the 110 and 63 kDa proteins and slowly convert the 43 kDa protein to one of 40 kDa (Baumann et al. 1985, Broadwell and Baumann 1987). The conversion of the 43 kDa protein to 40 kDa results in an activation of the toxin as indicated by a 54-fold decrease in the LC_{50} in tissue culture grown cells of *Culex quinquefasciatus* Say. Recently, 3 distinct genes coded for peptides immunologically related to the toxin have been cloned into *Eschericia coli* (Baumann et al. 1987). One 3.5 kilobase Hind III fragment has been recently sequenced and was shown to code for 2 peptides corresponding to the 63 and the 43 kDa proteins (Paul Baumann, personal communication). These results indicate that the previous suggestion that the 43 and 63 kDa proteins are derived from the 125 and 110 kDa proteins is untenable and that the 125 kDa (and its derivative the 110 kDa protein) constitute a distinct toxin.

Host ranges and environmental safety.

There are general differences in the host ranges of *Bti* and *Bs*, with the latter being the more specific, or having the narrower host range. The most notable difference between the two is that *Bti* is toxic to both mosquitoes and black flies, whereas *Bs* is toxic to mosquitoes only. There are also differences in the susceptibility of various groups of mosquitoes to the two types of microbial insecticides. *Aedes aegypti* is not susceptible to *Bs*, although some other *Aedes* species are susceptible to some degree, but less so than species of *Anopheles*, which are in turn, generally less susceptible than species of *Culex* (Yousten 1984). In general, *Bs* is less toxic to non-target organisms such as filter feeding nematocerous Diptera, than is *Bti*. *Bti* is toxic to a wide range of filter feeding Diptera of the suborder Nematocera, including mosquitoes, black flies, and some midges (Chironomidae). Predaceous aquatic insects are generally insusceptible to *Bti* and *Bs*. Culicine larvae are generally considered more susceptible to *Bti* than are anopheline larvae, but differences may be due to differences in feeding behavior rather than susceptibility to the toxins (Lacey and Undeen 1986).

Although some protein toxins of *Bti* released after dissolving of the parasporal body in high pH solvents are generally toxic to vertebrate and invertebrate cells, this is probably of little practical importance under most natural circumstances, because in nature, solubilization of the parasporal body usually takes place only in the highly alkaline gut of certain insects. Furthermore, no strains of *Bti* nor *Bs* examined so far produce the broad spectrum beta-exotoxin. There have been questions concerning the fate of toxins released after the death of mosquito larvae, and although attempts have been made to detect toxins in sediments underlying aquatic habitats treated with *Bti*, *Bs*, or both, no definitive results have so far been

Table 4.-Registered uses for *Bti* (information furnished by Ms. Barbara Kozusko, California Department of Health Services).

Product	EPA Reg. Number	Potency (Units/mg)	Remarks
Bactimos WP	43382-3	3500	Wettable powder
Bactimos FC	43382-8	1000	Flowable concentrate
Bactimos briquets	43382-10	400	Normal usage plus pre-flood
Bactimos granules	43382-16	175	Effective when emergent vegetation present
Bactimos pellets	43382-17	350	As above, plus aircraft dispersal
Teknar larvicide	11273-30	1500	Aqueous concentrate
Teknar granules	11273-38	260	As with Bactimos granules
Vectobac-WP	275-45	2000	Wettable powder
Vectobac-AS	275-52	600	Aqueous suspension
Vectobac-12 AS	275-66	1200	Aqueous suspension
Vectobac-G	275-50	200	Granules
Vectobac-SG	275-60	200	Sand granules

obtained to indicate that toxins can remain in these sediments in environmentally significant quantities (Katherine Donegan, personal communication).

Use of bacterial insecticides for mosquito control.

To date, only formulations of *Bti* are commercially available and are registered for use in mosquito control (Table 4). Formulations include wettable powders, aqueous suspensions, pellets, granules of various sizes and types, and slow release briquets. Formulations containing mixtures of *Bti* and other insecticides such as methoprene may be available in the future. Reports of large scale field trials with various formulations of both *Bti* and *Bs* are numerous (see Singer 1985 for a summary of trials with *Bs*; Lacey 1985 for *Bti*). Most of these reports support the idea that mosquito larvae can be killed by bacterial insecticides under real-world conditions of mosquito abatement. Success rates vary widely, however, with individual reports ranging from complete failure to complete success. This is not surprising, considering the relative complexity of this method of control in comparison with conventional chemical control. Factors known to affect the efficacy of mosquito control with bacterial insecticides include: species of mosquito, age of mosquito larvae, stage of mosquito larvae, water temperature, vegetative cover, degree of water pollution, especially presence of particulate pollutants, potency of insecticide, and amount of solar radiation (Lacey 1985). Water chemistry factors such as pH and concentration of cations such as Cl^- have not been shown to affect efficacy greatly within the range of values found in mosquito habitats (Lacey 1985).

Reports of detailed evaluations of mosquito control with bacterial insecticides under operational conditions are not nearly as common as reports of small plot experiments. Evaluations such as those of Parman 1986 and Kimball et al. 1986, however, have been consistent with findings from trials on a smaller scale.

Because bacterial insecticides are not equally effective under all environmental situations, several modifications in formulation and application have been developed. In situations where the water in which mosquitoes are breeding is polluted with high levels of suspended organic matter, dosage rates must be increased, often substantially (double or triple the usual rate). Granular and pelletized formulations of *Bti* are available where penetration is inhibited by emergent vegetation. To enhance the efficacy of *Bti* against surface-feeding anopheline larvae, formulations containing *Bti* and monomolecular films (Arosurf) have been used successfully. For use as pre-emergent treatment, slow release formulations of *Bti* are available.

Future research.

Biochemistry and toxicology.-- Considerable research is being conducted in California and elsewhere in an attempt to isolate, purify, and characterize the proteins associated with the parasporal bodies of microbial insecticides. Workers at UC Riverside are emphasizing strains of *Bt*, those at UC Davis-*Bs*. Although this represents a long-term approach to eventual improvement of microbial insecticides, it is an approach which is well justified. Any future attempts to understand the mode of action of these insecticides is dependent upon a thorough knowledge of the toxin or toxins present in the various species and strains of bacteria. A practical outgrowth of the studies may be the eventual production of purified toxins of far greater potency than existing formulations. This may permit the use of these agents in polluted environments presently unsuited for the use of bacterial insecticides. Since proteins toxic to mosquito larvae may be different than those having general cytotoxic properties, greater safety may also be realized.

We are just now scratching the surface of knowledge of the mode of action of these bacteria in killing mosquito larvae. Knowledge in this area may help in understanding the relationship between environmental factors and efficacy, host range, and the likelihood of physiological resistance. This in turn may permit establishment of effective strategies for insecticide resistance management. Dr. Sargeet Gill of the Department of Entomology, UC Riverside is studying the mode of action of *Bti* protein toxins in the mosquito midgut as well as in mosquito cell cultures. These studies are emphasizing the effect of the toxins on the cell membrane and the identification of receptor sites on membranes for the toxins. Studies such as these are critical to our understanding of host specificity and resistance development.

One of the most exciting areas of research involves attempts to genetically alter microbial organisms, and thus modify toxin-producing mechanisms. These studies may permit the eventual customization of bacterial strains for specific circumstances in terms of safety (both human and environmental), host range, site of application, and toxin yield. The number of environmental habitats where these insecticides can be used effectively may also be increased. Considerable progress has

already been achieved in this area, but much more remains to be done.

As noted above, we now know that the parasporal body of *Bti* contains four major proteins, those of 27, 65, 128, and 135 kDa. An immediate question that arises is whether all of these are essential to toxicity. If only one is, and existing evidence argues against this, then the simplest scenario is to engineer a bacterial strain that produces only this protein or its active form. If not, then using the methods of genetic engineering it should be possible to eliminate non-essential proteins, thereby increasing the toxicity of the parasporal body per unit weight. For example, suppose that only the 27 kDa and 128 kDa proteins are essential to toxicity. By deleting the genes for the 65 kDa and 135 kDa proteins and constructing a bacterial strain that only produces the 27 and 128 kDa proteins, we could potentially increase the toxicity per unit weight two-fold. To take this example further, we know that the 128 protein is cleaved by midgut enzymes to an active toxin of around 67 kDa. Thus, by modifying the gene for this protein so that it only produces the active toxin, we could further improve the toxicity per unit weight, perhaps to three- to four-fold that of the naturally occurring parasporal body. And considering the substantial evidence for synergism or potentiation between parasporal body proteins presented by Wu and Chang (1985) and Ibarra and Federici (1986), it may be possible to obtain even greater increases in toxicity per unit weight by altering the ratios of the proteins packaged in the parasporal body by the bacterium. Demonstration that any of these tactics is practical could lead to improved formulations of *Bti*.

There are similar possibilities for improving the toxicity of *Bs*. At present, only a small amount (<10%) of this bacterium's dry weight is toxin. The first objective, therefore, might be to improve the level of toxin production through classical genetic methods, or through genetic engineering. Another strategy made possible by the advances in genetic engineering would be to develop a bacterial strain that produces the most toxic proteins of *Bti* and *Bs* in the same cell.

The above examples are just a few of those made possible by the techniques of genetic engineering. While some may prove impractical or not cost-effective, this new technology offers enormous promise for the development of improved microbial larvicides.

Field studies.

Research is going on at UC Berkeley (Albany and Parlier Laboratories), UC Davis, and UC Riverside to determine the efficacy of bacterial insecticides in various ecological situations, and in combination with other mosquito control tactics. Studies conducted over the past few years have resulted in detailed recommendations for formulations and dosages of *Bti* (Table 5) and *Bs* (Table 6) in a variety of special habitats in California. Garcia (1986) has reviewed some of these data, with a plea for careful management of *Bti* use to avoid early development of physiological resistance. Although there has been no evidence of resistance to *Bti* in the field to date, there is little reason to believe that it will not eventually occur. It has been induced in a stored grain moth against *B. t. kurstacki* after a few generations of selection (McGaughey 1985), and in *Culex quinquefasciatus* against *Bti* after numerous generations of selection (Georghiou, unpublished).

There are still unanswered questions in regard to efficacy, safety, and integration of microbial insecticides into integrated mosquito programs. Badly needed are studies which determine the effect of pesticides introduced into aquatic environments in terms of all biological components present. Studies to determine the fate of toxins in aquatic environments are also needed. Are there indirect effects on other biological components, especially predators? Is overall control more effective when older rather than younger mosquito larvae are targeted, in spite of the fact that younger larvae are more susceptible? Are there cumulative mortality effects from the use of formulations combining conventional and microbial insecticides, and are such effects, if present, good? How can the presence of insecticidal particles be maximized in the larval feeding zone for various situations?

Human and Environmental safety.

As new formulations of microbial insecticides are developed, some having greater potency than present formulations, new questions concerning human and environmental safety will arise. As in the case of conventional pesticides, the burden of proof will be on the developer and manufacturer of such pesticides, but research results supporting claims of safety will be from users and user-supported groups.

Finally, it is necessary for mosquito abatement practitioners and applied researchers to participate

Table 5.-Customized applications of *Bti* in various habitats based on results of research conducted in California.

Habitat	Species of mosquito	Reference
Secondary-treated effluent applied to pastures	*Cx. tarsalis*	Garcia 1986
Algal mats in small streams	*An. franciscanus*	Garcia 1986
Waterfowl refuge ponds	*Ae. melanimon*	Garcia 1986
Rice fields	*An. freeborni* *Cx. tarsalis*	Kimball et al. 1986
	An. freeborni *Cx. tarsalis*	Kerwin & Washino 1983
Salt marshes	*Ae. dorsalis*	Garcia & Des Rochers 1980
	Ae. squamiger	Garcia et al. 1983
Dairy lagoons	*Cx. peus*	Mulla et al. 1980
Irrigated pastures	*Ae. nigromaculis* *Ps. columbiae*	Mulla et al. 1980
	Ae. nigromaculis	Mulligan & Schaefer 1985
Milo field	*Cx. tarsalis*, *Cx. quinquefasciatus*	Mulligan & Schaefer 1985
Alkaline ponds	*Cx. tarsalis*	Mulla et al. 1980
Wildfowl ponds	*Cx. tarsalis*	Mulligan & Schaefer 1982
Urban catch basins	*Cx. quinquefasciatus*	Parman 1986
Snow pools	*Ae. communis* *Ae. hexodontus*	Eldridge et al. 1986

Table 6.-Customized applications of *Bacillus sphaericus* in various habitats based on results of research conducted in California.

Habitat	Species of mosquito	Reference
Irrigated pastures	*Ps. columbiae* *Ae. nigromaculis* *Ae. melanimon*	Mulla & Darwazeh 1986
Date fields	*Ps. columbiae* *Ae. nigromaculis* *Ae. melanimon*	Mulla & Darwazeh 1986
Date field (pre-flood)	*Ps. columbiae*	Mulla & Darwazeh 1986
Dairy lagoon	*Cx. peus* *Cx. quinquefasciatus*	Mulla & Darwazeh 1986
Catch basins	*Cx. quinquefasciatus*	Mulligan & Schaefer 1980
Sewer plants	*Cx. pipiens*	DesRochers & Garcia 1983

now in strategic planning for the eventual development of new microbial pesticides. One can not expect molecular biologists to have the necessary perspective and detailed knowledge of mosquito control to unfailingly follow the most effective course of development. It is now that mosquito abatement personnel should examine important questions about future products, including:

1. In what way would it be desirable to modify species spectrum of activity? Broader? Narrower? To include species presently excluded? To exclude species presently included?

2. In what way should potency of the product be modified? For greater yield of toxin per unit of insecticide? Can production costs be lowered through this approach?

3. What formulations should be developed? Ones with greater persistence?

4. Among environmental sites not now suitable for treatment with bacterial insecticides (e.g. saline habitats), which ones should be targeted for adaptation of modified bacteria?

5. Is it practical or wise to develop microorganisms that will survive in mosquito habitats and through genetic engineering produce bacterial toxins at levels high enough to suppress mosquito populations effectively.

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ANOPHELINE BIOLOGY AND MALARIA: RIVERINE ECOLOGY OF *ANOPHELES PUNCTIPENNIS* SAY IN THE CENTRAL VALLEY AND SURROUNDING FOOTHILLS OF CALIFORNIA

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Introduction.

Approximately 300 to 400 cases of malaria occur in California each year. Most of these are attributed to sources other than local mosquito transmission (e.g., imported, induced). However, a series of 1986 outbreaks in several areas of the State appear to have been introduced autochthonous cases (MMWR, 35(43):679-681; Calif. Morb. 13, April 10, 1987). A unique aspect of these outbreaks has been the geographic locations where the cases occurred. With the exception of a single case in Yuba City in the Sacramento Valley, all were in areas where *Anopheles freeborni* Aitken, the western malaria mosquito, was either absent or did not occur in great abundance. There is much evidence that an undescribed *Anopheles* species closely related to *An. freeborni* may have been the vector in southern California (Barr et al. 1988) and in addition, Washino (1987) speculated that *An. punctipennis* Say may have been involved in the Fresno and possibly Merrit Island outbreaks. This paper outlines a projected program and preliminary progress report on a study recently initiated to study the biology of *An. punctipennis* and associated anophelines in the context of malaria control in the areas north of the Tehachapi range.

The objectives of the study were to 1) determine the seasonal history and abundance of *An. punctipennis* and other associated anophelines in riverine areas of the San Joaquin Valley and surrounding foothills of California; 2) collect and identify blood meal samples from anophelines to help understand host blood feeding patterns; 3) study the parity profile in the adult population as a basis for future life table studies for determining longevity and estimating daily survival rates; 4) determine the feasibility of mark-release-recapture study for life table and dispersal studies in future long-term studies; 5) collect and preserve genetic voucher material from a variety of geographic locations for establishing more precisely the validity of present systematic status; 6) establish laboratory colonies of various populations for future laboratory studies including those for malaria vector competence; and 7) investigate future malaria outbreaks in California and implement a variety of methods (e.g., ELISA) to assess the ability of the various anophelines to become infected and to transmit *Plasmodium vivax*.

Procedures.

1. Riverine study sites were established at Fresno (i.e., Skaggs) and Friant Dam in Fresno County, and Kingsburg and Woodlake in Tulare County. Two sites were in the heart of the San Joaquin Valley just east and west, respectively, of Hwy. 99 and the other two in the Sierra Nevada foothills. Adult mosquito populations were sampled from these sites from June 23, 1987, to October 1, 1987, through weekly collections from 6 ft. red boxes, CDC traps baited with CO_2, New Jersey light traps and human bait (i.e., landing rate collection). Collections at irregular intervals were also made from natural shelters and from malaise traps.

2. *An. punctipennis* collected from the above sites were brought back to the laboratory and processed to determine species, sex, metabolic state and parity status of the female. Bloodmeals from blood engorged females were frozen for precipitin testing at a later date to identify host.

3. Attempts were made using standard mosquito culture techniques to establish a laboratory colony from progeny of females collected from the above field sites in Fresno and Tulare Counties. Subsequent attempts were made utilizing forced mating to ensure insemination of female progeny of adult females collected from the foothills of the Sierra Nevada and Interior Coastal Range.

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Table 1.-Parous rates in the biting population of *An. punctipennis*, Fresno and Tulare Counties, CA, 1987.

Month	Parous rate & no. females dissected			
	Friant (324)	Skaggs (172)	Kingsburg (152)	Woodlake (75)
June	.30	.00	.00	.00
July	.34	.07	.13	.67
August	.38	.20	.28	.00
September	.32	.25	.44	.43
Total	.35	.19	.30	.29

Results and Discussion.

From a total of 1,113 females collected by all methods, a sample of 771 were dissected to determine parity profile of the summer populations. The profile of the biting segment of adult populations from all of the sites are summarized in Table 1. Collections from the Friant Dam site were most productive and yielded the most consistent results. The parity rates are not dissimilar to rates previously observed in *An. freeborni* populations in the Sacramento Valley further north (McKenna et al. 1973). However, such comparison would not be valid since the latter study involved samples from resting populations while the present study involved biting populations. Additional studies are necessary to confirm or deny the speculation that *An. punctipennis* exhibits greater longevity than *An. freeborni* in California. Blood engorged females were rare or absent in most collections so that material for blood meal identification was minimal.

During the summer months, landing rate collections during sunset/sunrise hours consistently yielded more *An. punctipennis* adults than any other collection methods. CDC traps baited with dry ice and New Jersey light traps were less productive. Collections from artificial (e.g., red box) and natural shelters (e.g., under bridge) or from malaise traps were totally unproductive. During the autumn months, however, collections from natural shelters (e.g., barns, bridges, etc.) did yield both active and dormant adult females.

Several attempts have been made to establish a laboratory colony with limited success. Past attempts in the mid-1960's were successful, but only with intensive effort. In our current trials, lack of mating was compensated for by forced mating, but females inseminated in this manner had poor survival. In our most recent trials, survival in the coastal population have improved and there is some indication that the colony is becoming established.

A collaborating microbiologist from the UCD School of Veterinary Medicine participated in a workshop at Walter Reed Institute of Medical Research on the application of ELISA technique to detect and identify *Plasmodium* infection in mosquitoes. A complete set of reagents, etc., is now available to implement an assay program in the event of an outbreak similar to that of 1986.

Acknowledgments.

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THE DISTRIBUTION OF AN UNDESCRIBED MEMBER OF THE *ANOPHELES MACULIPENNIS* COMPLEX IN CALIFORNIA

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ABSTRACT

Cytological and morphological evidence indicates the widespread presence of a new species of the *Anopheles maculipennis* complex in southern California. *Anopheles freeborni* was not found south of the Tehachapi Mountains. *Anopheles occidentalis* was not found south of Santa Barbara. Evidence indicates that the new species may be an important vector of malaria.

Introduction.

The *Anopheles maculipennis* group was the first complex of mosquitoes to be studied in depth. The species are so closely related that it is difficult or impossible to differentiate them morphologically, yet they differ greatly in vectorial capacity, so their correct identification is of the utmost importance. The complex at present comprises 13 species, 9 from the Old World and 4 from the New (White 1978).

In California, there are three members of this complex. *Anopheles freeborni* Aitken and *Anopheles occidentalis* Dyar and Knab are relatively well-known (Aitken 1945, Christensen 1968) but a third species is as yet undescribed. Hybridization and cytogenetic studies carried out by Fujioka (1986) and Menchaca (1986) respectively show that the undescribed species, referred to as "southern *occidentalis*" in the literature (Lewallen 1957), is only distantly related to true *An. occidentalis* but is rather close to, but specifically distinct from *An. freeborni*.

Morphologically, the three species are similar (Bohart and Washino 1978). Adults of true *An. occidentalis* have prominent pale scales on the wingtips (Fig. 1). This is in contrast to An. freeborni and the undescribed form, which have dark wingtips (Fig. 1). However, the pale scaling is easily removed by damage or wear and denuded specimens cannot be reliably distinguished. Also, reflection of light may cause the wingtips to appear white when in reality they are not. Obviously, caution must be used when employing this character for identification.

The only known method of identifying all individuals of the three species at present is the morphology of the sex chromosome. The X-chromosome of the undescribed species is unique in that it has a large terminal puff by which this species can be easily distinguished from the other two (Menchaca 1986, Morrison 1985). Another useful diagnostic feature is the fact that the X-chromosome of the southern form averages about 40 μm in length while those of the other two species average

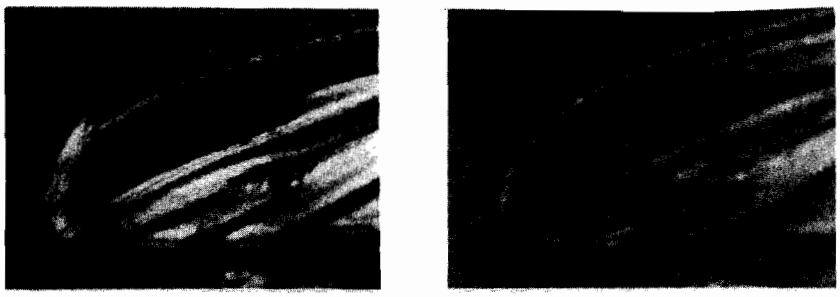

Figure 1.-Wingtips of *Anopheles occidentalis* (left) and "southern *occidentalis*". Arrow indicates pale scaling.

about 50 μm.

There are numerous records of *An. freeborni* and *An. occidentalis* from southern California, which we have been unable to evaluate (Aitken 1945, Seaman 1945, *Anopheles* collection records-California Department of Health Services). Therefore, the two main objectives of this study were: 1) to determine the distribution of "southern *occidentalis*", and 2) to determine whether either *An. freeborni* or *An. occidentalis* occurs in Southern California.

Methods.

In collaboration with mosquito abatement agencies, county health departments, and the California Department of Health Services, extensive larval and resting adult mosquito collections were made throughout southern California, with emphasis on coastal breeding sites. Limited collecting was done in the San Joaquin and Owens Valleys. Several adults from each site were mounted for morphological study, especially for the character of scaling of the wingtips.

Five to six day-old adults reared from field-collected immatures were blood-fed and then held for 29 hours at room temperature. Each ovary was dissected in modified Carnoy's solution, placed in a small drop of 45% glacial acetic acid on a siliconized coverslip for 1 minute, and stained with 0.5% lacto-aceto orcein for 5 minutes. Then a clean, nonsiliconized microscope slide was slowly lowered onto the coverslip. In order to disrupt the ovarian nurse cell chromosomes for analysis, the preparation was placed between several layers of bibulous paper and tapped or rubbed gently with the index finger. Preparations were then evaluated with a Zeiss phase contrast microscope at 160 and 400X magnifications.

Finally, attempts were made to establish a colony from each collection site.

Results.

A total of 95 collections was made. Appendix 1 gives localities by county and dates of collection. Immatures only were collected unless otherwise noted. Several collections did not contain any of the species listed in Appendix 1.

Our results indicate that "southern *occidentalis*" occurs from the San Luis Obispo area (San Luis Obispo County) southward to the Mexico-California border, a distance of approximately 560 km (Fig. 2). We have not looked for this mosquito in Mexico. Easterly, this species occurs inland at least to Lake Hemet (Riverside County), approximately 80 km from the coast. Altitude ranged from sea level to about 1200 m at Lake Hemet.

Collections of true *An. occidentalis* (Fig. 2) were made along the coast from Grover City (San Luis Obispo County) to Gaviota State Beach (Santa Barbara County). No specimens of true *An. occidentalis* were identified south of Gaviota State Beach. Two breeding sites, Gaviota State Beach and Hollister Ranch, were identified where "southern *occidentalis*" and *An. occidentalis* were sympatric.

One collection from Firebaugh in the San Joaquin Valley, four collections from Bishop and one collection from Lone Pine (both in the Owens Valley) were identified as *An. freeborni* (Fig. 2). No collections of *An. freeborni* were identified south of the Tehachapi Mountains.

Discussion.

John Belkin and William McDonald morphologically examined large numbers of larvae, pupae and adults of *An. occidentalis*, "southern *occidentalis*" and *An. freeborni*, apparently during the 1950's, and prepared keys for the differentiation of larvae and pupae of the three forms which were never published. The analyses of these workers were made available to us through the courtesy of Captain Michael Faran of the U.S. Army's Medical Entomology Project at the U. S. National Museum.

Belkin's collection of California anopheline mosquitoes shows *An. occidentalis* only coastally, from San Luis Obispo County north, and the southern form also only coastally, from Santa Barbara County south to San Diego County. His *An. freeborni* specimens are from many inland localities around the State, north of the Tehachapi Mountains. East of the Sierra Nevada Mountains, Belkin's southernmost *An. freeborni* specimens are from the northern part of San Bernardino County. Belkin's collections from southern California are all from coastal localities so we were surprised to find breeding populations in Duarte (Los Angeles County) and Lake Hemet, which are about 50 km and 80 km inland respectively. Our study cytologically confirms the results of Belkin and McDonald.

We found no cytological or morphological evidence of *An. occidentalis* south of Santa Barbara, although Aitken (1945) recorded this species from Ventura. Further support for our results is the fact, that, while we were able to easily colonize all of our "southern *occidentalis*" collections, we were unable to colonize any of our true *An. occidentalis* collections. In discussing the significance

Appendix 1.-Collection records--*Anopheles* Survey, UCLA School of Public Health. All collections from 1987 unless noted. (I+A) indicates immatures and adults collected.

"Southern *occidentalis*" only	
Los Angeles County	Collection Dates
Duarte-Van Tassel Canyon	V-13
Whittier Narrows Nature Center	V-19, VII-29
Triunfo Creek at Kanan Dume Road (I+A)	VI-25
Topanga Creek at Pacific Coast Highway	VI-25
Malibu Creek at Pacific Coast Highway	VIII-10
Las Virgenes Creek at Mulholland Highway	X-7
Orange County	
San Joaquin Marsh-Irvine (I+A)	May-Sept. 1985
Santiago Oaks Regional Park (I+A)	VII-17, VII-31
Newport Back Bay	VII-31
Veeh Lake	IX-4
Ventura County	
Moorpark-off Hitch Road (I+A)	V-21, VI-10
Fillmore Fish Hatchery (I+A)	VII-6, VII-15, VII-21, VIII-7,VIII-24
Fillmore-Pole Creek	VII-6, VIII-7
Foster Creek-Ventura River	VII-6
Camp Comfort-San Antonio Creek (I+A)	VII-6
Santa Paula Creek-off Route 150	VIII-7
Santa Clara River-along Route 126	VIII-7, VIII-24
Ventura County Fairgrounds-Ventura River	X-15
San Diego County	
San Mateo Point	VIII-25, IX-4
Dairy Mart Road, off Interstate 5	VIII-28
San Luis Rey Downs-San Luis Rey River	VIII-28
Rancho Carlsbad Mobile Home Park	VIII-28
Sorrento Valley Creek	VIII-28
Guajome Regional Park (I+A)	VIII-28
Riverside County	
Lake Hemet-Herkey Creek (I+A)	VII-2
Rubidoux-Carlson Park (I+A)	VIII-5
Rubidoux-Rancho Jurupa Park	IX-1
Santa Barbara County	
Goleta-Los Carneros Regional Park	IX-7
Hollister Ranch-Santa Anita Creek	IX-7
Refugio State Park	IX-19
Cuyama River-Hwy. 166 and Tepesquet Road Pk.	IX-20
San Luis Obispo County	
Arroyo Grande Creek-near Biddle Regional Park	IX-20
Anopheles occidentalis and "southern *occidentalis*" collected together	
Santa Barbara County	
Hollister Ranch-San Augustine Creek and Lake-7.8 miles from gatehouse	IX-7
Gaviota State Beach-at small bridge just before official park entrance	X-15
Anopheles occidentalis only	
San Luis Obispo County	
Oceano Memorial Park	IX-8, IX-19
Oso Flaco Lake	IX-19
Anopheles freeborni only	
Fresno County	
Near Firebaugh-old airport buildings near Althea and Prince Roads (Adults only)	VIII-15
Inyo County	
Bishop-flooded pasture across Highway 395 from golf course	IX-16
Bishop-east of town near intersection of Poleta and Van Loon Streets	IX-16
Bishop-Millpond Park	IX-16
Bishop-Shady Rest Trailer Park	IX-16
Lone Pine-in flooded pasture. Turn west at road just past Shell station and Frosty Stop at south edge of town.	IX-17

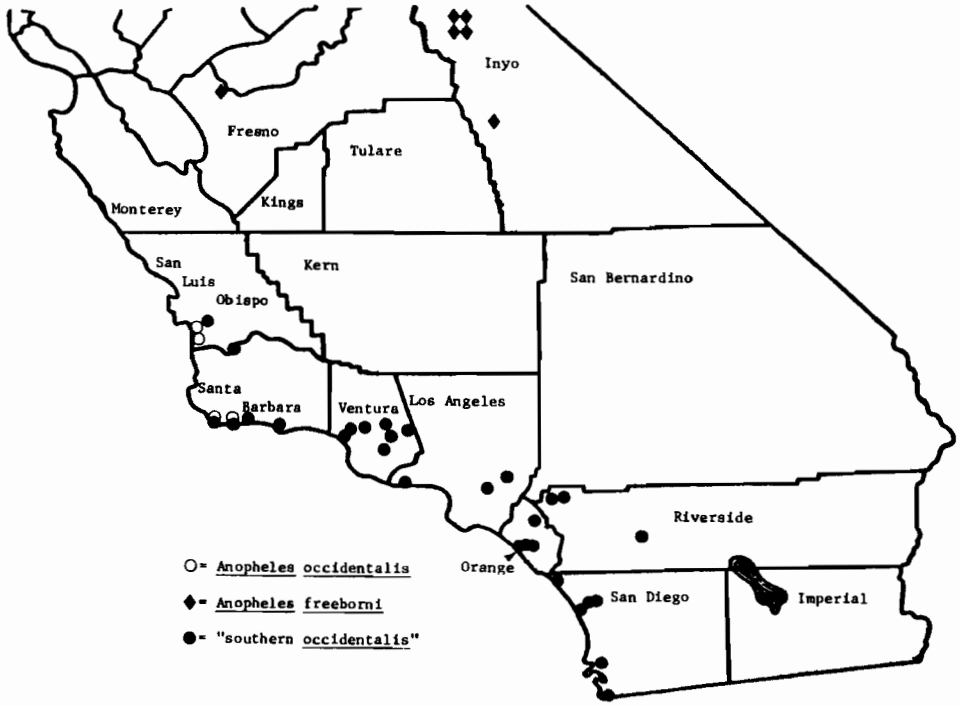

Figure 2.-Collection localities-*Anopheles maculipennis* complex in southern California-UCLA *Anopheles* survey, 1987.

of a similar observation for two species of the European *An. maculipennis* complex, Bates (1949) wrote, "it probably indicates that the mating habits of the two populations in nature are so different that individuals would never come in sexual contact with one another."

The area of overlap of the range of *An. occidentalis* and the new species (Fig. 2) is of taxonomic interest and value. If the criterion for specific distinctness is the flow, or lack of flow, of genetic material between populations, the proof of genetic distinctness could be found only in an area where the forms are sympatric. Since hybrids are easily recognized cytologically (Menchaca 1986), the degree of hybridization could be assessed in such areas.

In 1986, the largest outbreak of introduced autochthonous malaria in the United States since 1952 occurred in San Diego County, California. A total of 28 cases of laboratory confirmed *Plasmodium vivax* was reported (Centers for Disease Control 1987). Our results indicate that no other *Anopheles* are present in the area except *An. franciscanus* McCracken, a species that rarely attacks man and is therefore thought to be a poor vector of human malaria (Bohart and Washino 1978).

We have not taken *An. franciscanus* in numerous biting collections made in southern California, although adults of the new species commonly and abundantly bite man. Therefore, it is highly likely that the undescribed species was the vector in the San Diego County malaria epidemic.

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MORPHOMETRIC ANALYSIS OF THE EGGS OF AN UNDESCRIBED MEMBER OF THE *ANOPHELES MACULIPENNIS* COMPLEX IN CALIFORNIA

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ABSTRACT

Morphometric analysis of the eggs of an undescribed species of the California *Anopheles maculipennis* complex (Diptera: Culicidae) was performed. Eggs were obtained from the rearing of field collections made throughout the state. Comparisons are made with the other two members of the complex inhabiting California, *Anopheles freeborni* and *Anopheles occidentalis*. Morphometric parameters within any species did not appear to be dependent on the geographic origin of the collection. Although there may be slight differences between the eggs of the sibling species, the variations are not great enough to identify an egg reliably to species based on any single morphometric parameter.

Introduction.

Recent studies by Menchaca (1986) and Fujioka (1986) have provided evidence of a new member of the *Anopheles maculipennis* complex in California. Two members in this complex, *Anopheles freeborni* Aitken and *Anopheles occidentalis* Dyar and Knab, currently are recognized in California, with *An. freeborni* considered to be the prominent vector of malaria in the state (Moore et al. 1945). While *An. freeborni* has been collected throughout the state, *An. occidentalis* is believed to be restricted to coastal regions and only rarely takes human blood meals (Bohart and Washino 1978). Current research in our lab suggest that *An. freeborni* is limited to the central and northern portion of the state, and the new species is the dominant member of this complex south of the Tehachapi Mountains (Cope et al. 1988). This undescribed species has previously been called "southern *occidentalis*" (Lewallen 1957).

Adults of the three species are morphologically similar. Menchaca (1986) and Morrison (1985) have shown that the only definitive method of identifying species of this complex is by analysis of the sex chromosomes. This method is time consuming and necessitates proper equipment and trained personnel.

Mosquito taxonomists have long noted that the eggs of closely related species are often markedly different from one another, while the larval and adult stages may be indistinguishable (Christophers and Barraud 1931, Hinton 1967). Therefore, analysis of eggs often is a more valid method when identifying sibling species. Bates and Hackett (1939) have shown that the *Anopheles maculipennis* complex in southern Europe is comprised of species whose eggs are consistently distinguishable from one another. Surveillance of malaria vectors based on morphological characteristics of eggs in Brazil (Causey et al. 1944) and India (Reuben and Suguna 1983) has resulted in the description and naming of many previously unidentified anopheline species.

The objectives of this study were: 1) to describe the morphometric characteristics of the eggs of the undescribed species, and 2) to determine if a systematic method of species identification could be based on egg morphology for the *Anopheles maculipennis* complex occurring in California.

Materials and Methods.

Field collections were made of immature stages throughout southern California from June to October, 1987. Larvae were reared in the laboratory at room temperature in pans containing tap water. Pupae were transferred to one-gallon adult rearing cages. Mated females were offered a human blood meal. Collections were identified to species by ovarian nurse cell chromosomes following the methods of Menchaca (1986) and Morrison (1985).

Soon after identification of strains by chromosomal morphology, females were offered another blood meal. Fully engorged mosquitoes were randomly selected and aspirated into individual oviposition vials filled with tap water and lined with bibulous paper. Vials were kept at room temperature until oviposition occurred. Hydrological variations were standardized by allowing the eggs to float in the water for a minimum of 24 hours. The eggs were then transferred to moist filter paper and

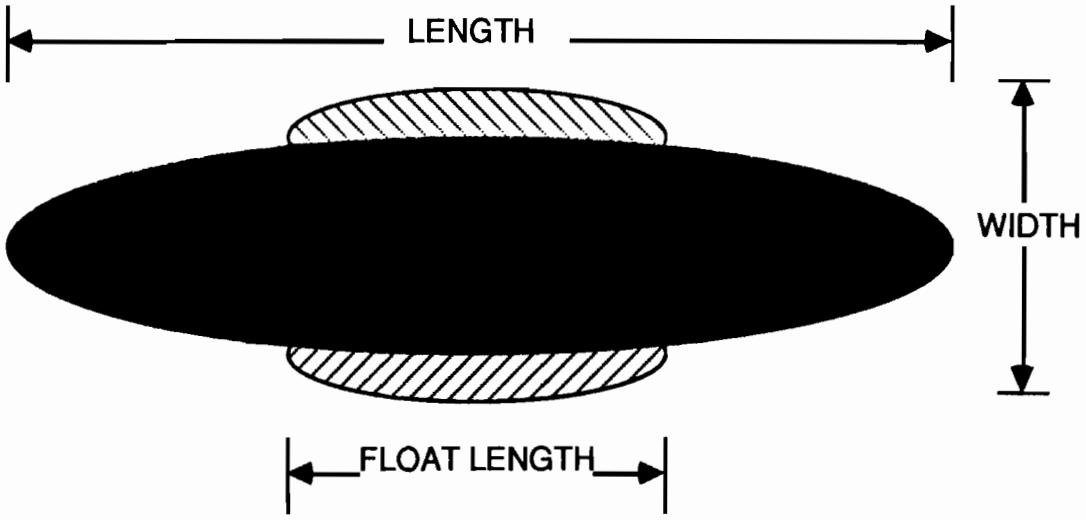

Figure 1.-Parameters measured of the eggs of the California *Anopheles maculipennis* complex.

examined under a dissection microscope at 50X magnification.

Ten eggs were randomly selected from each batch. Ten females were selected from each collection resulting in a sample size of 100 eggs per location. Some females, however, did not produce eggs, resulting in lower multiples of ten for some collections. Morphometric parameters measured include the length of the egg, the width of the egg inclusive of the bilateral floats, the length of the floats, and the number of chambers per float (Fig. 1). Measurements were taken blindly as each colony was assigned a number before analysis.

Statistical analyses were performed using a random unbalanced nested one-way analysis of variance for each parameter of interest (Netter et al. 1985). Analysis of the new species required nesting of the females within each collection location. Comparisons among the three species were analyzed by the same nested design with additional secondary nesting presented by the multiple collection locations within each species.

Results.

Larval collections identified as "southern *occidentalis*" were made from 21 locations extending south to the Mexico-California border in San Diego County, north to San Luis Obispo, and east to Lake Hemet (Riverside County). *An. freeborni* eggs were obtained from collections made in the San Joaquin and the Owens Valleys. True *An. occidentalis* was found only in collections made along the central coast from Gaviota (Santa Barbara County) to San Luis Obispo.

The eggs of the new species are similar to the others in the *An. maculipennis* complex in California. Independent of one another, the eggs lie horizontally on the water surface and are normally found in groups. The grouping may be induced by similar chorionic membrance surfaces and the surface tension of the water.

Collections were made from 21 locations from which 1380 eggs were obtained for analyses. The morphometric characteristics of the eggs of the undescribed species are shown in Table 1. The length averaged 0.61 mm with a range of 0.48 to 0.74 mm. The width was approximately one-third of the length, averaging 0.18 mm (±0.005 S.E.) inclusive of the bilateral floats. The number of chambers per float varied from 6 to 16 with a mean of 10.7 chambers. The float length to egg length ratio was fairly consistent at 0.23 (±0.006 S.E.). Statistical analyses suggest that there were no differences between collection locations within individual species. Therefore, it was possible to collapse across locations and compare the three species by each parameter regardless of collection location ($p>0.10$).

Morphometric parameters for the three species of the California *An. maculipennis* complex are shown in Table 2. Average egg length and width were identical for "southern *occidentalis*" and *An. freeborni*. *An. freeborni* had a slightly greater number of chambers per float in addition to a

Table 1.-Morphometric characteristics of the eggs of the undescribed species of the California *Anopheles maculipennis* complex. (21 collection locations, n = 1380 eggs).

	Egg Length (mm)	Egg Width (mm)	Chambers Per Float	Float Length /Egg Length
Mean	.61	.18	10.7	.23
S.E.	.02	.005	0.29	.006
Min - Max	.48 - .74	.12 - .23	6 - 16	.13 - .34

Table 2.-Morphometric parameters of the eggs of the California *Anopheles maculipennis* complex. Standard deviations are listed in parentheses.

	Egg Length (mm)	Egg Width (mm)	Chambers Per Float	Float Length /Egg Length
"Southern *occidentalis*" (n = 1380)	.61 (.03)	.18 (.02)	10.7 (1.7)	.23 (.04)
An. freeborni (n = 180)	.61 (.03)	.18 (.02)	12.3 (1.5)	.28 (.04)
An. occidentalis (n = 50)	.65 (.04)	.19 (0.1)	13.2 (1.9)	.27 (.05)

larger float length to egg length ratio. *An. occidentalis* eggs appeared to be larger than either "southern *occidentalis*" or *An. freeborni* in every variable except float length to egg length ratio, for which *An. freeborni* had the greatest value. Although differences were observed between the same parameter for each species, statistical analysis suggests that values of any one of the parameters taken independently cannot reliably identify an egg to its proper species.

Discussion.

Collections of each species were originally analyzed by their geographic origin due to the variety of ecologic foci inhabited by each. These foci ranged from sea level to an elevation of 1200 m at Lake Hemet, in addition to the latitude variations along the state. Downs (1951) has suggested that these ecologic variations may have a profound effect on the morphology of anopheline eggs. Our findings suggest that although these variations may be present in the field, they are negligible in the laboratory when oviposition and rearing techniques are standardized. Therefore, we were able to obtain morphometric values indicative of each species rather than each location.

The eggs of the three species examined appear to have similar morphological characteristics in California as described by Herms and Frost (1932), although it is not clear which species of the *An. maculipennis* complex they studied. Other researchers have suggested that when eggs of sibling species can be differentiated by morphometric parameters, the general structural appearance of the eggs is also readily distinguishable (Causey et al. 1944, Hinton 1967). Otsuru et al. (1976) have suggested that these gross phenotypic differences need not be present to indicate separate species.

Although differences in the mean values of the morphometric parameters for the three species are not statistically significant, there appears to be a noteworthy trend in the data. For each parameter measured, *An. occidentalis* had consistently greater values than did "southern *occidentalis*". The statistical insignificance may be due to an artifact of the data caused by a disproportionate sample size. Differences among these parameters would have to be of great magnitude to be detected in this sample, whereas subtle differences, although important, may be overlooked. It should be noted that the *An. occidentalis* eggs we obtained were infertile, possibly accounting for their larger size.

In this study, the analyses were performed on each morphometric parameter independently of the others. It may be helpful to construct a model which would consider all the variables in a single analysis. Research in our laboratory is currently investigating this possibility, in addition to increasing the size of the sample for the underrepresented species.

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COMPARATIVE ECOLOGY OF *AEDES DORSALIS* COMPLEX IN THE HOLARCTIC

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ABSTRACT

In the group of salt marsh mosquitoes, *Ae. dorsalis* has been classified nine times since first being described. In the Palearctic, the primary controversy has been whether *Ae. dorsalis* is a distinct species or is a subspecies of *Ae. caspius*. In the Nearctic, *Ae. melanimon* has long been considered a synonym for *Ae. dorsalis*. Definitely, both species are classified as a full species. When morphological characteristics of *Ae. dorsalis* and *caspius* from Yugoslavia were compared, intermediary types were found. Existence of the intermediary types was correlated with differing ecological conditions in the larval habitats, especially the salinity of the water. Variations in genitalia, which is generally a stable morphological character, may reflect more than variations due to ecological conditions.

Basic data of *Aedes dorsalis* Meigen, *Aedes melanimon* Dyar and *Aedes campestris* Dyar & Knab.

The taxonomy of mosquitoes belonging to the *dorsalis* group has long been discussed. The ancestor of this group probably arose in Central Asia and has extended its range into Western Europe and North America (Minar 1976, 1978). *Ae. dorsalis* Meigen, *Ae. melanimon* Dyar and *Ae. campestris* Dyar & Knab represent a group of closely related species in the United States. *Ae. melanimon* has long been considered a synonym of *Ae. dorsalis*.

Ae. dorsalis has Holarctic distribution, reaching Formosa and Mexico. This species has been reported in 30 states in the United States. Locus typicus is Berlin, West Germany (Knight and Stone 1977).

Ae. melanimon is distributed in the Western United States and Canada. Locus typicus is Bakersfield, CA. It is the sole member of the group with distribution in the Nearctic only (Knight and Stone 1977).

Ae. campestris is distributed in the western semi-arid plains of Canada, the U.S. and Mexico (Knight and Stone 1977). It was believed to be strictly Nearctic until Danilov (1980) reported the discovery of a population in the U.S.S.R. (Schultz et al. 1986).

These three species occupy mostly separate ecological niches in the same general environment and usually do not compete directly with one another.

In California, *Ae. dorsalis* is primarily a coastal, southeastern and northeastern species. The margins of lakes and bays can also be larval habitat sites (Bohart 1956). This species has been known to tolerate rather high salinity. The main larval habitats are characterized by salt-type plants, such as *Salicornia*, *Artiplex* and *Scirpus*. The larvae have been found in sources with a salt content of up to 12% and a pH range of 7.0 to 9.3 (Rees and Nilsen 1947 from Chapman 1960).

It is not unusual to find this species in freshwater, sometimes with larvae of *Ae. melanimon* which is an inland valley species (Bohart 1956). It occupies inland pastures, roadside ditches, potholes, sloughs and drainage ditches. The most important plant surrounding the breeding places of *Ae. melanimon* is Bermuda grass. Although *Ae. melanimon* is a freshwater species, it can be found with *Ae. dorsalis* in water with up to 1.8% salt content (Bohart 1956, Chapman 1960).

In California, *Aedes campestris* has been reported from Lassen and Modoc Counties, primarily in March and April. The breeding sites are shallow, sunlit pools that are definitely alkaline, with a pH of 8.4 and rich in organic matter (Gibson 1937, Rampel 1950, 1953 from Wood et al. 1979), as well as subarctic pools in birch and willow scrub at the tree line (Hockin et al. 1950 from Wood et al. 1979).

Aedes dorsalis, *Aedes melanimon* and *Aedes campestris* readily attack man, causing painful bites. They are a very important pest species in many areas due to their abundance. Most importantly, they have a relationship to certain arthropod-borne diseases, which make investigations concerning the status of the species essential.

Basic data about *Aedes caspius* Pall.

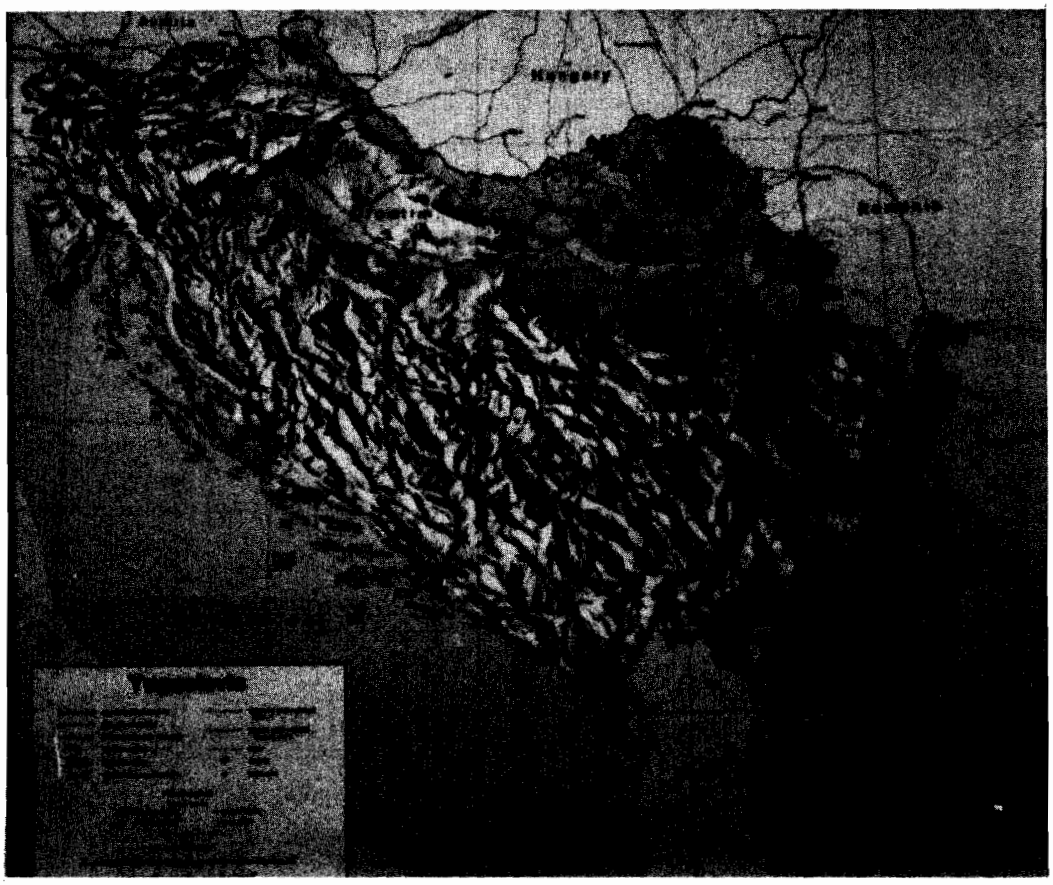

Figure 1.-Map of Yugoslavia with localities

● freshwater ponds or small lakes

★ flooded area near by the river Tisa

✱ salt lakes or swamps

Using the morphological approach to elucidate the taxonomic rank of *Aedes dorsalis*, this organism has been reclassified nine times since first being described by Meigen in 1830. The controversy occurs when considering whether *Ae. dorsalis* is a distinct species or is a subspecies of *Aedes caspius*.

Aedes caspius has Palearctic distribution, mainly coastal and inland saline areas. Type-locality is the Caspian Sea (Knight and Stone 1977).

In Dinarion Western Balkan (Yugoslavia), the distribution of *Ae. caspius* is connected with salt lagoons, swamps and pools near the sea, contrary to *Ae. dorsalis*, which is more of an inland species connected with salt lakes and swamps.

Gutsevich (1985) has proposed that the relationship between these species is in a rank of subspecies. He based his opinion on intermediary forms. These specimens are characterized by considerable variations of the scutel patterns. Also, some structural variations can be noticed in the basal lobes of the male genitalia.

Larvae of *Aedes caspius* occur in open or shaded, temporary or permanent water formed by snowmelt, flooded rivers or irrigations. The water contains some percentage of salt. The larval habitat is characterized by having little vegetation and a muddy bottom. Several generations of *Ae. caspius* may occur through the season, depending on the amount of rain and periodic flooding. The females are serious biters and often bite during the day. They may at times migrate up to 10 km. (Butsevich et al. 1971).

Materials and Methods.

Species of *Ae. dorsalis* and *Ae. caspius*, along with the intermediary types, were collected from the northeast area of Yugoslavia. Specimens have been collected from 15 localities: six are from freshwater ponds or small lakes (Obrovac, Mokrin,

Kumane, Zrenjanin, Vrsac and Deliblatska pescara); one is from a flooded area near the river Tisa (Ada); and eight are from salt lakes or swamps (Novi Becej, Melenci, Mali Siget, Elemir, Srpski Miletic, Svetozar Miletic, Taraska Dzuglja and Mesic) (Fig. 1).

The freshwater collection localities are situated near small towns or villages, except Zrenjanin (Carska bara), which is a bird refuge, and Deliblatska pescara which is a National park. They are mostly supplied by water seepage or rainfall and are surrounded by old willow trees and reed-like vegetation. Usually they produce two generations per season; one in the spring and the other after heavy summer rains or an increase in the seepage water. Temperatures varied from 11°C in the spring to 26°C in the summer. The water depth varied from 10 to 60 cm.

The salt lakes and swamps contained different amounts of salt (1% to 15%), were unshaded, and had edges that were overgrown with species such as *Scirpus*, *Salicornia* and *Carex*. In contrast to the freshwater habitats, they were usually shallow and consequently, warmer, especially during the spring. Two to four generations per season were produced, depending on the amount of rain and/or seepage water.

Adults used for morphological compilation were reared from larvae and pupae. A total of 122 females were examined from scutel variation, and 86 males for scutel and genitalia variation.

Results and Discussion.

Analysis of the female scutellar patterns showed 11 specimens were typical *Ae. caspius*, 55 were typical *Ae. dorsalis* and 56 specimens were of the intermediate types. The intermediates were divided into three types (type A, B and C) depending upon the markings on the scutum. Thirty-three of the 56 specimens were designated as type A, nineteen as type B and four as type C. Our data shows that 45.9% of the females examined were of the intermediary types (Fig. 2, Table 1).

Two male intermediary types were established from the relation of the basal lobe shape and position of the two main spines on the genitalia. Of the 86 specimens examined, 24 had the typical *Ae. caspius* genitalia, 37 were *Ae. dorsalis*, 15 were designated as type I and 10 as type II (Fig. 3, Table 1).

The most interesting part of the analysis was the comparison of the scutellur pattern and genitalia of the males. From 24 samples with a typical *Ae. caspius* hypopygium, only one had a typical scutel pattern for this species. The majority of the samples (20) were designated as intermediary type A, one as type B, and two as type C.

Out of 37 samples with a typical *Ae. dorsalis* genitalia, nine samples had the typical *Ae. dorsalis* scutel pattern and 28 samples were of type B. Among 15 samples with intermediary type I genitalia, 13 were type A, and two were *Ae. caspius*, in regards to the scutellar appearance. Five of ten samples with intermediary type II genitalia had the type A scutellar pattern, four were typical *Ae. dorsalis* and one was type B (Table 2).

According to this analysis, it is possible to conclude that the majority of the samples had the type A scutellar markings. Only one sample was typical *Ae. caspius* in regards to the genitalia structure and scutellar pattern, while 9 samples were *Ae. dorsalis*.

This kind of morphological analysis of *Ae. caspius* and *Ae. dorsalis* shows an incredible variability, not only in patterns and color of the thorax, but also in the structure of the genitalia, the supposedly most stable morphological character. While scutellar differences may be explained by different ecological conditions in breeding places (e. g., salinity, water temperature), the variations in genitalia may reflect more than intraspecies variation.

Our hypothesis was that scutellar variations are caused by the differing amounts of salt concentration in the water. Comparing specimens from freshwater and water with differing percentages of salt, we could not find any correlation of variation with water quality.

According to Gutsevich et al. 1971, intermediary types are rarely ever found in Western and Central Europe. The region where the material was collected from in the northeast part of Yugoslavia belongs to the southern part of Central Europe. We, therefore, could not completely agree with Gutsevich's statement, since 45.9% intermediary types were found on the basis of scutellar patterns in the 122 females sampled, and 29% of the 86 examined males were typed intermediary on the basis of genitalia variation.

Working on the Bohart collection of *Aedes dorsalis* complex collected mainly from coastal areas and nearby salt lakes or pools in California, an attempt was made to find a correlation between scutel appearance and genitalia as related to different larval habitats. Material was collected as eggs, larvae, or as pupae. In the laboratory, they were reared under conditions of differing temper-

Table 1.-Variations in female scutellar patterns and male genitalia observed in specimens collected from northeastern Yugoslavia.

				Intermediary types		
	N	*Ae. caspius*	*Ae. dorsalis*	A	B	C
Females	122	11(9%)	55(45%)	33(27%)	19(15.6%)	4(3.3%)
				I	II	
Males	86	24(28%)	37(43%)	15(17.4%)	10(11.6%)	

N = number of specimens examined.

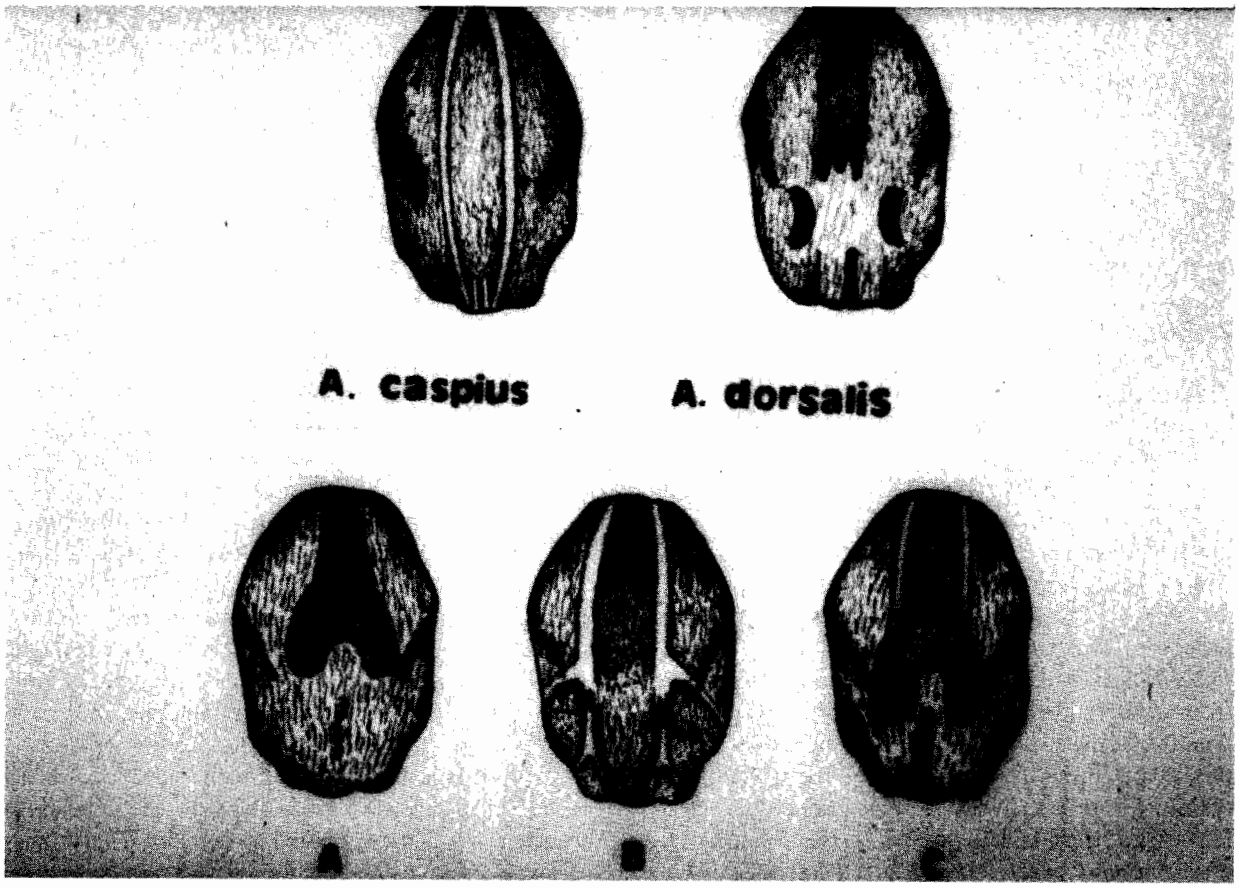

Figure 2.-Female scutel types.

Table 2.-Variations in male scutellar patterns vs. genitalia types observed in specimens collected from northeastern Yugoslavia.

Hypopygium	N	*Ae. caspius*	*Ae. dorsalis*	Intermediary types A	B	C
Ae. caspius	24	1(4.2%)	---	20(83.3%)	1(4.2%)	2(8.3%)
Ae. dorsalis	37	---	9(24.3%)	---	28(75.7%)	---
Type I	15	2(13.3%)	---	13(86.7%)	---	---
Type II	10	---	4(40%)	5(50%)	1(10%)	---

N - number of specimens examined.

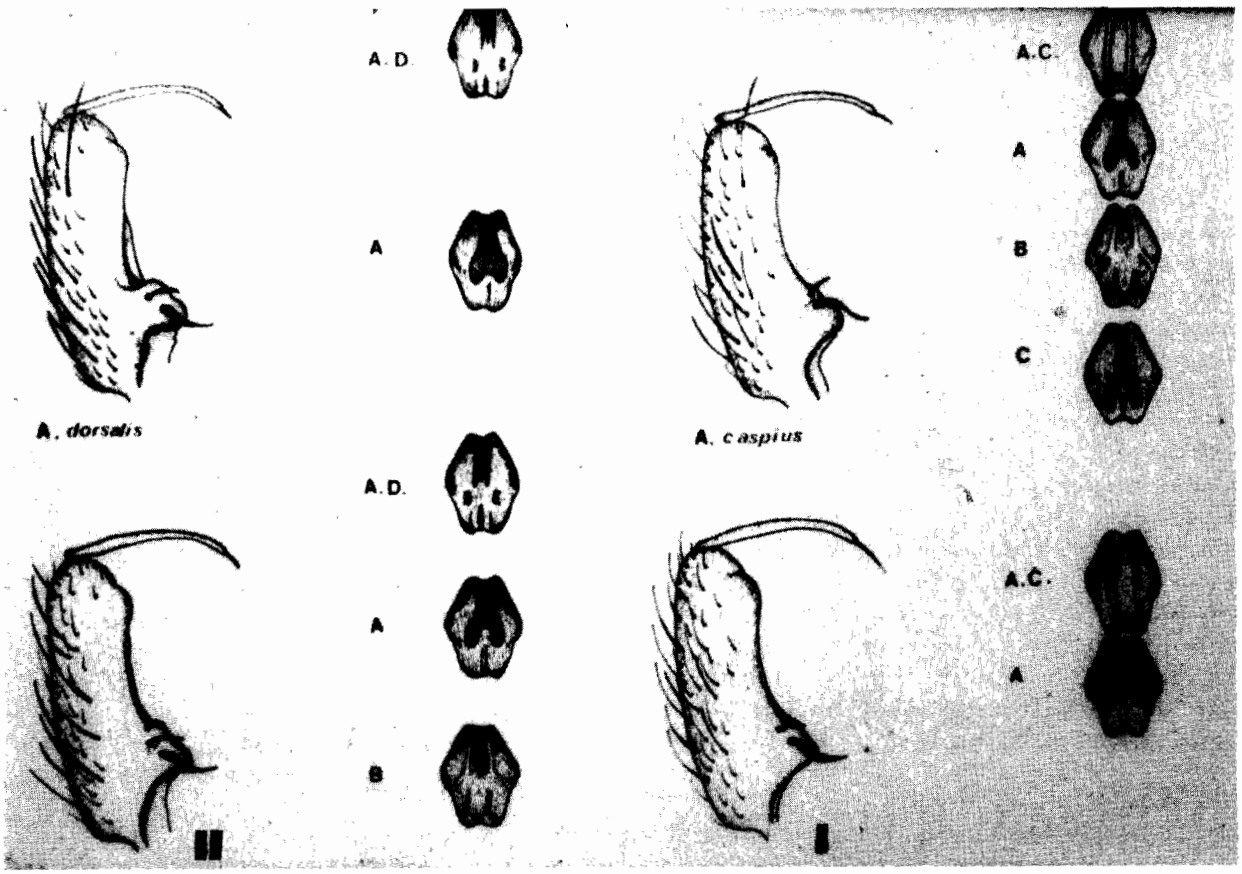

Figure 3.-Male scutel and genitalia types.

ature, salinity, and photoperiod. Variations in the scutel appearance of specimens collected from the different sites was noted.

Pupae collected from Winters, chilled for 6-8 days at 38-40°F, showed females with typical *Ae. caspius* and intermediary type B scutellar patterns. Female adults from fourth instar larvae collected at Grizzly Island and reared in water with 75% NaCl showed intermediary type patterns A and C. Material from Sears Point Road was collected as first instar larvae, reared in tap water at room temperature and showed a typical *Aedes dorsalis* scutum. Samples from Bolinas had the same appearance when first instar larvae were collected from water with 5% NaCl and were reared in tap water at 80°F. There was no difference in the male genitalia among specimens collected from the various localities and reared under the aforementioned conditions.

These results offer sufficient evidence to continue research of the *Aedes dorsalis* complex. One of the most acceptable ways to resolve this problem is to combine morphological and molecular taxonomic methods to distinguish different populations.

In the Holarctic region, the mosquito fauna is small compared with that of the tropics. Particularly characteristic to this region is a large number of species of the genus *Aedes*, subgenus *Ochlerotatus*, some of which have a wide, zonal distribution. *Aedes* (*Ochlerotatus*) *dorsalis* is widely distributed, originating from Central Asia, and reaching North America on the west and China and North Russia on the east. This typical wide, zonal distribution confirms the ecological tolerance of *Aedes dorsalis*. The means of penetration of *Aedes dorsalis* in Nearctic regions can not be explained by the recent wide distribution of the species. The immigration apparently originated from the West and East. The reason for this supposition is the presence of the species in high numbers in the tidal marshes along the Pacific coast, as well as on the eastern coast. The inland areas are also colonized with *Aedes dorsalis*, but the relative abundance is usually low, with the exception of salt pools. The distribution of *Aedes dorsalis* in the United States can be explained by the north-south orientation of high mountains and few salt pools in the inland areas which limit the inward spread from the coastal margins of the continent.

Conclusion.

The Holarctic salt marsh species, *Aedes dorsalis*, has the ability to successfully breed in either salt or fresh water. The hypothesis has been that salinity concentrations, along with other ecological features, caused different scutellar patterns and genitalia shapes in *Aedes dorsalis* and *Aedes caspius*. Comparing the scutellar patterns of 122 females and the scutum and genitalia of 86 males, no correlation could be found among the variations observed and the differing salinity of the larval habitats. The intermediary type of scutellar patterns found in this study consisted of 45.9% of the females examined. Examination of the male genitalia showed that 29% of the specimens were of the intermediary types. Due to the number of intermediary types that were collected from northeast Yugoslavia, these types may not be as rare in Central Europe as reported by Gutsevich (1985).

In analyzing the Bohart collection of *Aedes dorsalis* comp. from California, a great variability is noticed in the scutellar pattern and color, without any correlation to conditions of the larval habitat. There were no differences in the structural morphology of the male genitalia in specimens collected from various locations and reared under different conditions.

Comparing the distribution of *Aedes dorsalis* in the Palearctic and Nearctic, it is obvious that species preferred inland saline areas in the Palearctic region and coastal saline areas in the Nearctic. It also colonized freshwater localities in both hemispheres. The species population in freshwater localities, the Palearctic coastal areas and the Nearctic inland areas, is not as abundant as its preferred habitat.

More investigation on the basic biology and ecology of the *Aedes dorsalis* complex in the Holarctic is necessary to resolve the problem of relationship between these species and the taxonomic status of the intermediary types. This type of investigation should be combined with morphological and molecular methods of research.

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DEVELOPMENT OF THE MOSQUITO MICROSPORIDIAN PARASITE *AMBLYOSPORA CALIFORNICA* IN AN ALTERNATE COPEPOD HOST, *ACANTHOCYCLOPS VERNALIS*

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ABSTRACT

The microsporidian parasite *Amblyospora californica*, from *Culex tarsalis*, was able to infect an alternate copepod host, *Acanthocyclops vernalis*. After merogony, meronts developed into a number of sporonts which eventually became pansporoblasts containing 8 sporoblasts. The pansporoblast membrane was not seen in this study. Mature spores, confined in a sporophorous vacuole, were elongate and existed individually in the host tissue. Electron microscopy showed that spores contained a multichamber-like polaroplast in the anterior part and a posterior vacuole in which tangled "microtubules" were seen. The polar filament included an anterior straight portion and a posterior portion that was isofilar and consisted of 12-14 coils. A "parabody" resembling a bundle of folded tubules was close to one side of the polar filament. Presumably it was associated with the formation of the polar filament.

Introduction.

Microsporidians are among the most commonly observed parasites of mosquitoes. Every species of mosquito may have at least one parasitic microsporidian. Some of these have been studied from the standpoint of their effect on mosquito populations, especially those that are easily transmitted from one mosquito host to another such as *Nosema* or *Vavraia* (Kelly et al. 1981, Nugud & White 1985). Many microsporidians, however, have complex life cycles and are not easily transmitted from mosquito to mosquito; the genus *Amblyospora* is one such group of parasites.

Amblyospora was separated from the genus *Thelohania* by Hazard and Oldacre (1975) and placed in the family Amblyosporidae by Weiser (1977) due to its complex life cycle and unique transmission route. Parasitical members of this genus have been described from at least 67 species of mosquito hosts (Sprague 1977), although only 16 species have been described because of the difficulty in morphologically separating the forms found in differing host species. Species of *Amblyospora* have been recorded from 28 of 47 species of mosquitoes that occur in California (Hazard & Chapman 1977, Castillo 1980). Notwithstanding the widespread occurrence of these parasites, little is known of their biology or host relationships. Their effects on their hosts range from trivial to fatal. At one time, all were believed to be transmitted only vertically by the transovarian route since the spores from infected male larvae are not transmissible by mouth to uninfected larvae (Lord & Hall 1983). Horizontal transmission was unknown until the discovery in 1985 that copepods could act as alternate host (Sweeney et al. 1985, Andreadis 1985).

All of the known species of *Amblyospora* have two developmental cycles (Kellen et al. 1966a, Hazard & Brookbank 1984, Andreadis 1985). Infection in male larvae spreads from the oenocytes to the fat body and uninucleate spores are produced (Andreadis & Hall 1979). The infection in females is a benign schizogonic infection (Andreadis & Hall 1979). The parasites are restricted to oenocytes until the female feeds on blood; the hormones of the host mosquito seem to have an effect on the sporulation of the microsporidian during transovarial transmission (Andreadis & Hall 1979, Lord & Hall 1983, Hall & Washino 1986).

Amblyospora californica is a specific microsporidian parasite of *Culex tarsalis* (Kellen et al. 1966 a,b) which is a vector of St. Louis and Western equine encephalitis viruses in California. In this study, the possibility that *A. californica* is able to develop in an alternate copepod host, *Acanthocyclops vernalis*, was investigated.

Materials and Methods.

Infected mosquito colony.-An infected colony of *Culex tarsalis* Say was established from Harbor

Lake in Long Beach, but subsequently died out. A second infected colony was established from the Tule Indian Reservation, Tulare County and is presently being maintained in the laboratory. Most of the infected male larvae died before they pupated. Uninfected males from a healthy colony were introduced into the infected colony when the females pupated. Larvae from egg rafts laid by blood-fed females were reared in enameled pans (30 x 20 x 5 cm) in each generation.

Copepod colony.-Copepods (*Acanthocyclops vernalis*) used in this study were collected from a breeding site of *Culex tarsalis* in Harbor Lake, Long Beach, and were reared in enameled pans. A solution of cooked egg yolk in water was added to the pans once a week for food. Egg sacs produced by fertilized female copepods were released by killing the gravid females. Approximately 10 released egg sacs were placed in a pan for rearing. The egg-to-egg cycle of copepods required about two weeks at approximately 25°C.

Infection of copepods.-Colonized copepods were exposed to microsporidian spores that developed in infected male larvae. Individual copepods were squashed and stained with Giemsa from 7 to 21 days after exposure to the spores. The specimens were examined by phase-contrast microscopy. Other exposed copepods were collected for study by electron microscopy.

Electron microscopy.-Whole copepods were fixed in 2% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) at 4°C overnight. The specimens were post-fixed in 1% OsO_4 for 2 h; washed in a 1:1 mixture of buffer and distilled water; and dehydrated in an ethanol series. After infiltration with propylene, the specimens were embedded in Medcase (Epon 812). One-micron thick sections were stained with polychrome stain, containing toluidine blue and fuchsin, at 60°C and examined by light microscopy. Thin sections were cut on an LKB ultramicrotome; stained with uranyl acetate and lead citrate; and were examined with a Zeiss EM 109.

Results.

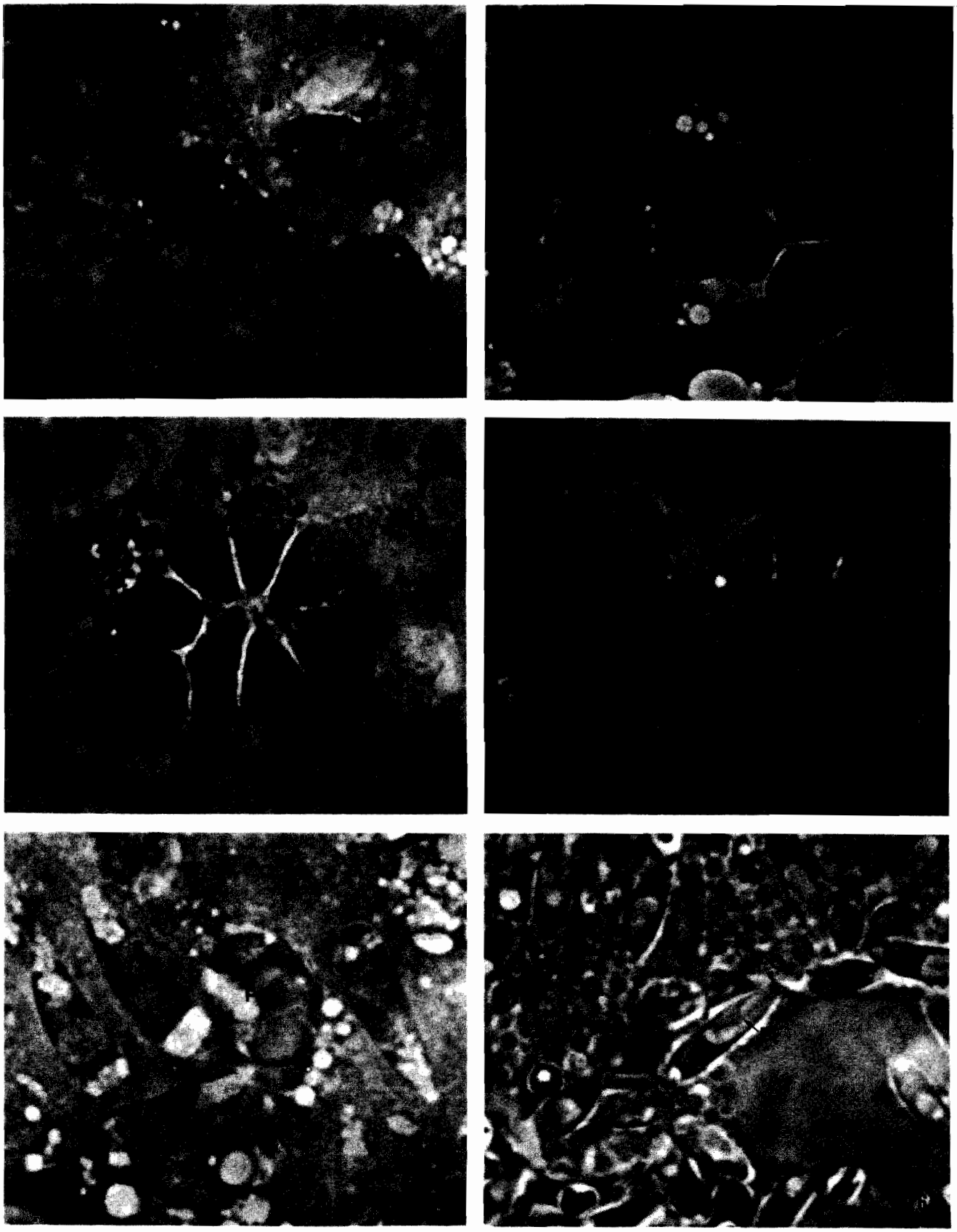
Nineteen of sixty (35%) exposed copepods were infected with *A. californica*, while none of the copepods in a control group was infected. Infected copepods appeared opaque white when observed under a dissecting microscope. Most of the copepods that were full of immature or mature spores had not died of the infection by the time they were examined.

The earliest developmental stages observed in the copepods were meronts which underwent repeated nuclear division to form a number of sporonts (Fig. 1). Each sporont divided to become a binucleate sporont (Fig. 1), then a quadrinucleate sporont (Fig. 2) and eventually a pansporoblast containing 8 sporoblasts (Fig. 3). The pansporoblast membrance was not seen in this study. The earliest sporoblasts were somewhat oval, approximately 11 μm in length and 4 μm in width (Fig. 3). They then formed a drop-shaped stage (Fig. 3). They elongated further, forming a later sporoblast stage (Fig. 4). The mature spores (MS), approximately 18 μm in length and 6 μm in width, were elongate and sometimes slightly curved (Fig. 5). Some possessed a long, sharp anterior part. In semi-thin sections, a polar cap (PC) was visible at the anterior end of the spore (Fig. 6).

Electron microscopy showed that the mature spore had a single nucleus (N) which was oval or round (Figs. 7, 9). The multichamber-like polaroplast (PP) occupied at least half of the anterior portion of the sporoplasm (Fig. 8). The posterior vacuole (PV) is located in the posterior part of the sporoplasm. Tangled tubules (T) were seen, in some sections, in the posterior vacuole (Fig. 7). There usually were some electron-dense large granules (G) around the posterior vacuole (Fig. 7).

The polar filament (PF) included an anterior straight portion and a posterior portion that was isofilar and consisted of 12-14 coils, each approximately 220 nm in diameter. They were posterior of the polaroplast (Fig. 7). The filament caps were found anteriorly, adjacent to the spore wall, in the sporoblasts and the mature spores (Figs. 6, 11). The polar filament was a hollow tubule containing an electron-dense core (Figs. 7, 9, 12). Several mini-sized coils (MC) appeared next to one side of the posterior part of the polar filament coils (Fig. 7). Their structure was similar to that of the regular coils.

A "parabody" (B), which resembled a bundle of folded tubules, was close to one side of the polar filament in many sections (Figs. 9, 10); its position was variable. The spore wall, about 34 nm thick, consisted of thin electron-dense outer (OL, 4 nm) and inner (IL, 4 nm) layers. The thicker, electron-translucent middle layer (MLM, 26 nm) was further divided into three sublayers by a slightly electron-dense sublayer in the middle (Fig. 12). A plasmalemma adjacent to the inner layer of the spore wall confined the sporoplasm and the or-



Figures 1-5.-Developmental stages of *Amblyospora californica* in *Acanthocyclops vernalis*. 2,000X.
Fig. 1.-Uninucleate and binucleate sporonts. Fig. 2-Uninucleate and quadrinucleate sporonts. Fig. 3.-Pansporoblast containing eight young sporoblasts. Fig. 4-Elongated sporoblasts. Fig. 5-Mature spores (MS) and a pansporoblast (PS).
Figure 6.-Semi-thin section of mature spores showing polar caps (PC), straight portion of polar filaments (PF) and posterior vacuoles (PV). 1,200X.

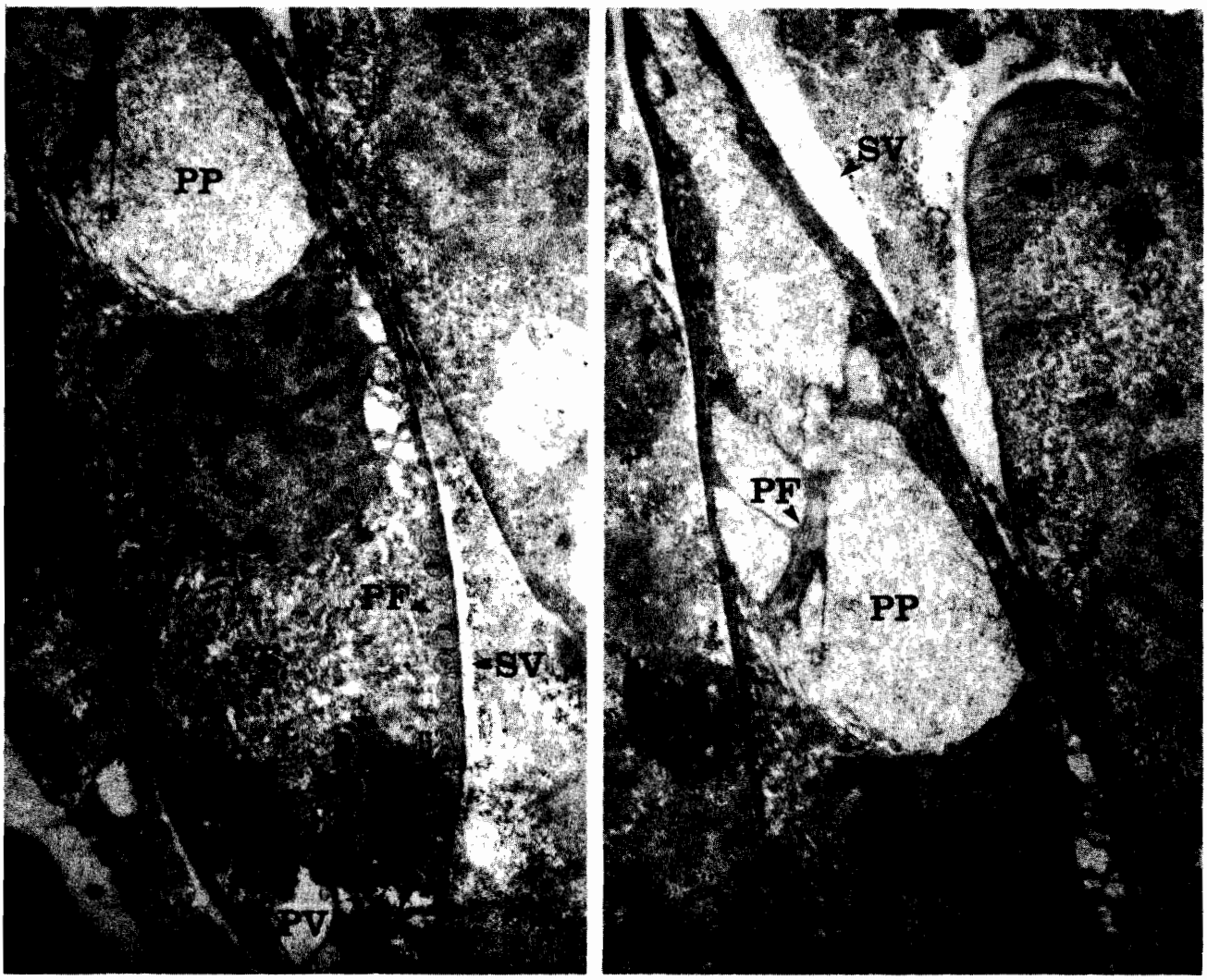


Figure 7.-Electron micrograph of a mature spore showing single nucleus (N), vesicle sporoplasm (VS), part of polaroplast (PP), polar filament (PF), mini-sized coils (MC), posterior vacuole (PV) with tangled "microtubules" (T), large electron-dense granules (G) and sporophorous vacuole (SV). 16,800X.

Figure 8.-Electron micrograph of the anterior part of a mature spore showing the multichamber-like polaroplast (PP). 16,100X.

ganelles. A sporophorous vacuole (SV), which consisted of a space between the vacuole and the spore wall, was visible in most sporoblasts and spores (Figs. 7, 8, 10, 12).

Discussion.

Transovarial transmission is important but seems not to guarantee the perpetuation of *Amblyospora*. In some species, such as that in *Aedes taeniorhynchus*, transovarial transmission is restricted to a single generation (Lord et al. 1981). Even for species exhibiting efficient transovarial transmission (ca. 90%), it was insufficient for continuation of the species (Andreadis and Hall 1979). Thus, another type of transmission is necessary to perpetuate these parasites (Sweeney et al. 1985).

It has been reported that copepods may serve as alternate or intermediate hosts of *Amblyospora*. *Amblyospora* sp. from *Aedes cantator* and *Amblyospora* sp. from *Cx. annulirostris* are able to develop in the copepods *Acanthocyclops vernalis* and *Mesocyclops albicans*, respectively (Andreadis 1985, Sweeney et al. 1985). Development of *A. californica* also has been reported in *Mesocyclops leuckarti* in Louisiana (Sweeney et al. 1985). The

present study reports that *A. californica* also is able to develop in the copepod *A. vernalis*, which was colonized from a breeding site of infected *Cx. tarsalis*. It is significant that copepods may play a role in this complex life cycle and transmission route. Thus far, however, we have been unable to infect mosquito larvae by exposing them to spores that developed in copepods. Whether or not *A. vernalis* serves as an intermediate host of *A. californica* remains unclear.

Apparently, in this species, three different types of mature spores could develop in their respective hosts. This degree of polymorphism may be one of the species characteristics of Amblyospora (Andreadis 1985). The absence of karyogamy and meiosis, which have been observed in male larvae of Amblyospora infected *Culex* mosquitoes (Hazard & Brookbank 1984, Chen 1988), indicate that a sexual phase is not involved; the spores produced in copepods may be haploid as are those produced in male larvae. Therefore, the ploidy restoration of *Amblyospora* sp. from *Cx. annulirostris* in the copepod phase postulated by Sweeney et al. (1985) may not occur in this group. The spores seem to be formed by merogony and subsequent sporogony, undergoing repeated nuclear divisions.

Pansporoblasts containing 8 young sporoblasts were seen in infected copepods. However, there was not evidence of the existence of a pansporoblast membrance. Instead, most sporoblasts and spores lay individually within a parasitophorous (sporophorous) vacuole similar to that appearing around the merozoites of *Plasmodium* within erythrocytes (Aikawa et al. 1978). Presumably, the individual young sporoblast, released from a pansporoblast, invaded host cells by forming a sporophorous vacuole, as *Plasmodium* does on erythrocytes. The mechanism, however, remains to be studied.

In spite of differences in spore configuration, the spores from copepods were similar in structure to those from male mosquito larvae. The multichamber-like polaroplast occupies approximately half of the spore and may assist the eversion of the polar filament. There are no lamellae in the polaroplast so the prominet polar cap is not formed by fusion of lamellae with the basal portion of the polar filament as described by Jensen and Wellings (1972). Presumably, it is the transformation of the basal portion of the polar filament. The mini-sized coils are assumed to be the tapering of the posterior end of the polar filament. Another characteristic structure, the "parabody", has not been shown in any other stage or any other species of microsporidian spores. The authors believe that this structure may be associated with the formation of the polar filament since it was usually close to one side of the filament coils. An understanding of its specific architecture and assembly mechanism would be valuable in elucidating its function in the spores. The "microtubules" in all or part of the posterior vacuoles exhibited, in some sections, a connection with the "parabody". Possibly they are an extension of the "parabody" and they may have a cytoskeletal function, as has been found in other species of protozoa (Tucker 1977).

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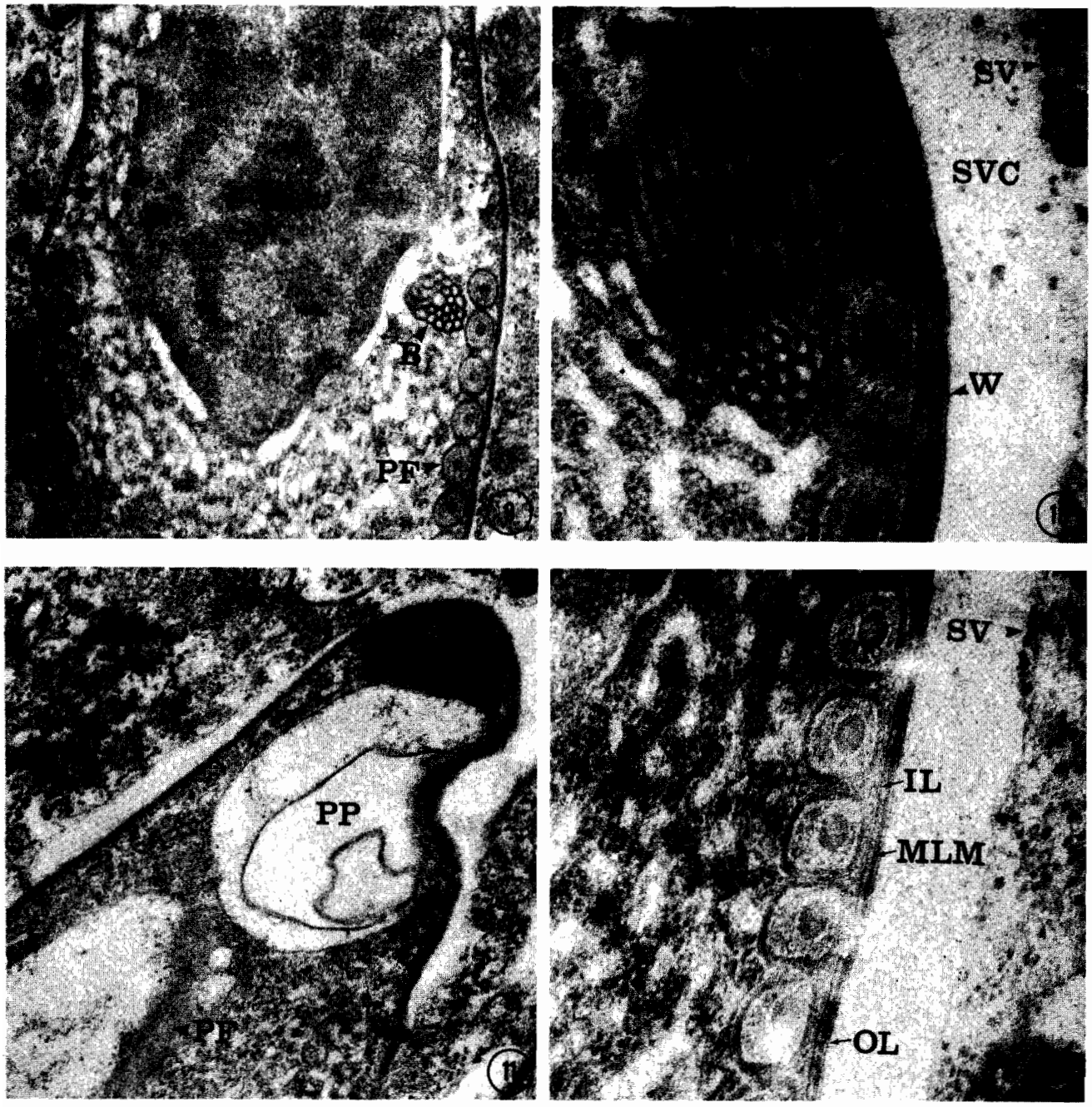

Figure 9.-Electron micrograph of a mature spore showing "parabody" (B) adjacent to one side of the coiled polar filament. 24,300X.

Figure 10.-A magnification of the "parabody" showing its configuration of a bundle of folded tubules. Sporophorous vacuole cavity (SVC) was seen between sporophorous vacuole and spore wall (W). 51,000X.

Figure 11.-Electron micrograph of a mature spore showing the polar cap anteriorly. 25,600X.

Figure 12.-Electron micrograph of a mature spore showing the triple-layered spore wall. It includes the outer layer (OL), the inner layer (IL) and the middle layer which was divided into three sublayers by an electron-dense sublayer in the middle (MLM). 67,000X.

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WATERFOWL MANAGEMENT AND MOSQUITO PRODUCTION IN DIKED SALT MARSHES: PRELIMINARY CONSIDERATIONS AND MESOCOSM DESIGN

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ABSTRACT

Diked salt marshes in the San Francisco Bay Area are important habitats for waterfowl. However, these habitats are also a major source of pest mosquitoes. This study has been designed to develop management strategies that will reduce mosquito populations in diked salt marshes and concurrently enhance wildlife habitat. For this research, twelve 0.024 hectare experimental ponds have recently been constructed at Grizzly Island Wildlife Area by personnel of the California Department of Fish and Game. Different flooding regimes will be used in these mesocosms to determine which regimes maximize the production of invertebrates that are important in the diets of waterfowl, minimize mosquito populations, and enhance pickleweed (*Salicornia virginica* L.) productivity.

Introduction.

The marshes surrounding San Francisco Bay support a diverse vertebrate and invertebrate fauna. Many of the marshes are tidal in nature. However, even a greater amount of marshland habitat has been removed from tidal action by the creation of dikes (Josselyn 1983). These diked marshes retain water only during the rainy season (usually November - April), although they are often artificially flooded earlier in autumn by government wildlife agencies or private duck clubs to provide habitat for waterfowl. These marshlands, particularly Suisun Marsh in Solano County, are considered a major overwintering resource for waterfowl of the Pacific flyway (Josselyn 1983). Diked salt marshes are considered to be a much higher-quality waterfowl habitat than tidal marshes, and in some coastal areas, brackish marshes can be more important to waterfowl than freshwater marshes (Chabreck et al. 1974).

Unfortunately, the seasonal flooding of diked salt marshes also creates severe mosquito pest problems. These marshes are habitats for the salt marsh mosquito *Aedes dorsalis* (Meigen), and are also the major sources of *Culex tarsalis* Coquillett, *Aedes squamiger* (Coquillett), and *Culiseta inornata* (Williston) in the California coastal region. This latter species is often considered to be zoophagous in most of its range, but when it develops in brackish water habitats it becomes an aggressive human biter (Bohart and Washino 1978). Most studies of habitat management for mosquito control in coastal California have concentrated on control of *Ae. dorsalis* in tidal marshes (Resh and Balling 1983a, 1983b). However, in the San Francisco Bay Area, diked salt marshes are more extensive than tidal marshes and they contain a wider range of mosquito problems.

The ecological importance of the diked salt marsh habitat has resulted in numerous restrictions on mosquito control practices. The increasing awareness of the importance of invertebrates, particularly pesticide-sensitive chironomid midges, in waterfowl diets (Murkin and Batt 1987) may result in state and federal waterfowl agencies and duck hunting organizations objecting to the use of even selective pesticides.

Diked salt marshes are highly manipulated ecosystems with the level of management reaching a peak in marshes managed for waterfowl production. Unfortunately, waterfowl management techniques rarely include mosquito control as a goal. Water-level manipulations are the primary tool used to manage waterfowl habitats. In many diked salt marshes, the sophisticated water-level management schemes that are available could also provide mosquito control. Needless to say, any management plan that is devised to limit mosquito production must also be beneficial to wildlife species for the directors of these habitats to be encouraged to alter their current programs.

Recent research has shown that invertebrates may be important in waterfowl diets (Murkin and Batt 1987). Hence, the enhancement of certain invertebrate populations may be beneficial to waterfowl. Many insects that are reported to be important in waterfowl diets, such as chironomid larvae (Order Diptera), hydrophilid larvae (Order Coleoptera), and corixid larvae and adults (Order Hemiptera), are major components of the invertebrate fauna of diked salt marshes. Other studies have shown that mosquitoes are less abundant

Figure A.-The series of 12 ponds constructed at Grizzly Island Wildlife Area.

in habitats that contain high numbers of other macroinvertebrates. For example, in Manitoba marshes, Murkin et al. (1982) have documented that marsh management practices that increase invertebrate production for waterfowl also resulted in decreased mosquito densities.

An appropriate habitat to examine how mosquito and wildlife management can be practiced concurrently is the pickleweed community of diked salt marshes. Diked marshes in the San Francisco Bay region contain several floral communities, but the major plant cover is pickleweed (*Salicornia virginica* L.). Although pickleweed is not an important plant food for waterfowl (Mall 1969), ducks are often found in flooded pickleweed areas, possibly feeding on the invertebrate fauna that occurs there. However, when diked pickleweed marshes are flooded, they become primary breeding sites for mosquitoes. As a result, the diked pickleweed marsh is an important habitat in terms of the mandates of both wildlife and mosquito control agencies.

The waterfowl benefits and the mosquito problems in diked marshes are related to seasonal flooding regimes. Therefore, this study has been designed to examine the effect of various flooding strategies on invertebrate and wildlife species in diked pickleweed marshes of the San Francisco Bay. The goal of this research is to develop a management strategy that will be based on answers to the following questions:

1. What flooding regime will maximize invertebrate populations that are beneficial to waterfowl?

2. What flooding regime will minimize mosquito production?

3. What flooding regime will maintain plant vigor and hence general marsh quality?

Mesocosm Design.

The effects of flooding regimes on invertebrate populations and pickleweed productivity will be evaluated in a series of small plot experiments. In August 1987, twelve experimental ponds (or mesocosms), each approximately 11 m by 22 m, were constructed for our use by the California Department of Fish and Game personnel at the Grizzly Island Wildlife Area in Suisun Marsh, Solano County. These ponds were built within a 30 m by 350 m area that is representative of the habitat of diked pickleweed marshes. The site was divided into a single row of twelve consecutive ponds that are isolated from each other by dikes 0.75 to 1.0 m high and 5.0 to 6.0 m wide (Fig. A). This arrangement was selected to allow water to be pumped mechanically from a parallel canal into each pond. The enclosed stands of pickleweed can be flooded to depths ranging up to 60 cm (the maximum depth currently used to manage diked marshlands for waterfowl).

Figure B.-A pond flooded to a 20 cm water depth.

Figure C.-A pond flooded to 40 cm water depth.

Pilot studies during autumn 1987 and winter 1988 have demonstrated that flooding of these ponds yields a succession of mosquito populations. *Aedes dorsalis* was present after the initial flooding in September; this was followed by *Cx. tarsalis* in October, and finally *Cs. inornata* in December through February. The dominant invertebrate taxa present were species of Corixidae, Chironomidae, Hydrophilidae, and Ephydridae. These studies also demonstrated that invertebrates and mosquitoes can be sampled effectively in these experimental habitats using standardized sweeps of an aquatic net, mosquito dip samplers, artificial substrates, sediment corers, and emergence cages.

However, these studies also indicated that large numbers of waterfowl were not attracted to

Figure D.-A pond flooded to 60 cm water depth.

the small plots. Therefore, the responses of waterfowl will have to be addressed through independent studies. For example, the invertebrates that are important to waterfowl can be determined from gut analysis of hunter-killed mallards and northern pintails. This information can be used to choose which invertebrate populations should be studied in detail in the experimental plots.

Most invertebrates that have been reported in waterfowl diets are benthic or epiphytic. Different flood regimes will affect them because of changes in substratum type and dissolved oxygen levels. In contrast, mosquito larvae occupy the water surface; they will probably respond differently to flooding depth than macrobenthos because of the changes in vegetative cover that different water depths will produce. In pilot studies, ponds were flooded over a range of depths that are currently used in waterfowl management: 20, 40, and 60 cm. The 20 cm depth has resulted in <25% open water (Fig. B); the 40 cm depth has resulted in approximately 50% open water (traditionally considered to be the most desirable cover for waterfowl) (Fig. C); and the 60 cm depth has entirely covered much of the pickleweed, resulting in >75% open water (Fig. D). The series of mesocosms will provide the template by which the effects of different water depths and different amounts of plant cover on mosquitoes and invertebrates may be evaluated experimentally.

In addition to invertebrate responses, the pickleweed vigor in each pond, which can indicate general marsh vitality, may also be affected by the different flooding regimes. Pickleweed quadrate samples (0.2 m^2, n=36) were collected in all ponds in August, 1987 to provide baseline data prior to any flooding. Similar samples will be collected each August (which is near the end of the growing season) to determine pickleweed productivity (using the techniques of Balling and Resh 1983). These data will then be analyzed to determine if flooding regimes cause significant changes in plant productivity.

Acknowledgments.

We thank Dennis Becker, Bob Smith, Andre Marechal, and Steve Ceniseroz of the California Department of Fish and Game for assistance and for constructing the ponds described in this paper; the California Waterfowl Association for providing a water pump; and Dennis Beebe and Carol Evkhanian from Solano County Mosquito Abatement District for their support of this project. This research has been funded by University of California Mosquito Research Funds.

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PROGRESS REPORT ON THE USE OF REMOTE SENSING DATA TO SURVEY MOSQUITO LARVAL ABUNDANCE IN CALIFORNIA RICE FIELDS

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During the 1987 growing season, a study of the relationship between various environmental factors and the abundance of *Anopheles freeborni* larvae in rice fields was carried out in two areas of Sutter County, California. Sampling sites, approximately 2000 square meters in area, were established in 104 rice fields prior to or immediately after flooding in late April and early May. Water depth was recorded weekly from May 18 through September 3, 1987. Weekly maximum and minimum water temperatures were recorded beginning May 18 in 29 rice fields (24 in South Sutter and 5 in the Sutter Basin). Conductivity and pH were measured from June 29 through August 28. Water quality parameters (such as Ca, Mg, B, Na, and Cl) were measured from water samples taken from each of 104 fields on August 17-21. Rice canopy development, aquatic weed diversity, phytoplankton abundance and rice management practices were monitored from May 18 through September 3, 1987; the methods used to measure these factors are described in Rejmánková et al. (1988).

The abundance of *Anopheles freeborni* mosquito larvae was estimated from June 29 through September 3, 1987. Ninety dips using a standard one-pint dipping cup were removed weekly from each field. Approximately 20-30 additional dips were taken every two weeks from July 13 through September 3 to assess the natural predator diversity and abundance in each field.

Remotely sensed data (i.e., reflected visible and infrared, emitted infrared and thermal wavelengths) were acquired with a Daedalus Thematic Mapper Simulator (TMS) mounted on NASA's ER-2 aircraft, the Landsat Thematic Mapper, and the Spot Multispectral Scanner on 10 flight dates from April 24 through August 6, 1987.

The results show that the larval abundance of *An. freeborni* was low in most of the 104 field sites monitored: only 46 fields exceeded a 10 week average of 0.1 larva per dip. The total number of adults emerging from each field throughout the season (adult production) was estimated from the cumulative total number of fourth instars over 10 weeks. Estimated abundance of fourth instar larvae was used as a maximum likelihood estimate of emerging adult numbers. The following assumptions were made: 1) a constant efficiency rate for dippers to collect mosquito larvae from a given area; 2) the numbers estimated in each sample area were representative for the whole field.

The results show that adult production was variable among fields with values ranging from 0 to >20 million mosquitoes per field (mean = 2.39 million adults over 10 weeks). However, the expected number of adults emerging from most fields was low: as many as 67 fields were estimated to produce less than 2 million mosquitoes per field over the whole season. Total adult production was dominated by a small subset of fields: 16 fields resulted in 50% of the total *An. freeborni* adults produced and 33 fields resulted in 75% of the total number of adults.

Identification of field characteristics that are associated with high mosquito production may allow mosquito control efforts to be more efficient and effective. Preliminary analysis of the relationship between remote sensing data and mosquito production suggests that vegetation characteristics, identified and tracked with aircraft or satellite-borne sensors during early season rice development, may be used to forecast larval mosquito production in late summer.

Estimates of plant development over time, using a ratio of infrared to red reflectance, showed that those fields with low numbers of mosquito larvae tended to develop slower than those fields that had high larval abundance. The greatest difference in the amount of emerged plant canopy, as detected using remote sensing data, was in June. Eventually, in mid-July, as the density of the total vegetation cover approached 100%, the difference among fields disappeared.

A canonical discriminant model, which uses the full spectral range of the remote sensing data,

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was developed to predict mosquito abundance. All 104 field sites were ranked according to average larval abundance then divided in half: the lower 52 sites were identified as the low group and the higher 52 sites were identified as the high group. Using remote sensing data acquired on June 19, the discriminant function correctly identified 42 out of 52 sites to have high mosquito abundance and 38 out of 52 sites to have low mosquito abundance. Given these results, we feel encouraged that remote sensing may provide for a regional predictive survey tool for the detection of optimal mosquito habitat among commercial rice fields in northern California. Studies of other environmental parameters, such as water chemistry and crop management practices, are continuing.

Acknowledgments.

We greatly appreciate the assistance of E. E. Kauffman of the Sutter-Yuba Mosquito Abatement District and J. Williams of the University of California Cooperative Extension. This research was supported in part by NASA Contract/Grant No. ID 840037 and USDA Research Grant CR 806771-02, RF-4148A, and Special Mosquito Augmentation Fund, University of California.

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AQUATIC VEGETATION IN RICE FIELDS AS A HABITAT FOR *CULEX TARSALIS* AND *ANOPHELES FREEBORNI*

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Background and Objectives.

Rice fields represent a complicated system of interactions involving higher aquatic plants and algae, mosquitoes, and their natural predators, both invertebrates and vertebrates. The ecology of mosquitoes associated with California rice fields, *Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken, has been the subject of intensive research over the past 25 years (Washino 1980). A 1985 joint study of investigators from the University of California at Davis (UCD) and the National Aeronautic and Space Administration (NASA), using available information on mosquito population, rice cultivation and remote-sensing of wetlands, suggested that vegetation characteristics, identified and tracked with aircraft sensors during early season rice development, could be used to predict larval mosquito populations later in the season (Pitcairn et al. 1987). More thorough investigation of the vegetation, on a ground level, was needed.

The main objective of our research was to monitor changes in the abundance and diversity of aquatic vegetation occurring in rice fields from the beginning of flooding in May, until harvest in September. The vegetation analysis, together with other data characterizing the individual fields, was used to identify and predict favorable mosquito habitats.

Methods.

Two study areas were chosen: South Sutter, near the town of East Nicolaus, and the Sutter Basin, near the town of Robbins. Both areas are located in Sutter County in the lower Sacramento Valley of California. Permission for entry was acquired from 19 farmers for 69 commercial rice fields in South Sutter and from 10 farmers for 35 commercial rice fields in the Sutter Basin. Quantitative monitoring began May 18 and continued through September 3, 1987.

The abundance of aquatic plants was estimated weekly in three 25 square meter plots in each field from May 18 through July 9, 1987, and every two weeks thereafter. Three vegetation layers were distinguished: emersed, floating and submersed. Total percent cover of each layer was estimated and the contribution of each species to each layer was assigned a value on the seven-degree Braun-Blanquet scale (Mueller-Dombois and Ellenberg 1974). Higher aquatic plants were identified to species level, macroscopic algae to genera. Phytoplankton abundance was assessed weekly by fluorometric measurement of chlorophyll-a concentration from June 29 through August 28, 1987.

Rice height and phenological stage were monitored weekly from May 18 through September 3, 1987. At the end of August (the time of expected maximum biomass), rice plants were sampled in five 0.1 square meter rings taken from each field. The number of tillers, leaf area, and biomass were recorded. In 30 fields with high aquatic weed abundance, individual species were sampled at the time of their maximum biomass (usually the beginning of August).

The abundance of *Culex tarsalis* and *Anopheles freeborni* larvae were estimated from June 29 through September 3, 1987; the methods used are described in Pitcairn et al. (1988).

Information on management practices (i.e., rice variety, herbicide combinations and timing, fertilizer application, water source) was acquired for each field.

Several methods of numerical classification and ordination were employed for data evaluation. Single linkage and sum of squares clustering (Ward 1963) were applied on Euclidean and chordal distance matrices (Pielou 1984). Reciprocal averaging was used for ordination of individual fields and species. ANOVA was used for finding significant relationships between particular groups of fields delineated by the above mentioned methods, individual herbicide combinations, and mosquito larvae density. Nomenclature of vascular plants follows Mason (1957).

Results and Discussion.

Twenty vascular aquatic plant species and five genera/groups of macroscopic algae were com-

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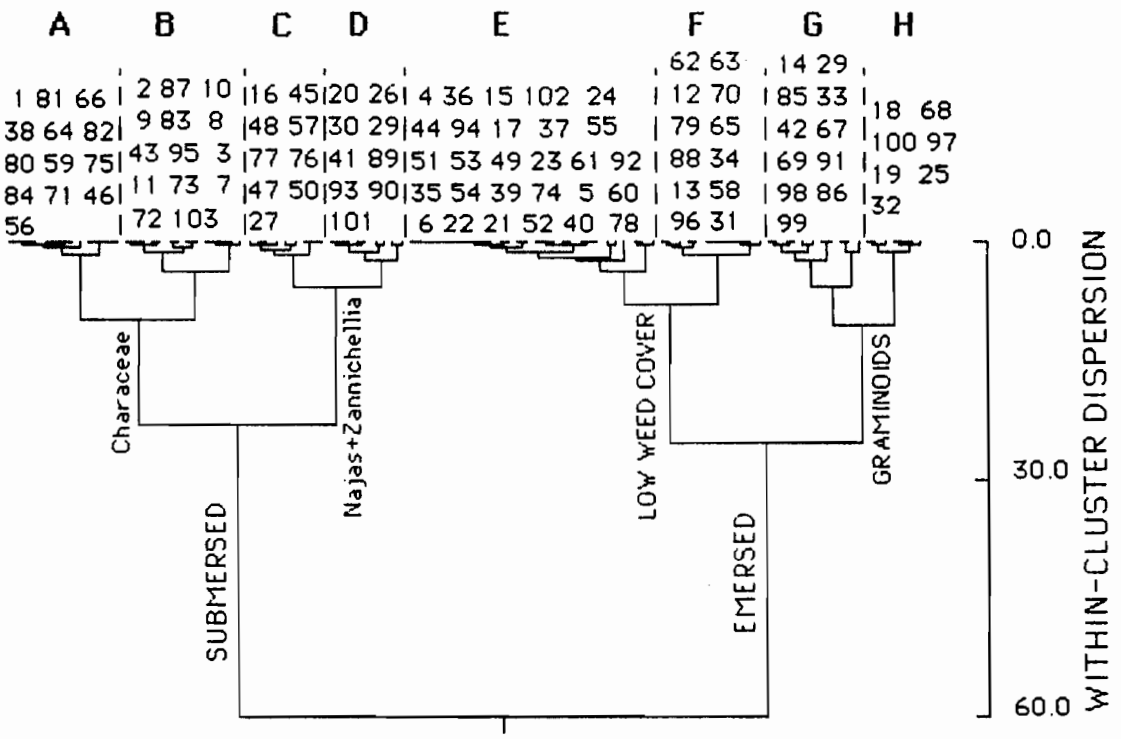

Figure 1.-Sum of squares (Ward) clustering of rice weed communities after chordal transformation. Numbers correspond to the field numbers, letters indicate eight distinct clusters characterized by different species compositions and proportions. Fields belonging to cluster H (dominated by *Cyperus difformis*) had a significantly higher density of *Culex tarsalis* larvae.

monly found in our study sites. Fourteen of them were emersed, i.e., the same growth form as rice: *Echinochloa crus-gali, Leptochloa fascicularis, Scirpus mucronatus, Eleocharis obtusa, Cyperus difformis, Alisma triviale, Sagittaria longiloba, S. montevidensis, Echinodorus berchtoldi, Heteranthera limosa, Ammania auriculata, Dopatrium junceum, Lyndernia dubia, Polygonum lapathifolium*; five species/species groups were submersed: *Zannichellia palustris, Najas guadalupensis, Chara* spp., *Nitella* spp., *Elatine californica*; and the remaining species/species groups were floating: *Lemna minor, Bacopa rotundifolia, B. eisenii,* filamentous algae, *Gloeotrichia* and other blue green algae.

The most common emersed weeds were: *Sagittaria montevidensis* (79 fields), *Heteranthera limosa* (62 fields) and *Ammania auriculata* (51 fields). The most common submersed weed was *Elatine californica* (93 fields), followed by *Chara* spp. (53 fields) and *Najas guadalupensis* (32 fields). *Gloeotrichia, Anabaena and Nostoc* were the most frequently occurring genera of blue green algae. *Spirogyra, Mougeotia*, and *Zygnema* were the most common genera of filamentous green algae.

Typically, herbicides were applied twice (occasionally three times): the first application was about a week after seeding, the second one was about 35 days after seeding. The following combination of herbicides were the most commonly applied: 1) Bolero and Basagran (28 fields), 2) Bolero and MCPA (20 fields), and 3) Ordram and Basagran (21 fields).

The multivariate methods grouped the fields together according to weed species abundance and proportions. Figure 1 was constructed by sum of squares (Ward 1963) clustering method applied on chordal distances between plant communities of examined fields using the data from the end of July. The ANOVA revealed a significantly higher density of *Culex tarsalis* larvae ($p < 0.01$) in the cluster of fields (H) characterized by high dominance of *Cyperus difformis*. ANOVA also showed that most of the fields in this cluster were treated by the Ordram/Basagran herbicide combination.

For each of the two mosquito species, one field with extremely high larval density (Fig. 2) was excluded from the following correlation analysis

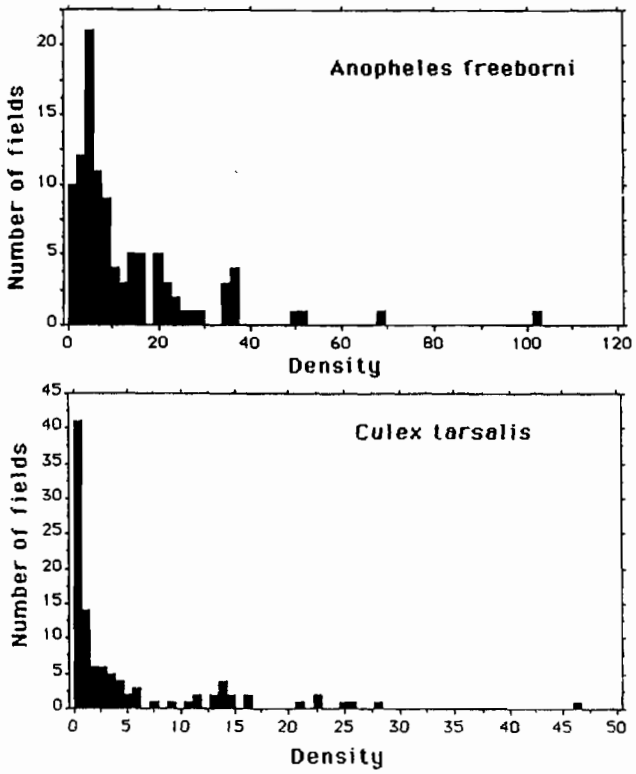

Figure 2.-Number of fields in each of 60 density categories of *Anopheles freeborni* and *Culex tarsalis* larvae. Density is expressed as a number of larvae per 90 dips.

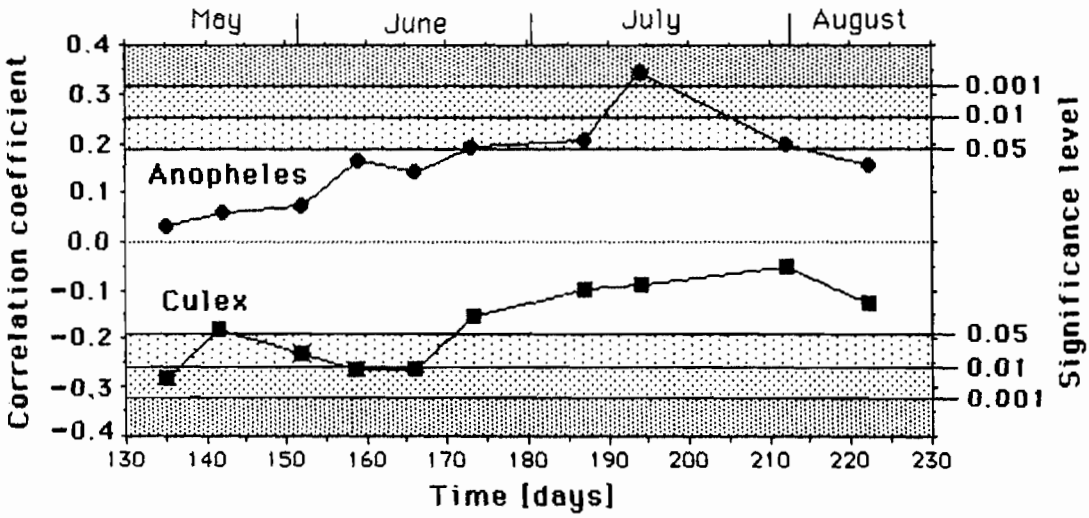

Figure 3.-Correlations between mosquito larval density and rice cover in 103 fields in the course of the 1987 growing season.

using Grubbs' (1969) test for detecting outliers. In the remaining 103 fields, *Anopheles freeborni* was significantly positively correlated with rice cover in the second half of the growing season and *Culex tarsalis* was significantly negatively correlated with the rice cover in the first half of the growing season (Fig. 3).

Our preliminary conclusion is as follows: The main factor determining the mosquito larvae abundance in rice fields is the development of rice plants. From the point of view of the rice field plant community, rice is the prevailing species and does not allow the other species to exert their effect. The exception may be fields with strong domination (the other dominant being at least equally abundant as rice itself) of other emergent weeds, e.g., *Cyperus*.

Acknowledgments.

We greatly appreciate the collaborative efforts of E. E. Kauffman of the Sutter-Yuba Mosquito Abatement District and J. Williams of the University of California Cooperative Extension. This research was supported in part by University of California Mosquito Research Funds, NASA Contract/Grant No. ID 840037, and USDA Research Grant CR 806771-02, RF-4148A.

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THE RELATIONSHIP BETWEEN THE ABSOLUTE POPULATION DENSITY AND SWEEP NET SAMPLES OF NOTONECTIDS IN CALIFORNIA RICE FIELDS

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ABSTRACT

Estimates of relative and absolute density of notonectids (predominantly *Notonecta unifasciata* with a few *Buenoa scimitra*) in rice fields were compared using regression methods. An equation, $X = Y + 0.32 - 0.276$, allows estimation of absolute density from relative density data (sweep net counts). *N. unifasciata* spent only one generation in Fresno rice fields and emigrated from rice fields around June 20, but *B. scimitra* tended to remain in the fields.

Accurate estimations of the total numbers of mosquito larvae and its major predators in a given field are essential steps for the development of integrated mosquito management programs. However, since it is usually impossible to count all the larvae and predators in the field, it is necessary to estimate the absolute population density from relative sampling data. Stewart and Schaefer (1983) estimated absolute density of *Culex tarsalis* Coquillett larvae in rice fields by calculating data from dipper sampling. Miura et al. (1982) and Stewart and Miura (1985) estimated population density and growth rate of mosquitofish, *Gambusia affinis* (Baird and Girard) from data using a capture-recapture method. Populations of damselfly nymphs in rice fields were estimated by Miura and Takahashi (unpublished manuscript) using dipper sampling data.

The objective of this study was to estimate absolute population density of notonectids in rice fields and to define the relationship between notonectid population density and growth of rice plants.

Materials and Methods.

A series of 8 to 11 hectare commercial rice fields in western Fresno County were used for this study. The fields are flooded each year in early May with canal water and usually drained in September.

Sweep Net Efficiency.-Aluminum sheet enclosures measuring 1 m^2 x 30 cm high (Stewart and Schaefer 1983) were used in this study. Ten enclosures were placed at random in a rice paddy and 5 sweep net samples (1 m length) were taken at 3 to 5 minute intervals from different areas of each enclosure with a sweep net (25-cm diam ring with 1-mm mesh net). Each sweep net sample was counted for notonectids in shallow white pans and returned to the collection site. After all sweep net counts were made from the enclosures, absolute densities within the enclosures were estimated by either counting all notonectids or by a removal method (Southwood 1966, Wada 1962, Miura 1980). The sweep net efficiency study was repeated four times during May, June and July.

To evaluate the relationship between sweep net counts (Y_i) and absolute population density from each enclosure (X_i), the data obtained were examined by the correlation method and linear regression (Ostle 1954).

Field Population Survey.-Three rice fields in the study area were used to study seasonal population changes of notonectids. Twenty sweep net samples (1 m length) were taken along each of 2 transects which roughly divided each field into 3 equal parts, thus a total of 40 samples were taken from each field on each sampling day. The population census was taken weekly, starting June 6 and terminated on August 17, 1983.

Emigration Census.-A rectangular, box-shaped screen trap, measuring 30 x 40 x 100 cm was used to census daily emigration of notonectids from rice fields. The trap was placed in the exit weir boxes of 2 of our study fields (nos. 4 and 5) in the morning and retrieved the next morning. All contents were placed in a gallon jar containing 75% ethanol and counted at the laboratory. This study started on June 5 and terminated on July 19, 1983.

Results and Discussion.

Notonectids collected by sweep net in the early season were predominantly (ca. 90%) *Notonecta unifasciata* Guerin with very few *Buenoa*

Table 1.-Relationship between average sweep net collection of notonectids and absolute population density in 1-m^2 enclosures (absolute density was estimated by a removal method).

Enclosure no.	Avg. per sweep	Number removed	Absolute number estimated by: Regression line	Absolute number estimated by: Zippin's chart	Percent captured
1	18.0	77	79	79	97.5
2	5.0	20	20	20	100.0
3	5.3	23	26	28	88.5
4	19.3	71	89	82	86.6
5	4.0	21	21	22	100.0
6	17.0	55	56	58	98.2
7	18.7	81	85	85	95.3
8	14.3	34	40	39	85.0
9	13.3	45	45	47	100.0
10	14.3	40	42	42	95.2

scimitra Bare. As the season progressed, more *B. scimitra* were collected.

Sweep Net Efficiency.-Table 1 shows the relationship between mean sweep net collection and absolute population density within 1-m^2 enclosures as determined by the removal method. Fig. 1 shows the relationship between the numbers of notonectids per sweep (on the Y-axis) and the accumulated number of catch (on the X-axis). The regression line can be drawn visually and the total population of notonectids can be estimated by the intercept of the regression line on the X-axis, as indicated by the arrow. The estimated number can be double-checked with the estimate using Zippin's charts (Southwood 1966). The removal method is a relatively simple and fairly accurate estimate of animal population size inhabiting small confined areas. Wada (1962), Miura (1980) and Mogi et al. (1984) used this method to estimate mosquito population densities breeding in small confined areas, such as fertilizer pits, storm drains, catch basins and crab holes.

Fig. 2 shows the average mean number of notonectids collected by sweep net from the enclosures and the corresponding absolute population estimates. The correlation coefficient (r) was 0.9713, highly significant at the 1% critical level; there was a corresponding increase in numbers sampled with increasing absolute population density.

Fig. 3 illustrates the relationship among notonectid population density, number of emigrating notonectids from rice fields and rice plant growth phases. There was only one notonectid population peak, appearing in early June, when rice plants were still in the tillering stage. Subsequently, the notonectid density fell to almost zero by the middle of July when rice plants were in the late jointing stage, (65 to 75 days after planting). During this time tillering slows down, the internodes begin to enlarge, thus the plants grow taller rapidly and the notonectid population almost disappears. This sudden population decline is primarily due to the mass emigration of notonectids from the rice fields (Fig. 3); the effluent trap set at field no. 4 on June 20 yielded ca. 15,000 third through fifth *N. unifasciata* nymphs in a single day. No *B. scimitra* were found in the trap. The cause of this mass exodus is due to the elimination of the notonectids preferred habitats (i.e.-open, unimpeded water surfaces) by the rapidly growing rice plants. We have noted this phenomenon before with notonectids (Miura et al. 1984) which appears to occur annually in late June (personal communication-D. A. Reed, Manager, Fresno Westside Mosquito Abatement District).

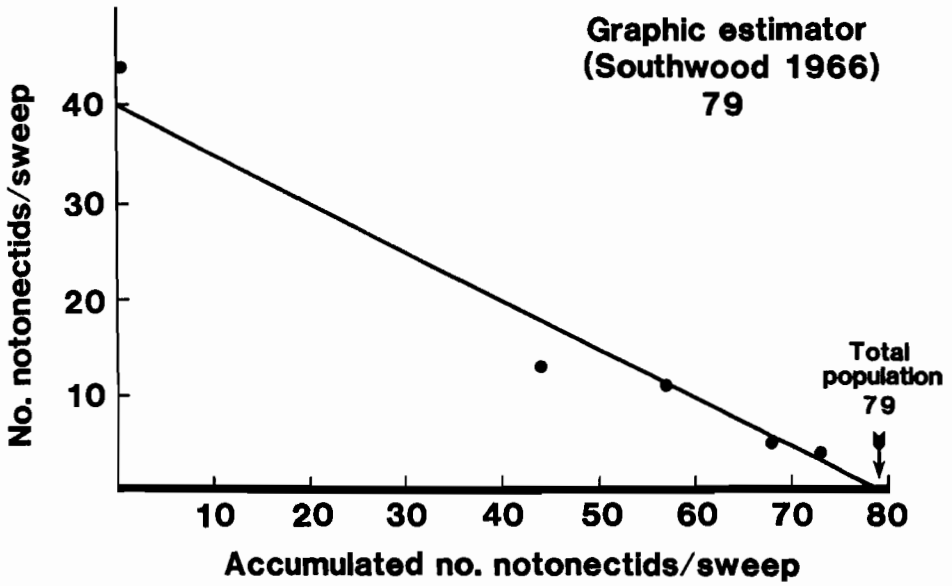

Figure 1.-A regression line to estimate absolute number by the removal method.

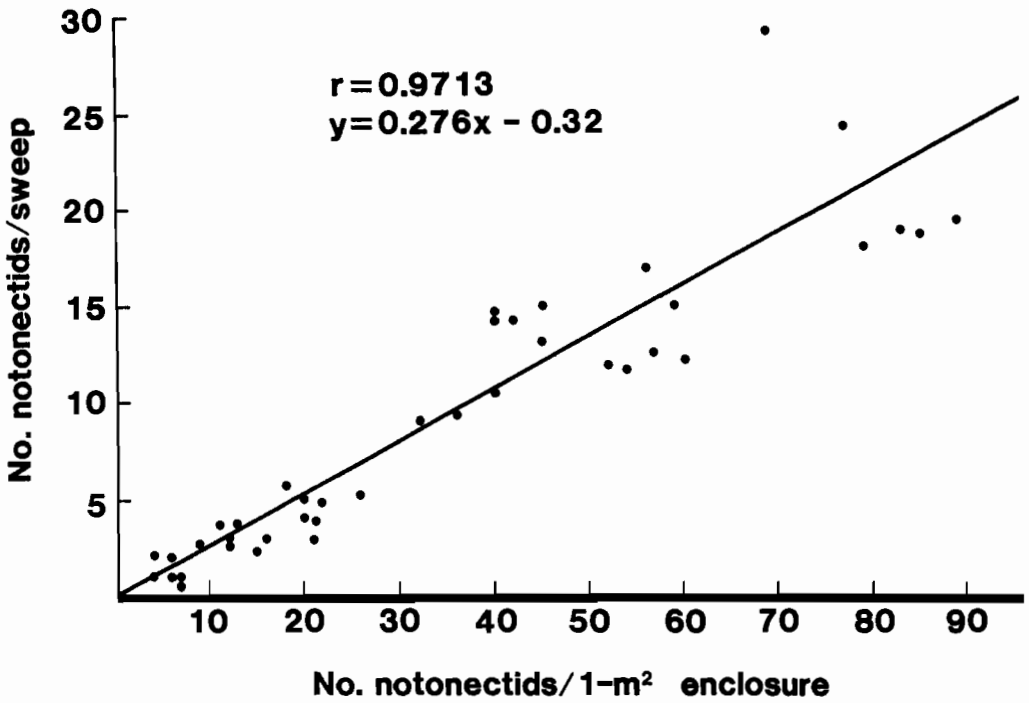

Figure 2.-A scatter diagram showing correlation between sweep net counts (Y) and absolute numbers (X) and regression line.

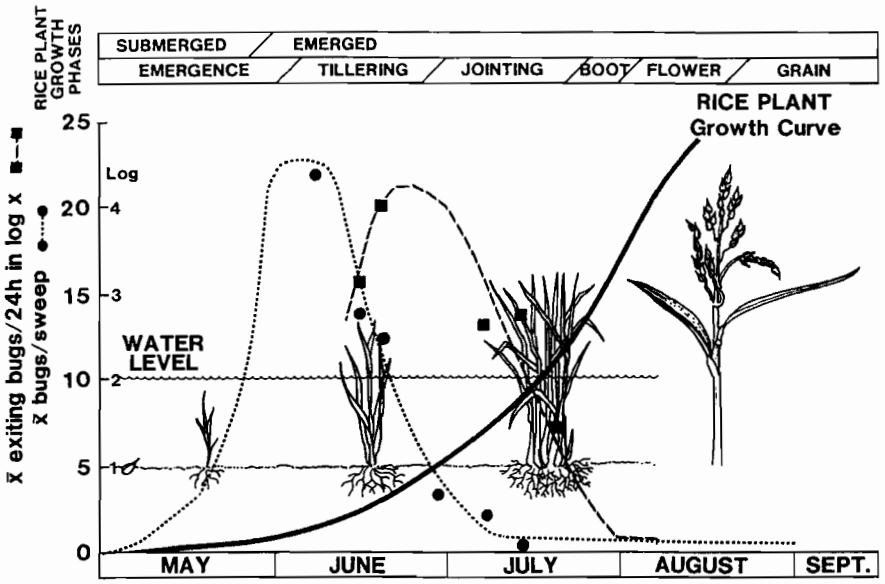

Figure 3.-The relationship among mean average numbers in notonectid sweep net counts, mean number exiting notonectids from rice fields and rice plant growth phase.

Conclusion.

The number of notonectids captured by sweep net were significantly correlated with the absolute population density (r = 0.9713). Therefore, the regression equation, $Y_i = 0.2755X_i - 0.32$, is useful to estimate the absolute density of notonectids in rice fields.

N. unifasciata spent only one generation in these rice fields and almost all of them emigrated from the fields during the rice plants rapid growth phase (jointing stage), about 70 days after planting. *B. scimitra*, on the other hand, tend to remain in the fields and congregate in sparse and open pockets when the rice plants begin their rapid growth.

Acknowledgment.

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DEVELOPMENT AND SURVIVAL RATES OF IMMATURE STAGES OF *CULEX TARSALIS* (DIPTERA: CULICIDAE) IN CENTRAL CALIFORNIA RICE FIELDS

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ABSTRACT

Laboratory and field studies extending over a 5-year period on the development and survival of immature stages of *Culex tarsalis* Coquillett in the San Joaquin Valley rice fields is summarized. The median time required from eclosion to pupation varied inversely with water temperature. The rate of development seems to be determined by mean temperatures, whether or not temperatures were constant or fluctuating. The mean emergence rate was 2.1% in general areas and 13.6% in areas of concentrated mosquito population. The most important mortality factor was predation by polyphagous predators such as *Gambusia affinis*, Odonata, Notonectidae and Coleoptera (Dytiscidae, Hydrophilidae). The rate of predation was 60 to 75%.

The development of an integrated riceland mosquito management program requires an accurate understanding of the nature in which various control agents will act on the ecosystem, as well as a thorough understanding of the biology, ecology and population dynamics of the target mosquito species.

The objectives of this study were to estimate patterns of immature growth rates of *Culex tarsalis* Coquillett at various temperatures, under laboratory conditions and in rice fields, and to estimate the survival rates of immature stages under actual rice field conditions.

Methods and Materials.

Estimation of developmental rates.-Immature *Cx. tarsalis* developmental rates were examined under constant temperatures in the laboratory and under fluctuating temperatures in small rice plots.

Mosquitoes used in this study were obtained from our laboratory colony which had been colonized from wild-caught *Cx. tarsalis* in Bakersfield, California.

Constant temperature studies.-Experimental temperatures of 5, 15, 20, 25, 30, and 35°C were used to obtain estimates of developmental rates in the laboratory. All experiments were conducted under a photoperiod regime of 13L:11D in Percival®E-30B environmental cabinets. Ten egg rafts (<1 h-old) were held in a custard cup with 100 ml of temperature-adjusted tap water. Approximately, 150 to 200 newly hatched larvae were randomly pipetted into a white enamel pan (30 x 18 x 5 cm) with 1 liter of tap water conditioned to rearing temperatures. Larvae were fed 2 ml each of 10% baker's yeast, 5% liver powder suspension, and finely ground rodent chow daily. The amount of rodent chow given was determined by the stages and number of larvae in the pan. The pupae were transferred to a glass storage jar (100 mm diam X 80 mm) with 250 ml of temperature-adjusted tap water; the emerged adults were sexed and counted.

Observations were made 3 times a day (8-h intervals). Growth rate calculations were based on midpoint hour between each observation time. Natural mortality, which occurred at each stage, and temperature were also recorded. This study was replicated twice.

Fluctuating temperature study.-A preliminary study was conducted in Fresno, CA in 1984. Twenty or more age-known egg rafts (<1 h-old) were placed in a floating plastic cage with a fine nylon screen bottom (200-mesh) in a small rice plot (1 m^2). Upon hatching, the larvae were poured in the rice plot. Ten dips of water samples containing immature stages were collected daily using a white enameled dipper (450 ml) with a long handle. All collections were preserved in 70% alcohol for later examination. In the laboratory, all larvae were examined under dissecting microscopes to determine their stages. Growth rate estimation was based on midpoint hour between each collection time. All molts into a given stage were plotted as a cumulative frequency vs. time and the median molts were estimated graphically. Water temperatures were monitored by a recording thermograph with the

sensing probe floating just beneath the water surface.

During the 1985 rice growing season, another study was conducted to determine the growth rate and natural mortality of the immature stages of *Cx. tarsalis* under varying temperatures. Experimental rice plots at the University of California, Kearney Agricultural Center (KAC), Parlier, CA were used. Ten or more age-known (<1 h-old) egg rafts were placed in the floating plastic cages in the rice plot. Upon hatching, 10, 20, 25 or 50 larvae were randomly pipetted into separate floating predator-exclusion cages (plastic cylindrical cages measuring 15 cm deep x 12 cm diam) with two 84 cm^2 underwater windows covered with 200-mesh nylon screen and also screened on top. The water depth in the cages was 10 cm and the internal water surface was ca. 100 cm^2. Six cages were used on 3 separate occasions during the study period. On the 1st run, 10, 20 and 50 larvae were placed in each cage with 2 replicates. In subsequent tests, 25 larvae were used in each cage with 6 replicates. The cages were inspected daily for molting and mortality at the plot, however, early stages (i.e., 1st and 2nd stages) required examination with dissecting microscopes in order to identify exuviae. The contents of each cage were condensed in fine nylon mesh screens and brought into the laboratory. Caged larvae were fed ca. 50 mg of finely ground Tetramin®fish food daily. Growth rate estimation was based on midpoint hour between observations. A frequency distribution of molting was made from data, and duration was calculated.

Estimation of survival of natural populations.- Immature stages of mosquitoes were sampled with dippers (450 ml) from 3 different rice fields in the San Joaquin Valley: in 1981, a medium sized field (16 ha) was sampled in Kern County; in 1982, a large field (90 ha) in Kings County; and in 1983, a small field (8 ha) in Fresno County. All fields were sampled yearly from June to September. Generally, samples were examined and counted in the field, but set samples of 20 or 60 dips were taken from areas where pre-sampling had indicated a heavy concentration of immature stages ("hot-spots"). All "hot-spot" samples were concentrated (Husbands 1969) and taken to the laboratory for species and stage identification under dissecting microscopes.

The collection data were then analyzed for time-specific survivorship using the methods described by Service (1976) and by Reisen and Siddiqui (1979). Survival rates at each stage were estimated from the survivorship curves by using the formula: initial no. of stage 1/ total no. of 1st stage.

Results and Discussion.

Estimation of growth rates.

Constant temperatures.-The duration for each immature stage and the total time from hatching to adult emergence are given in Table 1. Within the temperature range of 20 to 30°C, the duration of 4th stage larvae was longer than the other stages and the 2nd stage was the shortest. Egg rafts kept at 5°C did not hatch during the 54 day observation period; some 1st stage larvae that hatched from the 15°C regime were transferred to the 5°C cabinet, but all died within 16 days.

There have been several reports pertaining to the duration of immature stages of *Cx. tarsalis* under controlled conditions. The period from hatch to pupation has been reported for 36.4-9.3 days at 12.8-35°C, respectively (Bailey and Gieke 1968), 22.9-10.5 days at 19-24°C, respectively (Rosay 1971) or 20-10 days at 18-34°C, respectively (Reisen et al. 1984). Our data generally agreed with these reports (Table 1.).

Miura et al. (1978) with calculations based on the data of Bailey and Gieke (1968), indicated that immature stages of *Cx. tarsalis* had a developmental zero point of 6.67°C (44°F) and degree-days of 217.7. Reisen et al. (1984) reported it at 5.3°C and 268.8. In our present study, developmental zero point was 9°C and degree-days was 190.25. The maximum temperature at which aquatic stages proceed (upper threshold point) was not determined experimentally, but estimated empirically as 35°C. Egg rafts kept in the 35°C cabinet were hatched and developed to 3rd stage larvae, but no larvae proceeded to the 4th stage in our experiment. Bailey and Gieke (1968) reared larvae successfully at 95°F (35°C), but with a high rate of mortality (93.7%). Reisen et al. (1984) reared larvae at 34°C; a few larvae pupated, but none emerged.

A growth rate curve (phenological curve of Podolsky 1984) was constructed from the growth rate study with constant temperatures (Fig. 1a). In general, the larvae developed disproportionately slower at the lower temperatures than at the high temperatures (i.e., the larvae took 3 or more times longer to complete the aquatic stage at 15°C than at 30°C).

Fluctuating temperatures.-Tables 2 & 3 show the results of growth rate studies under fluctuating temperatures. The duration of each stage is similar to that under constant temperatures; the 4th stage was the longest and the 2nd stage was the shortest.

Table 1.-Median duration (days) for development of immature stages of *Culex tarsalis* at constant temperatures in the laboratory, each test was duplicated twice.

Stage	Temperature (°C)					
	5	15	20	25	30	35
Eggs	54.0[a]	5.30	2.80	2.00	1.40	1.30
1st stage larvae	16.0[b]	4.15	2.87	1.74	1.93	1.21
2nd stage larvae	--[c]	4.39	2.48	1.30	1.38	.89
3rd stage larvae	--	6.85	3.38	2.00	1.62	1.25
4th stage larvae	--	8.97	5.09	3.48	2.85	--[c]
Pupae	--	9.85	3.22	2.20	1.61	--
Hatch to pupation	--	34.21	17.04	10.72	9.37	--

[a] Egg rafts kept at 5°C not hatched.

[b] 1st stage larvae hatched at 15°C were transferred to 5°C but all died.

[c] -- tests were terminated because all larvae died.

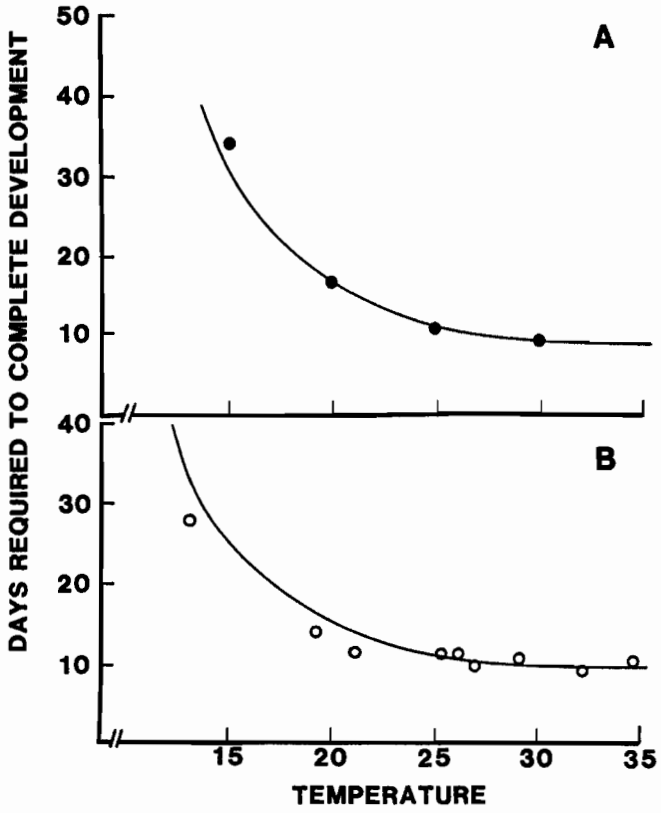

Figure 1.-Growth curves for the immature stages of *Cx. tarsalis*. A. Under constant temperatures. B. Under fluctuating temperatures. Points denote mean temperatures.

Table 2.-Median duration (days) for development of eggs, larvae and pupae of *Cx. tarsalis* in varying temperatures in a small rice plot at the Fresno Airport, 1984.

Stage	Temperature (°C)					
	13	19.2	25.5	26.3	27 [b]	32.3
	(8-18)[a]	(16-28)	(18.5-33.5)	(18.5-35)	(23-31)	(17.5-35.5)
Eggs	4.5	2.5	1.1	1.1	1.4	- -
1st stage larvae	4.4	2.7	2.0	2.0	1.7	1.4
2nd stage larvae	5.8	4.8	4.2	3.0	1.0	1.6
3rd stage larvae	5.2	2.5	2.0	2.0	1.5	1.3
4th stage larvae	10.1	2.7	1.8	2.0	3.3	2.3
Pupae	2.5	1.3	1.4	2.3	2.3	2.3
Hatch to pupation	28.0	14.0	11.4	11.3	9.8	8.9

[a] Numbers in parentheses are minimum and maximum temperatures during the growing period.

[b] This temperature range was conducted in the laboratory.

Table 3.-Median duration (days) for development of larvae and pupae of *Cx. tarsalis* in varying temperatures in a small rice plot at the Kearney Agricultural Center, 1985.

Stage	Temperature (°C)		
	19.6	25.8	28.8
	(17.9-21.2)[a]	(22.8-28.7)	(22.9-34.6)
1st stage larvae	2.4	1.5	1.8
2nd stage larvae	1.2	1.3	1.2
3rd stage larvae	2.0	2.1	1.5
4th stage larvae	3.6	3.8	3.4
Pupae	2.2	2.1	2.0
Hatch to pupation	11.4	10.8	10.2

[a] Numbers in parentheses are minimum and maximum temperatures during the growing period.

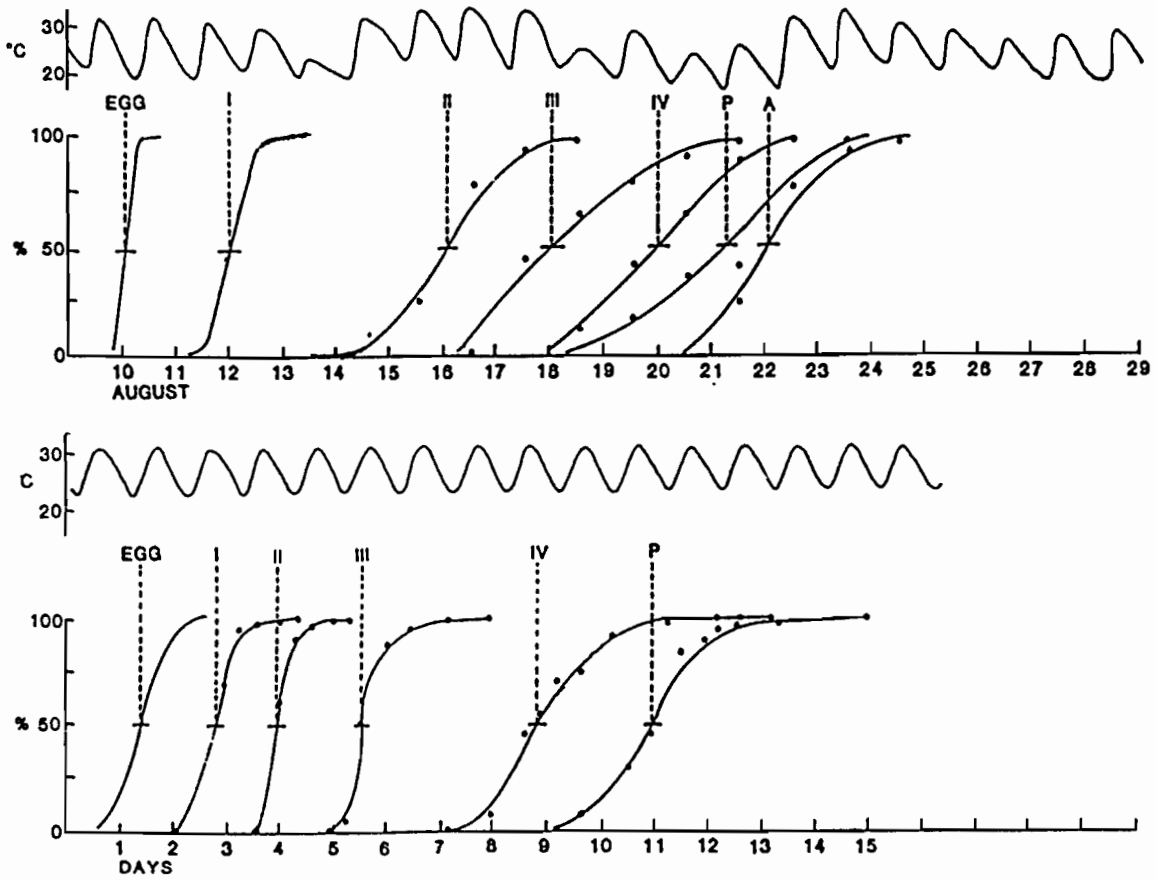

Figure 2.-Cumulative frequency distribution of development time for the immature stages of *Cx. tarsalis* showing daily fluctuating temperatures and median point for each stage.

However, in outdoor conditions the temperature (and hence larval growth) was influenced by many weather factors such as rain, wind and cloud coverage. On August 13 and 14, 1984, there was an abnormally cold rain. The late 1st and early 2nd stage larvae exposed to the cold went through a prolonged 2nd stage (Fig. 2).

A growth rate curve from the fluctuating temperature studies at the Fresno Airport and the Kearney Agricultural Center is shown in Fig. 1b. Surprisingly, the two growth rate curves, one based on constant (Fig. 1a) and the other on fluctuating (Fig. 1b) temperatures, are almost identical. It seems that the mean of daily fluctuating temperatures might be considered equivalent to constant temperatures. The growth rate is approximately the same when the constant temperature is equal to the mean of the fluctuating temperatures. The same was reported by Milby and Myer (1986) when they reared *Cx. tarsalis* in a deep shaded pond (constant temperature) as opposed to a shallow unshaded pond (fluctuating temperature). They concluded that the growth rates were determined by mean temperature, regardless of temperature fluctuation.

Estimation of survival rates.

Constant temperature.-Mortality in our constant temperature study (Table 4) was considerably high. However, it is reasonable to assume that this degree of high mortality would occur when larvae are subjected to unnatural conditions such as constant temperatures combined with artificial lights and high larval densities. Despite the low survival rates, the duration required to complete the aquatic life cycle agreed closely with those published by Bailey and Gieke (1968), Hagstrum and Workman (1971), Rosay (1972) and Reisen et al. (1984).

Fluctuating temperatures in predator-exclusion cages.-The stage-specific age distribution and survivorship curve of *Cx. tarsalis* reared under

fluctuating temperatures in predator-exclusion cages was constructed using the data obtained from the KAC rice plot study (Fig. 3). Stage durations were determined, by using our data and other published data (Bailey and Gieke 1968, Hagstrum and Workman 1971, Rosay 1972, Reisen et al. 1984), to be 2.4 days for 1st, 1.6 for 2nd, 2.3 for 3rd, 3.7 for 4th stage larvae and 1.9 for the pupal stage. The survivorship curve constructed was nearly a straight line, indicating a constant rate of mortality during the immature stages (i.e., no greater probability of dying at one stage group than at another). When reared under optimal temperatures, with sufficient food supply, and in predator-free rearing cages, immature survival rate was 74.7%. Mogi et al. (1984) reported that emergence rates of *Culex vishuni* Theobold in Philippine rice fields ranged from 95 to 40%, averaging ca. 70%.

Natural populations.-Four species of mosquito larvae belonging to two genera were collected from rice fields in the San Joaquin Valley, They were *Cx. tarsalis*, *Cx. quinquefasciatus* Say, *Anopheles freeborni* Aitken, and *An. franciscanus* McCracken. *Cx. tarsalis* was the most abundant in each field (90% or more); relatively few *An. freeborni* and *An. franciscanus* were collected (in the latter part of rice growing season); and only 2 specimens of *Cx. quinquefasciatus* were collected (Kern County rice fields).

Although the sampled rice fields produced relatively few mosquito larvae, larval densities often varied greatly from one area to another, amongst fields and even within a field. Areas which produced higher than normal densities, as a matter of convenience, were termed "hot-spots" (Table 5, Fig. 4). Each collection contained larvae of all stages,

Table 4.-Mortality (%) during development of larvae and pupae of *Cx. tarsalis* at constant (laboratory) and fluctuating (rice plots) temperatures.

Temp.	Mean mortality (%)						No. Larvae
°C	I	II	III	IV	P	Total	Used
	-------- Constant temperature (laboratory) --------						
5	100	- -	- -	- -	- -	100.0	375
15	28.3	43.7	23.0	3.7	1.0	99.7	514
20	14.0	11.7	42.3	11.3	4.3	83.6	710
25	7.7	2.7	24.0	14.0	3.7	52.1	976
30	21.0	13.0	18.3	14.7	14.0	81.0	799
35	35.3	23.3	29.3	12.0	- -	100.0	150
	-------- Fluctuating temperature (rice plots) --------						
19.6 (17.9-21.2)	3.0	4.0	6.0	15.0	3.0	31.0	150
25.8 (22.8-28.7)	1.0	2.0	2.0	9.0	3.0	17.0	150
28.8 (22.9-34.6)	3.1	1.7	4.0	11.3	8.0	28.1	160

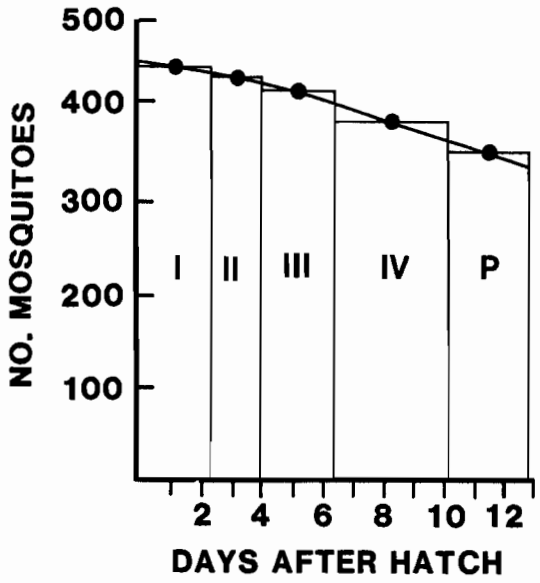

Figure 3.-Age distribution and survivorship curve for the immature stages of *Cx. tarsalis* reared in predator-exclusion cages in rice fields. Points denote number of immatures at the temporal midpoint of each age group.

generally in decreasing numbers with advanced stages. A few pupae were collected intermittently, thus indicating relatively stable age structures. Age distribution and survivorship curves were constructed to determine age distribution and survival rate of natural populations in the rice fields (Table 5, Fig. 4). In a routine larval survey, all collected specimens were tabulated separately, by stage, in the field. However, to construct age distribution and survivorship curves, 1st and 2nd stage larvae were analyzed separately only from collections examined under microscopes in the laboratory. Mortality was the most intense in the early stages in the Fresno and Kern County rice fields. In the Kings County rice fields, highest mortality occurred during the 3rd larval stage. Once larvae reached the 4th stage, survival to adults was high. This was especially true in "hot-sport" areas (Fig. 4). Occurrences of mosquito producing "hot-spot" areas in rice fields are well-known and many speculations have been proposed to explain this phenomenon: ovipositional preference of female mosquitoes (Kato 1955), high content of nitrogenous compounds attract females to oviposit (Schaefer et al. 1982), and abundant larval food material in the fields (Nakamura et al. 1971). Miura et al. (1983) contended that exposure of submergent vegetation (*Najas* sp., *Chara* sp.) to air, by lowering the water level in Fresno County rice fields, had created habitats conclusive for larval abundance (i.e., the habitats thus created excluded most of the normally effective predators). Improper cultivation practices, such as inadequately prepared soil, can result in "hot-spots". In Kings County, we sampled many isolated, small, shallow pools created from uneven land preparation which bred larvae at high densities. Kern County rice fields which were irrigated with a mixture of sewage and well-water resulted in an abnormally fast growth of rice plants; these plants lodged in several large areas. The fallen plants created a habitat which was nutrient-rich and excluded predators, and we sampled many larvae in these habitats.

The mean emergence rates of *Cx. tarsalis* in the San Joaquin Valley rice fields were 1.5% in general areas and 14.3% in "hot-spots". Conversely 98.5% and 85.7%, respectively, of 1st stage larvae hatched in those areas, died during the aquatic stages. The main cause of this reduction was probably due to predation. Mortality due to natural enemies (the difference between the emergence rates of the predator-free cages and those of the natural population) was estimated to be 60 to 75%. Natural enemies, therefore, are the most important factors in rice field mosquito control in this area. Mogi et al. (1984) reported that the mean adult

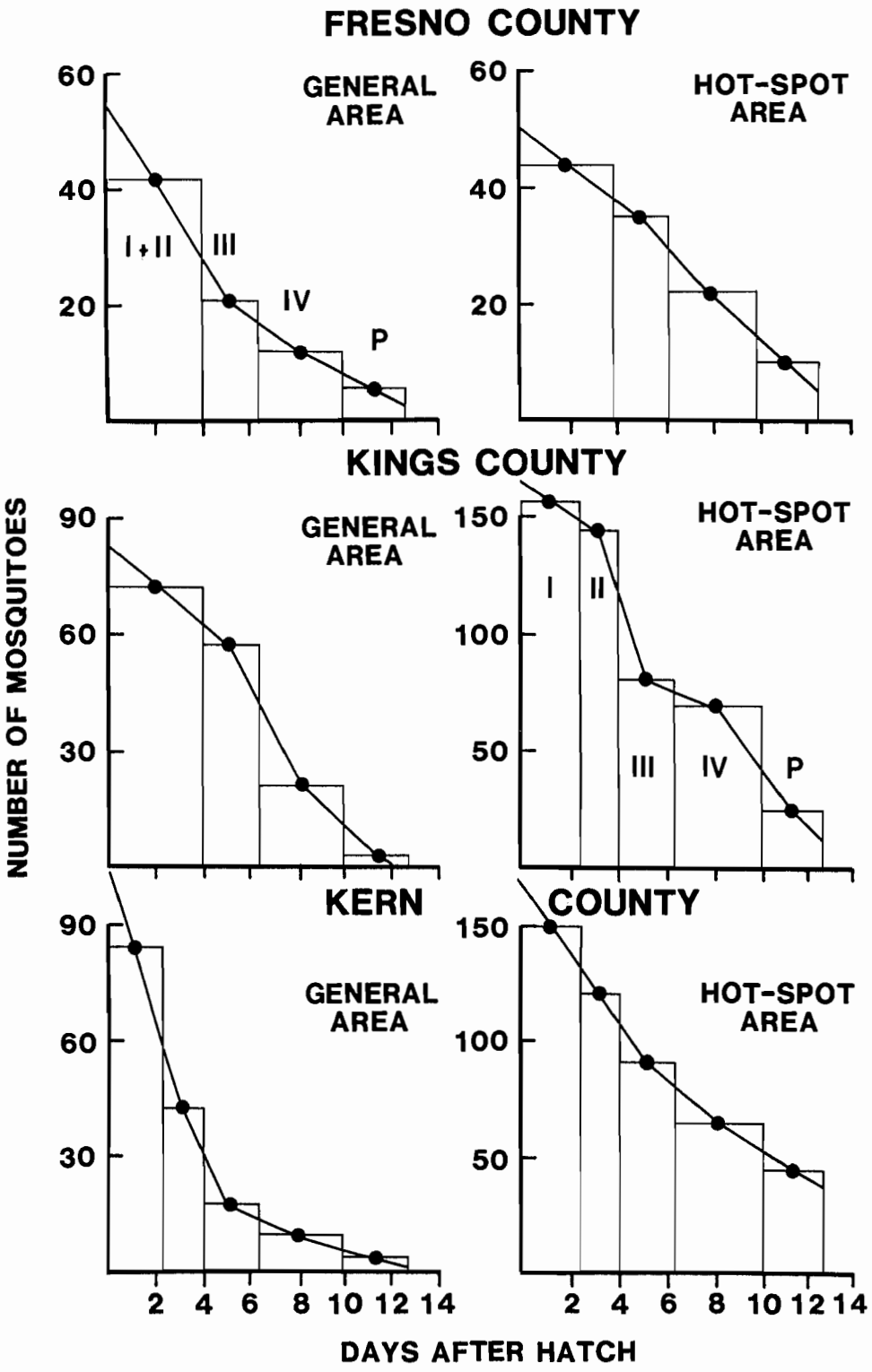

Figure 4.-Age distribution and survivorship curves for the immature stages of *Cx. tarsalis* in rice fields of the San Joaquin Valley.

Table 5.-Survival rates of *Culex tarsalis* immature stages in the San Joaquin Valley rice fields.

Rice field	Stage	Total No.	Median Age No.[a]	Initial No.[b]	Survival Rate
			Fresno County		
General	I + II	164	41.0	56	0.500
	III	48	20.9	28	0.286
	IV	44	11.9	16	0.143
	P	12	6.3	8	0.054
	A	- -	- -	3	
"Hot-spot"	I + II	174	43.5	50.6	0.751
	III	81	35.2	38.8	0.593
	IV	79	21.4	30	0.296
	P	20	10.5	15	0.099
	A	- -	- -	5	
			Kings County		
General	I + II	284	71	81	0.778
	III	133	57.8	63	0.518
	IV	73	19.7	42	0.148
	P	5	2.6	12	0.000
	A	- -	- -	0	
"Hot-spot"	I	380	158.3	167.5	0.896
	II	230	143.8	150	0.716
	III	179	77.8	120	0.463
	IV	266	71.9	77.5	0.269
	P	44	23.2	45	0.075
	A	- -	- -	12.5	
			Kern County		
General	I	202	84.2	106.5	0.577
	II	67	42.9	61.5	0.310
	III	41	17.8	33	0.141
	IV	30	8.1	15	0.056
	P	5	2.6	6	0.009
	A	- -	- -	1	
"Hot-spot"	I	360	150	170	0.765
	II	195	121.8	130	0.647
	III	212	92.2	110	0.471
	IV	233	63	80	0.318
	P	86	45.3	54	0.235
	A	- -	- -	40	

[a]Total no./stage duration.
[b]Estimated by linear interpolation.

Table 6.-List of predators collected from rice fields in the San Joaquin Valley, California.

Collection Method	Taxon	Location: Fresno County	Location: Kern County	Location: Kings County[a]
Dipper (no./dip)		100 dips	200 dips	- -
Eucopepoda				
	Cyclopidae (2 gen, 2 spp)	20.110	68.325	+ + + +
Odonata				
	Zygoptera (2 gen, 2 spp)	.641	.265	+
	Anisoptera (2 gen, 2 spp)	.030	.150	+
Hemiptera				
	Notonectidae (2 gen, 2 spp)	.060	.010	+
Coleoptera				
	Dytisciade (5 gen, 7 spp)	.110	.055	+
	Hydrophilidae (2 gen, 3 spp)	.090	.195	+
Minnowtrap (no./trap)		183 traps	120 traps	- -
Odonata				
	Zygoptera (2 gen, 3 spp)	2.565	.150	+
	Anisoptera (2 gen, 2 spp)	.808	13.750	+ +
Hemiptera				
	Belostomatidae (1 gen, 1 spp)	.423	.552	+
	Notonectidae (2 gen, 2 spp)	8.704	.969	+ +
Coleoptera				
	Dytiscidae A. (5 gen, 7 spp)	3.119	5.818	+ +
	Hydrophilidae A. (2 gen, 3 spp)	3.081	40.531	+ +
Cyprinodontiformes				
	Peociliidae			
	Gambusia affinis	13.165	52.146	+ + +

[a]No scheduled sample was taken, relative abundance of predators are indicated by the signs (+); (+) sparse, (+ +) few, (+ + +) moderate, (+ + + +) many.

emergence rate of *Culex* sp. in Philippine rice fields was 0.9% and that reduction by natural enemies was 50 to 90%.

Rice fields create a unique aquatic environment. They are man-made, shallow, temporary bodies of water which support only those species which can annually immigrate through canal systems or by flight. Despite this restriction, many aquatic organisms establish themselves and develop in enormous numbers in a relatively short time. Our previous studies (Miura et al. 1981) show that 62 species or species groups can be collected regularly and another 23 taxa collected occasionally from Fresno County rice fields.

Many of these organisms are predators of mosquito larvae, and hence, reduce the mosquitoes' chances for survival. Table 6 shows a list of predators collected from rice fields in the San Joaquin Valley. *Cyclops vernalis* (Crustacea), the most commonly found predator in dipper collections, have been shown to prey on early stages of mosquito larvae (Miura et al. 1984). About one-third of all aquatic insects we sampled in rice fields are predacious to mosquito larvae. Although the relative abundance of these predators varied from field to field, certain predators always seemed to be very well represented. For example, members of the suborder Zygoptera (damselfly), while moderately represented in dipper and trap sampling, were more noticeable in area sampling of 1-m^2 enclosures, in which a range of 400 to 800 nymphs were consistently captured. In Kern and Kings Counties, our sampling yielded more Anisoptera (dragonfly). In many fields, Notonectidae were the most abundant in the early rice growing season, but as rice stands rose above the water surface, most emigrated via irrigation water and we observed no other generations. Also abundant in these fields were aquatic Coleoptera (Dytiscidae and Hydrophilidae), which have been reported to be very important mosquito predators (Washino 1969, Hazelrigg 1974).

Perhaps the most effective of all mosquito predators is the mosquitofish (*Gambusia affinis*), which was introduced into California in 1922, and is planted annually by many mosquito abatement districts as part of their regular mosquito control program. The effectiveness of mosquitofish as a predator is well-documented (Washino and Hokama 1967, Bay 1969, Hoy and Reed 1970, Hoy et al. 1972, Miura et al. 1984, Stewart et al. 1985). Many of the Kern and Kings rice fields supported a large number of mosquitofish, and very few mosquito adults successfully emerged from those fields (Table 5, Fig. 4).

A microsporidian infection [*Amblyospora californica* (Kennen and Lipa 1960)] was reported from this area. But of all mosquito collections examined, no infected larva was found, indicating that this infection has a minor role in regulating *Cx. tarsalis* populations in the San Joaquin Valley rice fields.

In determining the effect of predation on mosquito larvae in rice fields, the weakest aspect has been a quantitative assessment of predation. Further detailed studies are necessary to elucidate the role of these predators in mosquito larval population regulation.

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FISH PREDATION ON MOSQUITOES IN WASTEWATER: EFFECTS OF THERMAL STRESS AND INCREASED FISH COMMUNITY COMPLEXITY

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ABSTRACT

We investigated the interactions of two fish species and their mosquito prey, and the effects of thermal stress on these interactions. Amargosa pupfish (*Cyprinodon nevadensis*) and mosquitofish (*Gambusia affinis*) were placed in 32 210-L tanks, resulting in four replicates of eight treatments: cool temperature (range 18-31°C), tanks with no fish, pupfish, mosquitofish, and both fishes combined, and hot (23-36°C) tanks with the same four treatments. The tanks had thick growths of Sago pondweed (*Potamogeton pectinatus*) to simulate wastewater marshes. Mosquito (*Culex pipiens*) egg rafts were planted in each tank at rates approximating four egg rafts per day. Emergence rates were monitored for two months. Pupfish and mosquitofish reduced mosquito emergence to low levels compared to no fish treatments. Thermal stress reduced emergence in the absence of fish, increased emergence in the presence of a single fish species, and had no effect in the presence of both fish species. Our results show that thermal stress can act to increase emergence in the presence of either mosquitofish or pupfish, suggesting that thermal stress inhibits the ability of fish to reduce mosquito emergence. No increased emergence in the presence of both fish together in the high temperature environment suggests that these fishes complement each other's mosquito control efficacy in thermally stressful environments. These results suggest a complex interaction between fish and mosquitoes mediated by environmental conditions, the outcome of which depends on fish community complexity.

PHOTOPERIODIC EFFECTS ON REPRODUCTION IN CALIFORNIA MOSQUITOFISH, *GAMBUSIA AFFINIS*

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ABSTRACT

We examined the effects of short photoperiods in dispelling reproductive refractoriness in the mosquitofish, *Gambusia affinis affinis*. Sixteen mature females and five males were randomly distributed into each of 16 38-liter aquaria held at 25°C. Short-day treatments were applied for periods of 4, 8, 12, and 16 weeks: fixed short-day photoperiods were 3L:21D (3 hours light:21 hours darkness), 6L:18D, 9L:15D, and decreasing daily photoperiods duplicated ambient autumn photoperiods. At four-week intervals, one aquarium from each treatment was changed to photoperiods duplicating the natural daily increases, beginning with that of the winter solstice (Dec. 21), to stimulate reproduction. Gravid females were moved to brooding cups suspended in each aquarium and allowed to give birth. Each reproductive event and the number of resulting fry was recorded.

Fishes kept on the 9L:15D short-day pretreatment for 12 weeks showed a rapid and marked reproductive response after transfer to lengthening days. While 84% of the females gave birth prior to the seventh week of increasing-day treatment, a cumulative total of 94% was achieved after 11 weeks at a final daylength of 11.6 hours. In comparison, fishes kept on the natural photoperiod pretreatment for 12 weeks showed a much slower reproductive response, which did not begin until after 5 weeks on increasing days. A cumulative total of 44% of the females in this treatment reproduced after 11 weeks of lengthening days. We concluded that the 9L:15D pretreatment photoperiod was more effective than ambient photoperiods at dispelling reproductive refractoriness. Conversely, the 3L:21D and 6L:18D photoperiods were no more effective than natural photoperiods at dispelling reproductive refractoriness.

ISOLATION OF A NEW STRAIN OF *LAGENIDIUM GIGANTEUM* AND IMPLICATIONS FOR CONTROL OF FLOODWATER AND OTHER RAPIDLY DEVELOPING MOSQUITO SPECIES

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ABSTRACT

The oomycetous fungus *Lagenidium giganteum* has been evaluated in the field against a number of multivoltine mosquito species primarily in California and North Carolina. These trials have documented the ability of the fungus to recycle for weeks or months following a single application in larval habitats supporting culicine and anopheline species. Continuous oviposition or sporadic inundation of breeding sites with species occurring in succession between early spring and late fall is often sufficient to support appreciable levels of fungal activity.

Occasional field evaluations against *Aedes* spp. breeding in pastures and other areas subject to periodic flooding have resulted in sporadic results, probably due primarily to insufficient zoospore release in organically rich environments. Induction of zoosporogenesis, which is necessary for larval infection, is usually the limiting factor in the initiation of epizootics in field trials with *L. giganteum*. Not all floodwater species breed in water characterized by high organic content. These should be amenable to control by the asexual stage of the fungus which releases zoospores in 6 to 24 hours following dilution in a suitable water source. It has been assumed, however, that multi-season control of rapidly developing floodwater and univoltine species was not feasible since the sexual (oospore) stage of the fungus, which is responsible for persistence of the parasite in the absence of suitable hosts and when breeding sites seasonally dry out, could not germinate on a time scale matching that of mosquito egg hatch and larval development.

In late May, 1987, a routine collection of *Aedes melanimon* was made in the Butte Sink area north of Colusa, California. Over 5000 late instar larvae and pupae were collected and returned to the laboratory for physiological studies related to *in vivo* oosporogenesis and to attempt to colonize this species. Upon return to the laboratory, a number of moribund larvae which had been set apart from the remaining immatures upon collection were examined. A fungus morphologically indistinguishable from *L. giganteum* was readily identified in the dead individuals, and within 48 hours, over 85% of the collected *Ae. melanimon* had died from infection by this fungus.

Field studies have been carried out in the Colusa area using one strain of *L. giganteum*, designated the California (CA) strain, since the early 1970's. Although the epizootic occurred over 20 kilometers from the nearest site of application, it is possible that the new isolate of the fungus, designated the Butte Sink (BS) isolate, was derived from the former strain and fortuitously introduced into the area by natural or accidental transfer. Following isolation of the BS strain in axenic culture, a number of physiological tests involving lipid biosynthetic and metabolic capabilities clearly differentiated this isolate from the CA strain. Whether these basic matabolic pathways are immutable or subject to change by prolonged *in vitro* maintenance or *in vivo* cycling in e.g. aedine vs. culicine hosts is not known. Investigation on the relation of these two isolates to others from the southern United States, including one North Carolina isolate from which the CA strain was presumably derived, is continuing. These comparative studies will provide information on the factors regulating in vivo reproduction and bear directly on the recycling capabilities of the *L. giganteum* in the field.

Isolation of an apparently new strain of this mosquito parasite from a population of floodwater *Aedes* greatly expands the potential operational range of *L. giganteum*. One main advantage of using a biological control agent is its potential for prolonged efficacy against its target host following a single application. Documentation of this natural epizootic suggests that under proper physical and/or chemical conditions, the dormant oospore stage can germinate on a time scale comparable to rapidly hatching and developing floodwater and univoltine species. Multi-season control of these mosquitoes must now be considered a possi-

bility, albeit a little understood phenomenon. The physiology of oospores of the BS isolate is being investigated for possible clues to more predictable and rapid germination of these dormant and long-lived propagules.

ACTIVITY OF SLOW-RELEASE FORMULATIONS OF THE IGRS FENOXYCARB AND ALTOSID AGAINST MOSQUITOES AND NONTARGET AQUATIC ORGANISMS[1]

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ABSTRACT

Slow-release briquette formulations of two insect growth regulators, fenoxycarb and methoprene (Altosid®),were evaluated against larvae of *Culex tarsalis* Coquillett in 30 m^2 field ponds. Fenoxycarb briquettes were prepared from a plastic base material, and those of methoprene were prepared from charcoal. Three formulations each of fenoxycarb briquettes and of methoprene were evaluated. Two of the fenoxycarb formulations were effective for more than two weeks at the high rate of 0.1 lb AI/acre. The methoprene charcoal briquettes also yielded similar results at the high rate of 12 lbs of the formulations/acre (0.5 lb AI/acre). None of the formulations tested displayed marked impact on macroinvertebrates prevailing in test plots through the duration of the experiment.

Introduction.

A number of IGRs have been reported to possess a high level of activity against several species of stagnant and floodwater mosquitoes. However, these materials, in general, have failed to provide long-lasting control due to their rapid degradation on exposure to sunlight and other environmental factors in field situations (Mulla and Darwazeh 1975, Schaefer and Dupras 1973). In order to increase the longevity of methoprene (Altosid®),a briquette type formulation was developed and tested against several mosquito species in a wide range of habitats. At the rate of 1 briquette/100 ft^2, excellent control of *Aedes taeniorhynchus* (Wiedemann) was obtained for 28 and 31 days when applied as pre- and post-hatch treatments respectively (Rathburn and Boike 1977). In other studies, these briquettes were equally effective in clear and polluted waters, and excellent control of *Culex quinquefasciatus* Say and *Culex peus* Speiser was obtained for 44-60 days in catch basins, septic tanks, dairy wash lagoons and irrigation ditches (Dunn and Strong 1973, Stewart 1977).

Recently, the IGR fenoxycarb (R013-5223), a carbamate type compound, produced excellent control of *Aedes melanimon* Dyar, *Psorophora columbiae* (Dyar and Knab) and *Culex tarsalis* Coquillett at the rates of 0.01, 0.025 and 0.25 lb AI/acre respectively (Mulla et al. 1985). Field evaluation of granular formulations of fenoxycarb produced excellent control of *Aedes nigromaculis* Ludlow and *Ae. melanimon* at the low rate of 0.005 lb AI/acre. Against *Cx. tarsalis*, these IGRs, as expected, were 5-10 fold less active than against floodwater mosquitoes. However, they yielded satisfactory control of *Cx. tarsalis* at the rate of 0.025 - 0.1 lb AI/acre, with no apparent ill effects on nontarget organisms prevailing at time of treatment (Mulla et al. 1985, 1986).

In order to extend the duration of efficacy of these highly active IGRs, slow-release formulations in briquette form were developed and provided by the manufacturers for evaluation. The present studies were conducted to evaluate the initial activity and longevity of fenoxycarb and methoprene briquette formulations against mosquitoes in experimental ponds, to determine the optimum rate of application for each formulation, and to assess their impact on selected nontarget organisms prevailing in the ponds at time of treatment.

Methods and Materials.

Several types of briquettes were tested at the Aquatic and Vector Control Research Facility of the University of California, Riverside, located in the Coachella Valley of southern California. This facility, described elsewhere (Mulla et al. 1982), consists of 64 ponds constructed in eight rows, each pond measuring 18 x 18 ft (30 m^2), and with vegetation cover of bermuda, nut and crab grasses ranging in lengths from 8-15 cm. Water (pH 9.4) to each pond was supplied from an artesian well

[1]These studies were supported by the State of California Special Funds for Mosquito Research.

through underground pipeline, and water depth was maintained constant (30 cm) by float valves.

Three types of briquettes of similar size (7.5 g each) of fenoxycarb were provided by Maag Agrochemicals, Vero Beach, FL., and each briquette contained 500 mg AI (6.6%) of fenoxycarb. These briquettes were tested and designated according to their color: gold, white and black. Three formulations of methoprene briquettes of various weights, 0.74 g, 7.48 g and 21.2 g, were provided by Zoecon Industries, Dallas, TX, each containing the same concentration (4%) of the active ingredient.

The fenoxycarb briquettes were applied at the manufacturer's recommended high rates of 1 and 2 briquettes/100 ft^2 (0.445 and 0.89 lb AI/acre) for the purpose of achieving extended control. Three and six briquettes of each formulation were distributed evenly in each pond to ensure good coverage, utilizing two replicates for each rate of application. Methoprene briquettes were also applied to duplicate ponds at the rates of 20 and 40 g of each type per pond (0.25 and 0.5 lb AI/acre): 1 and 2 briquettes/pond of 21.2 g, 3 and 6 briquettes of 7.48 g, and 30 and 60 briquettes of 0.74 g. Along with each test, 2 ponds were left untreated as checks. All aquatic stages of *Cx. tarsalis* were present in good numbers (15-20/dip) in all ponds at time of treatment.

Procedures utilized in assessing the initial activity and subsequent longevity of briquettes against mosquito larvae and nontarget organisms were reported earlier (Mulla et al. 1985). In brief, 5 dips per pond were taken prior to treatment, 2 and 7 days after treatment, and every week thereafter until the end of the experiment. The 5 dips were concentrated into one sample, preserved in 65% ethyl alcohol, and organisms present were counted and identified under a dissecting microscope in the laboratory.

For determination of inhibition of adult emergence, 40 4th-instar larvae of *Cx. tarsalis* were collected from each check and treated pond, and 20 larvae were placed in each of 2 sentinel cages. Cages utilized in these studies were described by Mulla et al. (1974), and were floated freely in the ponds. Larvae were collected and placed in the floating units 2 days after treatment, and once every week thereafter until the end of the experiment. Isolated organisms in the cages were inspected twice weekly, and dead larvae, pupae and adults were counted and removed. When no living organisms remained in the cages, percent inhibition of adult emergence (%EI) was determined. Mean (%EI) calculation was based on the average number of adults surviving from the 4th-instar larval isolates in the check and treated ponds. Due to mortality in the checks, (%EI) values were corrected according to Abbott's formula (Abbott 1925): % activity = $\frac{X-Y}{X} \times 100$, where x is mean % survival in the check, and y is mean % survival in the treated.

Throughout the duration of these studies, water temperature was monitored with a minimum-maximum recording thermometer, and mean minimum-mean maximum readings are shown in the Tables.

Results and Discussion.

Oviposition of *Cx. tarsalis* in the ponds was continuous, and the larval population in both treated and check ponds persisted at a high level, averaging 10 4th-instar larvae/dip during the duration of these studies (data omitted). However, dead pupae were observed in the treated ponds 2 days after treatment and beyond. Since both IGRs do not induce mortality until pre-pupal and pupal stages (Mulla and Darwazeh 1979, Mulla et al. 1985, 1986), larval assessment by dipping in a stagnant water habitat where oviposition is continuous does not provide an accurate method of evaluation. Therefore, treated larvae should be isolated to determine delayed action in post-larval stages.

In the isolation units, the gold briquettes of fenoxycarb failed to produce adequate initial or extended control of *Cx. tarsalis* at the rates of 1 and 2 briquettes/100 ft^2 (0.44-0.89 lb AI/acre). However, the white briquettes caused complete inhibition of adult emergence at the lower rate (1 briquette/100 ft^2) for one week, while the high rate (2 briquettes/100 ft^2) remained active for more than 2 weeks. The black briquettes produced similar results at both rates applied (1 and 2 briquettes/100 ft^2), causing complete inhibition of adult emergence for 2 weeks (Table 1).

The white briquettes appear to be the most effective of the three briquette formulations of fenoxycarb, producing adequate control of *Cx. tarsalis* for 5 weeks at the high rate of 2 briquettes/100 ft^2 (0.89 lb AI/acre, data omitted). Beyond 2 weeks post-treatment, due to contamination, mortality in the checks was greater than normally observed (40-50%). Therefore, data obtained 3, 4 and 5 weeks after treatment were excluded from the Tables.

The smallest briquettes of methoprene (0.74 g) produced excellent control of *Cx. tarsalis* for one week at both rates of 20 and 40 grams/324 ft^2 pond (0.25 and 0.5 lb AI/acre) (Table 2). Both rates,

Table 1.-Evaluation of fenoxycarb (Pictyl) briquettes against *Culex tarsalis* in experimental ponds.[a]

Post-treatment (days)	(%) Cumulative mortality and (% EI) in larval isolates[b]								
	1 briquette/100 ft²			2 briquettes/100 ft²			Check		
	Larvae	Pupae	(% EI)	Larvae	Pupae	(% EI)	Larvae	Pupae	(%EI)
				Gold briquettes					
7	21	9	18	44	9	48	15	0	15
14	16	8	13	19	5	14	10	3	13
				White briquettes					
7	5	95	100	10	90	100	15	0	15
14	5	63	75	5	93	100	10	3	13
				Black briquettes					
7	29	68	100	13	87	100	10	5	15
14	9	86	100	8	92	100	10	3	13

[a] Water temperature mean min. 17.7° - mean max. 27°C.

[b] No adult mortality in the check was noted; however, mortality in emerging adults in the treated was (0-10%).

Table 2.-Evaluation of Altosid briquettes against *Culex tarsalis* in experimental ponds.[a]

Post-treatment (days)	(%) Cumulative mortality and (% EI) in larval isolates[b]								
	20 g/pond			40 g/pond			Check		
	Larvae	Pupae	(% EI)	Larvae	Pupae	(% EI)	Larvae	Pupae	(%EI)
				0.74 g briquettes[c]					
2	21	63	86	4	81	84	1	11	12
7	10	90	100	25	61	93	9	0	9
14	21	36	42	18	51	67	26	8	34
				7.4 g briquettes[d]					
2	21	48	76	5	80	89	1	11	12
7	10	73	88	24	73	98	9	0	9
14	39	28	52	41	48	89	26	8	34
				21.1 g briquettes[e]					
2	5	46	51	24	55	85	1	11	12
7	25	40	76	34	58	98	9	0	9
14	43	38	83	18	64	94	26	8	34

[a]Water temperature mean min. 17° - mean max. 26.6°C.

[b]No adult mortality in the check was noted; however, mortality in emerging adults in the treated was (0-14%). % EI was corrected according to Abbott's formula.

[c]Thirty and 60 briquettes/pond.

[d]Three and 6 briquettes/pond.

[e]One and 2 briquettes/pond.

however, produced mediocre control beyond 2 weeks after treatment. The medium sized briquettes (7.4 g) produced 88% inhibition of adult emergence for one week at the low rate (20 g/pond), while the high rate (40 g/pond) was effective for 2 weeks, and level of control declined markedly thereafter. The larger sized briquettes (21.2 g) were somewhat more effective, yielding good inhibition of adult emergence for more than 2 weeks at the high rate of 40 g/pond. At the low rate of 20 g/pond, unsatisfactory results were obtained 2 days post-treatment, but yielded 76 and 83% inhibition of adult emergence, 7 and 14 days after treatment.

Studies on nontarget macroinvertebrates showed that mayfly naiads (*Callibaetis pacificus* Seeman), diving beetle adults (Dytiscide and Hydrophildae) and Ostracods (*Cypridopsis* sp. and *Cyprinotus* sp.) were present in all the ponds prior to treatment, and their numbers followed similar trends in both the treated and check ponds during the entire duration of these studies (Table 3). Diving beetle larvae were absent from all the ponds prior to treatment, but began to appear in somewhat equal numbers in treated and check ponds 2 weeks after treatments and persisted in all the ponds until the termination of the experiments (Table 4).

From the data presented, it appears that fenoxycarb and methoprene (Altosid) briquettes had no noticeable adverse impact on mayfly naiads, diving beetle larvae and Ostracods at all rates applied. Dragonfly naiads, libellulid (*Tarnetrum corruptom* Hagon) and the aeshnid (*Anax junius* Drury), were absent at time of treatment, but began to appear in low numbers in the check ponds and in the ponds treated with the gold type briquettes of fenoxycarb 2 and 3 weeks after treatment. No dragonfly naiads were observed in the samples obtained from treated ponds with the white and black briquettes (Table 3). Due to their low numbers in the checks, no definite conclusion can be drawn regarding the impact of these formulations of fenoxycarb on the development of dragonfly naiads.

In earlier studies, fenoxycarb did not exhibit adverse effects on dragonfly naiads at the mosquito larvicidal rate of 0.1 lb AI/acre (Mulla et al. 1985, 1986). Further studies, however, are warranted to study the impact of slow-release formulations at high initial rates on dragonfly naiads and other organisms which cohabit in the breeding sites of stagnant water mosquitoes.

No apparent harmful effects on dragonfly naiads were observed in the ponds treated with Altosid briquettes. These organisms were found in equal numbers in the treated and the check ponds 3-4 weeks after treatment (Table 4).

In summary, it can be stated that the white and black briquette formulations of fenoxycarb were active for more than 2 weeks at the rate of 2 briquettes/100 ft^2 (0.89 lb AI/acre). In recent studies (Mulla et al. 1986), a 0.2% sand core granular formulation of fenoxycarb rendered good control of *Cx. tarsalis* initially, but lost its activity within 7 days after treatment at the rates of 0.05 and 0.1 lb AI/acre. For extended control using slow-release formulations, the rates of application has to be increased. In conclusion, the white briquettes seemed to be slightly more effective, and could effectively control stagnant water mosquitoes for 3-5 weeks at the rate of 2 briquettes/100 ft^2). It should be pointed out that the extended control may be due to the high rate of application rather than a slow-release from the formulation. Studies on such high rates using granular formulations are warranted.

The larger sized briquettes of Altosid (21.2 g) showed higher efficacy than the other 2 formulations tested, inhibiting adult emergence for more than 2 weeks at the rate of 40 g/pond (0.50 lb AI/acre). Here, as in the case of fenoxycarb, the residual activity could be the result of high dosages rather than the formulations. Comparative studies on longevity of briquettes and SR10 formulations of Altosid are essential.

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Table 3.-Impact of fenoxycarb (Pictyl) briquettes on nontarget organisms in experimental ponds.[a]

Nontarget organisms	Mean no. of nontarget organisms/5 dips pre- and post-treatment (days)[b]											
	1 briquette/100 ft²				2 briquettes/100 ft²				Check			
	Pre	7	21	28	Pre	7	21	28	Pre	7	21	28
Gold briquettes												
Mayfly naiads	26	22	25	14	25	26	27	19	61	136	34	18
Diving beetle adults	11	12	3	10	8	7	5	8	3	4	1	5
Diving beetle larvae	0	0	2	1	0	2	2	3	0	1	2	1
Dragonfly naiads	0	0	1	2	0	0	3	6	0	0	5	4
Ostracods	27	88	179	102	0	56	56	39	70	81	35	147
White briquettes												
Mayfly naiads	38	5	1	12	56	41	26	1	61	136	34	18
Diving beetle adults	6	5	3	4	3	3	2	4	3	4	1	5
Diving beetle larvae	0	0	1	2	0	0	1	2	0	1	2	1
Dragonfly naiads	0	0	0	0	0	0	0	0	0	0	5	4
Ostracods	20	28	189	347	0	0	140	105	70	81	35	147
Black briquettes												
Mayfly naiads	59	9	9	6	114	84	12	3	61	136	34	18
Diving beetle adults	11	3	2	4	7	1	0	8	3	4	1	5
Diving beetle larvae	0	1	1	2	0	0	1	2	0	1	2	1
Dragonfly naiads	0	0	0	0	0	0	0	0	0	0	5	4
Ostracods	0	21	350	245	0	14	252	56	70	81	35	147

[a]Water temp. as in Table 1.

[b]Data for 14-day post-treatment are excluded.

Table 4.-Impact of Altosid briquettes on nontarget organisms in experimental ponds.[a]

Nontarget organisms	Mean no. of nontarget organisms/5 dips pre- and post-treatment (days)[b]											
	20 g/pond				40 g/pond				Check			
	Pre	7	21	28	Pre	7	21	28	Pre	7	21	28
0.74 g briquettes												
Mayfly naiads	153	17	17	21	136	47	26	21	229	34	41	81
Diving beetle adults	21	10	3	2	22	5	3	6	12	5	2	2
Diving beetle larvae	0	1	1	1	0	2	2	1	0	0	2	4
Dragonfly naiads	0	0	8	14	0	0	9	18	0	0	1	14
Ostracods	28	193	39	81	14	42	63	121	25	116	32	175
7.4 g briquettes												
Mayfly naiads	209	86	56	31	106	19	25	28	229	34	41	81
Diving beetle adults	34	9	1	1	15	3	1	3	12	5	2	2
Diving beetle larvae	0	1	2	3	0	1	4	3	0	0	2	4
Dragonfly naiads	0	0	10	15	0	0	10	3	0	0	1	14
Ostracods	18	60	28	105	14	187	140	315	25	116	32	175
21.2 g briquettes												
Mayfly naiads	97	36	29	32	83	17	29	33	229	34	41	81
Diving beetle adults	13	3	3	5	19	4	5	5	12	5	2	2
Diving beetle larvae	0	3	1	2	0	1	2	4	0	0	2	4
Dragonfly naiads	0	0	10	12	0	0	7	19	0	0	1	14
Ostracods	3	56	25	105	39	49	32	236	25	116	32	175

[a]Water temp. as in Table 2.

[b]Data for the 14-day post-treatment omitted.

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PREDICTION OF SPRAY DEPOSIT PATTERNS AND DISPERSION CHARACTERISTICS FROM AERIAL APPLICATIONS OF *BTI*

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Background and Objectives.

The uniformity of the aerial spray distribution pattern and the associated swath spacing used by the aerial applicator are key factors that are related to the success or failure in the use of *Bacillus thuringiensis* var. *israelensis* (*Bti*) for control of mosquito larvae in irrigated pastures and rice fields. There is a multitude of complex variables that can critically affect the spray distribution pattern. The objective of this project was to evaluate the use of the AGDISP model to optimize the selection of important application variables that can be controlled by the aerial applicator. The results could potentially enhance efficacy, reduce quantity required, and/or ensure more consistent control of mosquito larvae.

Procedures.

The research was coordinated with a Mosquito Abatement District (Sutter-Yuba County MAD) that had successfully utilized aerial applications of *Bti* during 1986 and planned to expand its use in 1987. The first phase was to gain some confidence in the use of the AGDISP model to predict spray distribution patterns for this type of application. Field trials were conducted to measure the swath distribution patterns under typical field conditions and operational variables selected by the aerial applicator. A fluorescent dye (Rhodamine B) was added to the undiluted *Bti* formulation (Sandoz, Teknar). The application was made with a Cessna Agwagon equipped with two Micronair AU5000 rotary atomizers. Each AU5000 was adjusted to a blade angle of 65° and a flow rate of 2.6 L/min (0.7 gpm). The system provided an application of 0.3 L/ha (4 oz/A) with a swath spacing of 55 m (180 ft) and an airspeed of 190 km/hr (115 mph). The drop size distribution was measured with our particle size measurement system (PMS) in our wind tunnel. Spray deposits were collected on Mylar plastic sheets (697 cm^2). Deposits were extracted and measured with a fluorometer (Turner). Next, the AGDISP program was used to predict the distribution pattern based on the application parameters and results compared with the measured deposits. The second phase was to calculate the spray pattern for a wide range of application variables. From the computer simulations, we selected conditions that substantially improved the theoretical spray distribution pattern.

Results and Discussion.

Figures 1 and 2 illustrate the experimental spray distribution pattern and AGDISP simulation for a single spray pass over an irrigated pasture in Sutter County, respectively. The pass was made north to south with a wind velocity of 2.1 m/s from 150° which produced a very low crosswind component of 1.0 m/s. This was a typical critical condition where many fields are longer in the N-S direction and winds frequently in an southerly or northerly direction. The low crosswind would likely produce narrow patterns with a greater chance for gaps or streaks in the treatment. The deposit pattern on both graphs represent the crosswind from left to right. The composite ground deposition shown in Figure 2 was based on the drop size distribution measured in our wind tunnel with a PMS laser instrument. The drop size used in the simulation is shown in the deposition legend of Figure 2. A comparison of the predicted and measured patterns was very encouraging. For example, the measured pattern showed two peaks at -7.5 and +12.5 m from the aircraft center line (0), and an overall swath width of approximately 27 m. The predicted results showed peaks at -7.5 and 12 m and an overall swath width of about 25 m. The results generally indicate that under the above conditions, a large gap exists directly in the center of the pattern, and with a flight spacing of 55 m (180 ft), there would also be areas of very low deposits between each pass.

A series of simulations were completed to evaluate the effect of drop size, nozzle location, crosswind, turbulence level, length scale, vortex decay, and initial spray variance.

Figure 3 illustrates a series of drop trajectory calculations designed to determine the maximum

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Figure 1.-Measured spray deposition pattern for Agwagon application with two Micronair nozzles and a 1.0 m/s crosswind from left to right.

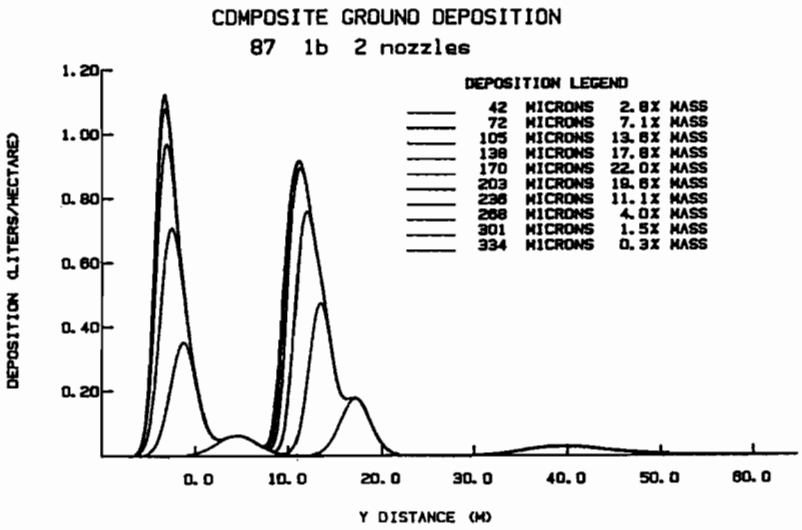

Figure 2.-AGDISP simulation of spray deposit pattern for application similar to above conditions.

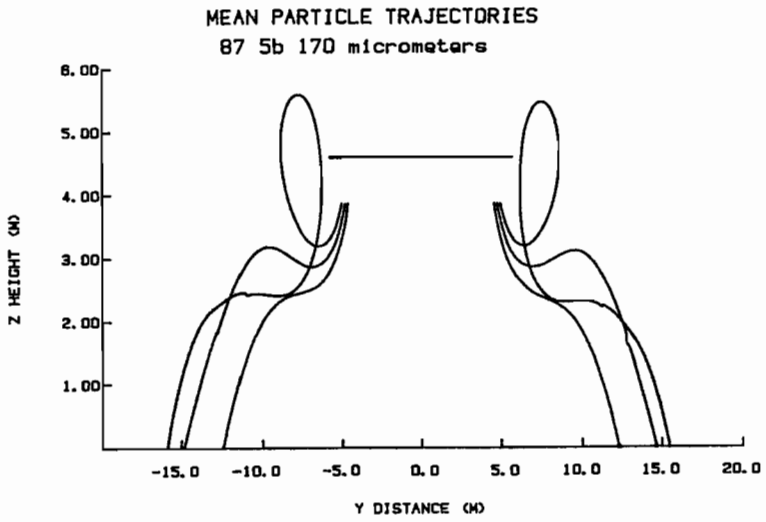

Figure 3.-Trajectories of 170 μm drops released at ±4.6, ±4.8, and ±5.0 m from center line of Agwagon aircraft with zero crosswind.

outboard location of nozzles such that 170 μm drops would not be entrained in the vortices. The release points were ±4.6, ±4.8 and ±5 m from the center line. The ±4.8 m location would produce maximum swath width without entrainment in the wing tip vortices.

Figure 4 illustrates the effect of drop size (138, 170 and 236 μm) on the trajectories of particles released at five locations; 0, ±2.4 and ±4.8 m from the aircraft center line.

Figure 5 shows the theoretical composite ground deposition for the drop spectra produced by the Micronair nozzle (65°). The nozzle locations are the same five positions as discussed above. The simulation was for "0" crosswind. The results indicate some high peaks may occur, but no real wide gaps are evident. The maximum swath width is approximately 30 m (100 ft).

Figure 6 shows the composite ground deposit for the same aircraft treatment as in Figure 5, except with a crosswind of 1.8 m/s. As shown, the overall swath width remains about the same, 30 m, but most of the gaps in the pattern are filled in and the entire pattern was displaced about 12 m downwind.

In general, the results are very encouraging and indicate that the AGDISP program can provide excellent guidelines to improve the uniformity of spray deposit patterns. It appears that substantial reductions in application rates may be possible if the distribution pattern can be improved. We plan to simulate the use of 8 to 9 small flat fan nozzles in an effort to improve the critical distribution pattern for a zero or very low crosswind. Further work is needed to evaluate mosquito larvae control with the proposed systems.

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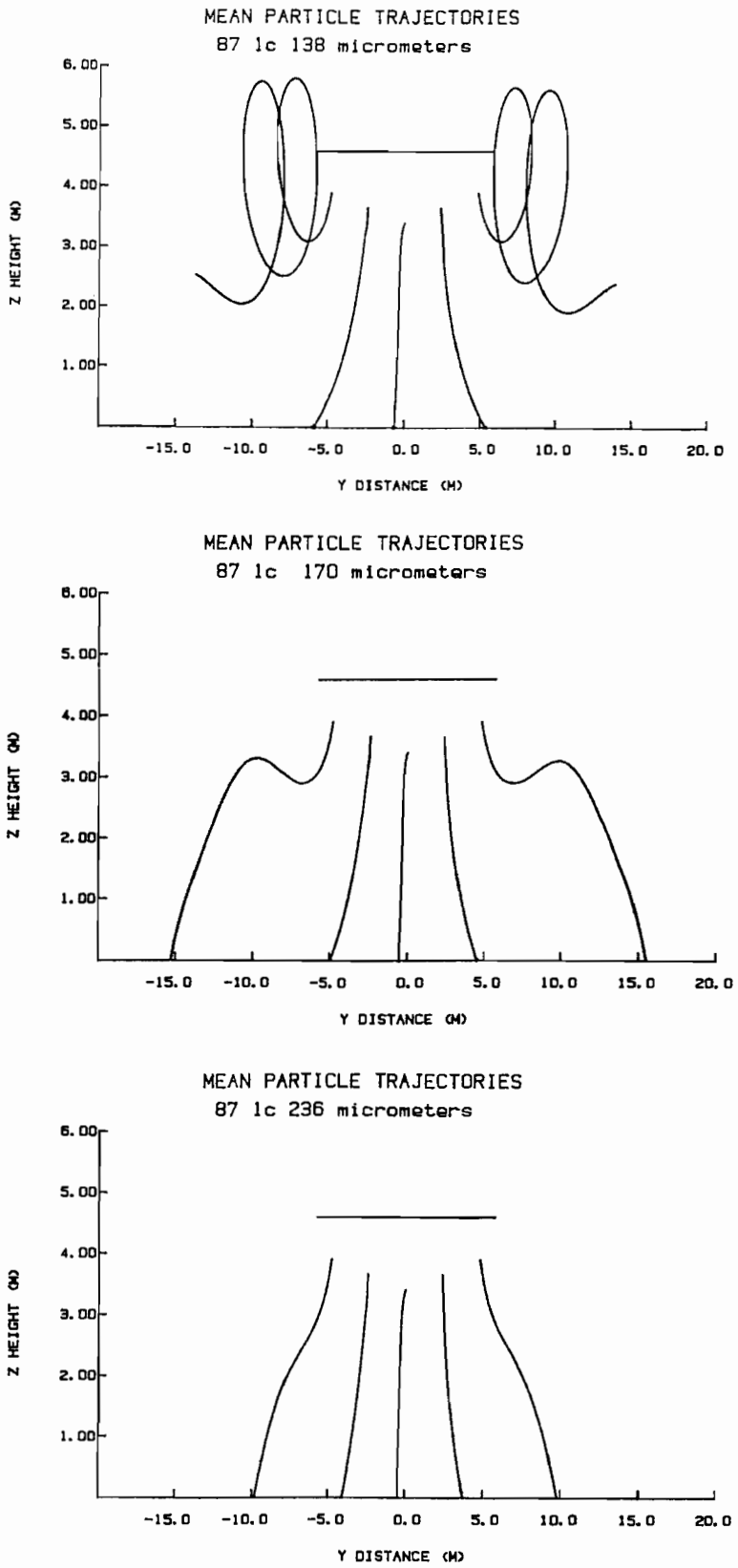

Figure 4.-Trajectories of 138, 170 and 236 μm drops released from 5 proposed nozzle locations, 0, ±2.4, ±4.8 m from aircraft center line.

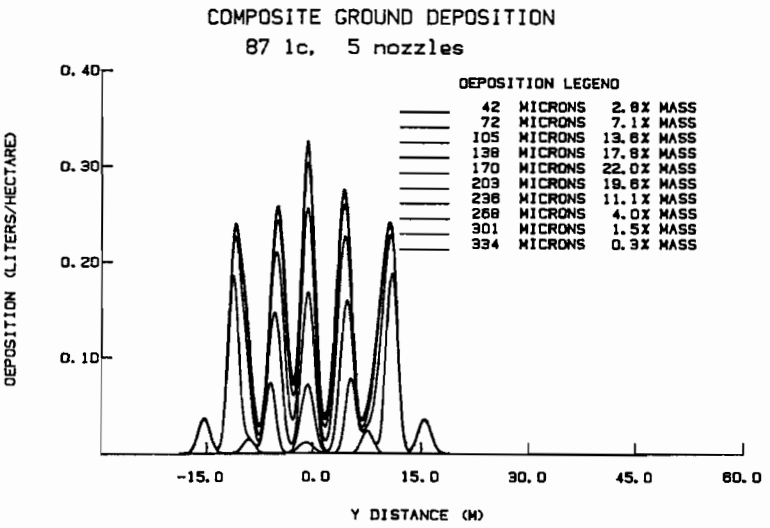

Figure 5.-Theoretical composite spray deposit pattern for Micronair nozzles located at 0, ±2.4, ±4.8 m from aircraft center line with no crosswind.

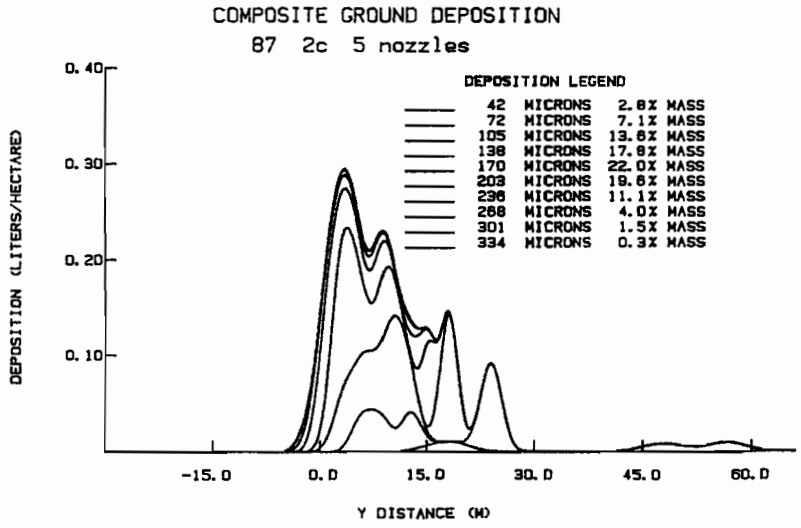

Figure 6.-Theoretical composite spray deposit pattern for Micronair nozzles located as above (Fig.5), but with a crosswind of 1.8 m/s.

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.

Vicki L. Kramer was the recipient of the award at the 56th Annual Conference held in San Mateo. The finalists were James A. Ferrari and Truls Jensen.

A COMPARISON OF MOSQUITO POPULATION DENSITY, DEVELOPMENTAL RATE AND OVIPOSITIONAL PREFERENCE IN WILD VERSUS WHITE RICE FIELDS IN THE CENTRAL VALLEY

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ABSTRACT

The population density of immature mosquitoes, the developmental rate of *Culex tarsalis* larvae and the ovipositional preference of *Cx. tarsalis* adults were compared in wild versus white rice fields in the Central Valley of California. The mosquito population was significantly greater in the wild than in white rice fields during the last few weeks of the wild rice growing season. *Culex tarsalis* larvae pupated 4 days earlier in wild than in white rice. The faster developmental rate was apparently due to greater nutrient availability in the wild rice system as water temperatures were similar in the wild and white rice fields. *Culex tarsalis* adults did not exhibit an ovipositional preference for a particular type of rice field water.

Introduction.

Wild rice is native to Minnesota and southern Canada and has been cultivated by Indians for centuries. It has been grown commercially since the mid-1960s and was introduced into the Central Valley of California in 1977 (Winchell and Dahl 1984). White rice has been grown in California for more than 75 years, and the vast acreage of standing water creates breeding habitat for *Culex tarsalis* Coquillett (the encephalitis mosquito) and *Anopheles freeborni* Aitken (the western malaria mosquito) from April through October. Some areas of white rice cultivation in the Central Valley have been converted to wild rice and, although cultivated in a similar manner, the plants have several differences. Wild rice has a shorter growing season (100 vs. 150 days), grows much taller (2.5 vs. 1 m) and has a fuller canopy than white rice. Fewer pesticides are applied to the wild rice crop. The differences between the plants could affect mosquito populations in the two rice systems.

Preliminary investigations in 1986 (Kramer and Garcia 1987) indicated that mosquito larvae were more abundant and developed more rapidly in wild than in white rice fields, implying a larger emerging adult population. Such results have important implications for mosquito control in Central Valley rice fields. The purpose of this study was to further evaluate and substantiate the 1986 findings, and to provide additional information on immature mosquito population densities, the development rate of *Cx. tarsalis* larvae and the ovipositional preference of *Cx. tarsalis* adults in wild versus white rice fields.

Materials and Methods.

Two sites (2 km apart) consisting of adjacent wild rice and white rice fields were selected for study near Nicolaus, Sutter County, in the Central Valley. Three checks (each 1 to 2 ha) of each rice type were monitored at each site. All fields were seeded in early May; at site 1 the wild rice checks were seeded 4 days prior to the white, and at site 2, the white was seeded 2 weeks before the wild. The wild rice was harvested in mid-August and the white rice in October. Water was supplied to all fields from the Sacramento River.

The mosquito population was monitored on a weekly basis beginning in mid-June by taking 150 dips (400 ml each) around three sides of each rice check (450 dips per rice type at each site). Dip samples were concentrated and the larvae counted and identified at each site. Population density differences were analyzed for each sampling date using Student's t-test ($P<0.05$).

The white rice field at site 1 was bordered to the north by a wild rice field planted 2 months earlier than the fields under study; the wild rice field monitored at this site bordered the white rice to the east. Twenty dips were taken among 4 checks of the earlier planted wild rice field on June 22 to check mosquito activity in the area. The remaining fields at both sites were bordered by white rice or fallow fields.

In 1986, *Cx. tarsalis* larvae in uncovered fine mesh floating cages developed more rapidly in wild than in white rice although water temperatures were similar (Kramer and Garcia 1987). These

cages did not exclude falling plant materials, such as grain chaff, pollen and other debris. In addition, the fine mesh screen may have excluded nutrient inflow. To assess the effect of these factors on larval development, *Cx. tarsalis* larvae were placed in floating cages similar to those used in 1986 (mesh 0.3 mm aperture, 1300 ml capacity) and divided into four treatment groups: cages without covers, cages with covers (a fine mesh screen) and field water added, cages with covers and food added, and cages with covers (nothing added). Thus, the uncovered cages allowed plant debris to fall into the cages, field water was added to replenish nutrients potentially excluded by the mesh, food was added to assess the role of supplemental nutrients on larval developmental time, and the covered cages served as controls.

Four cages (one of each treatment) were randomly placed 1 m apart and 2 m from each levee. Thus there were 6 replicates (2 per check) of each treatment in each rice type at each site. Five newly hatched *Cx. tarsalis* larvae, obtained from a recently established Sutter/Yuba Mosquito Abatement District (MAD) colony, were placed in each cage on July 24. The larvae were monitored every 1-3 days and the instar and number surviving recorded. On each monitoring date, 5 dipfuls (ca. 2000 ml) of field water were slowly poured into the appropriate cages. Food (a mixture of ground rabbit pellets and liver powder) was added to the designated cages (ca. 1 g/cage) when the larvae were released into the cages and once per week thereafter.

Water temperature was continuously monitored with an on-site weather logger (OWL 87[TM1]). This device digitally records data from up to eight temperature probes onto a battery-powered portable computer. One weather logger was placed at each site and a temperature probe placed in each check next to the floating cages. Water temperatures were recorded hourly on the computer. Water temperatures inside and outside the cages were measured on July 31 to determine whether temperatures recorded by the probes were representative of temperatures inside the cages.

The developmental rate of *Cx. tarsalis* larvae was also monitored at the Sutter/Yuba MAD to compare lab and field developmental rates. Larvae were reared in tap water, given ample food and observed daily. Air temperature in the rearing room was a relatively constant 27°C (80°F) with a light:dark photoperiod of 16:8 hours.

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The ovipositional preference of *Cx. tarsalis* adults was evaluated by placing water of each rice type and tap water in three lab colony cages (ca. 250 adults/cage, 50% female) and counting the number of subsequent egg rafts daily for 3 weeks. The water was renewed every 3 to 4 days with water brought directly from the rice fields. The *Cx. tarsalis* colony was established just prior to the study.

Plant height and water depth were measured weekly. A water sample from each rice check was collected on August 3 and analyzed within 24 hours for nitrate and phosphate content, hardness, alkalinity, conductivity, turbidity and pH.

Results and Discussion.

The mosquito density at site 1 was significantly greater in the white rice from mid-June to mid-July and in the wild rice in early August (Fig.1). The relatively high population in the white rice may have been due to the extensive *Cx. tarsalis* breeding (0.33 larvae/dip in late June) in the earlier planted wild rice field to the north. Adults emerging from this field may have oviposited in the adjacent white rice field. The decrease in larval abundance in the white rice field with the drainage of the wild rice field on July 1 supports this hypothesis. The white rice mosquito population in June was 86% *Cx. tarsalis* and 14% *An. freeborni*. In July and August, the larval population in both rice systems was almost entirely *An. freeborni*. The mosquito population in the wild rice field began to increase when the plants were near their maximum height of 2.5 m.

At site 2, there was little breeding until mid-July when the mosquito population rapidly increased in the wild rice checks (Fig. 2). Wild and white plant heights at this time were 2.5 and 0.9 m respectively. The larval mosquitoes were significantly more abundant in the wild rice in late-July and early August than in the white rice. Seventy-five percent of the population was *Cx. tarsalis*. Lodging (falling) of some of the wild rice plants in late July may have contributed to the sudden increase in mosquitoes by providing refugia for mosquito oviposition and development. Casual observations in Lake County wild rice fields support this contention. The water was apparently contaminated with an unknown substance on August 4 (as indicated by the sudden mortality of sentinel larvae) and thus monitoring was discontinued.

Maximum mosquito population density in the wild rice field at site 1 was 0.37 larvae/dip and at site 2, 1.04/dip. The mosquito population in the

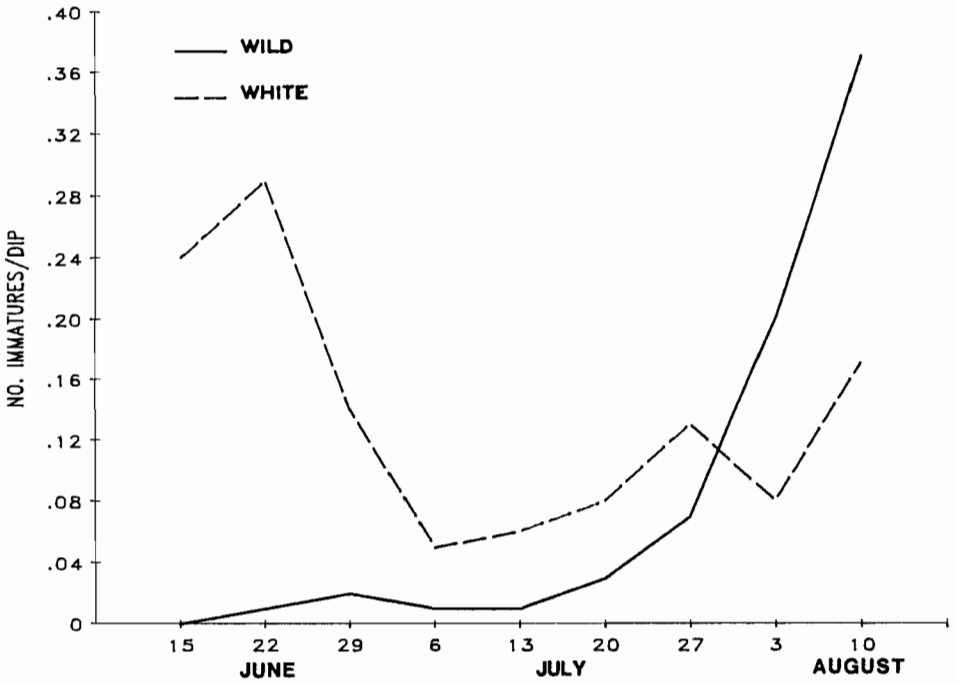

Figure 1.-Population densities of mosquito larvae in wild and white rice fields, site 1, Sutter County, 1987.

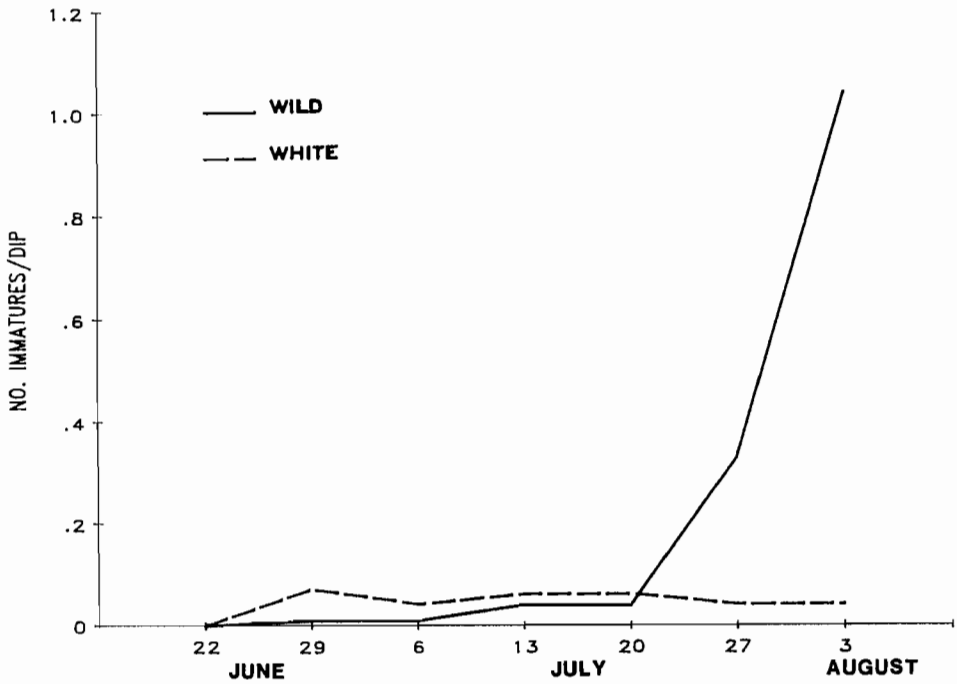

Figure 2.-Population densities of mosquito larvae in wild and white rice fields, site 2, Sutter County, 1987.

wild rice fields, therefore, exceeded the Sutter-Yuba MAD white rice treatment threshold of 0.08 larvae/dip from late July through early August. This period coincided with greatest plant height, flowering and pollination. Thus, differences in the structure of the wild and white rice plants were at their maximum. When the plants were relatively similar during the early stages of growth, the wild rice larval population was less than or approximately equal to the white rice larval population.

In the wild rice at site 1, the *Cx. tarsalis* larvae in covered and uncovered cages and those with field water added developed at approximately the same rate (Fig. 3). Larvae given supplemental food pupated 2 to 3 days earlier than the other cage types. In the white rice, larvae given food pupated 4 days earlier than the other treatment groups which developed at about the same rate (Fig. 4). The lack of difference among the non-supplemental food treatments indicates that new plant matter falling into the cage and the mesh screening of the cage do not substantially affect the developmental rate of the larvae.

Since the covered, uncovered and field water added treatments in each rice system at site 1 developed at about the same rate, these groups were combined for each rice type to compare the developmental rate of *Cx. tarsalis* in wild versus white rice fields. Most of the larvae in the wild rice system pupuated 13 days post-hatching and in the white rice, 17 days post-hatching (Fig. 5). The developmental rate was, therefore, 4 days faster in the wild rice checks than in the white. Most of the *Cx. tarsalis* larvae given supplemental food pupated 11 days post-hatching in the wild rice and 13 days post-hatching in the white rice (Figs. 3, 4). Overall mortality rate in the wild rice was 37%, and in the white, 43%. Mortality was especially high among first instars.

At site 2, the developmental rate study was terminated 7 days post-hatching due to water contamination and high larval mortality. At this time, 42% of the larvae without supplemental food in the wild rice were third instars and 58% were fourth instars. In the white rice, almost 100% of the larvae were third instars. Larvae given supplemental food were all fourth instars in the wild rice while in the white rice, 84% were third and 16% were fourth instars. Although the larvae did not reach pupation, the developmental trend is similar to that of site 1; *Cx. tarsalis* larvae developed more rapidly in the wild system than in the white.

Larvae developed more rapidly in the laboratory than in the rice fields, requiring 7 to 10 days from first instar to pupation. Other laboratory studies (Bailey and Gieke 1968) found *Cx. tarsalis* to require an average of 9.1 days for larval development at 27°C (80°F). They found the temperature threshold for complete larval development to be 10°C (50°F). At this temperature, the larvae grew to fourth instar but did not pupate. Calculating degree-days based on this threshold, the larvae needed a minimum of 210 degree-days to pupate in the rearing room where the water temperature was 27°C.

The water temperature at site 1 averaged 21.4°C (70.6°F; range 18.6 to 23.9°C) and 21.0°C (69.8°F; 17.5 to 24.4°C) over the course of the study in the wild and white rice, respectively. The wild rice water was, therefore, an average of 0.4°C warmer than the white rice water. In the laboratory, *Cx. tarsalis* larvae required an average of 13.2 days for development at 21.1°C (Ibid). This is comparable with the wild rice developmental time (13 days). Degree-days accumulated from first instar to pupation were ca. 267 in the wild rice and 336 (17 days) in the white rice. At site 2, the wild rice water (21.8°C. range 19.4 to 23.8°C) was an average of 0.7°C cooler than the white rice water (22.5°C, 19.6 to 26.0°C). Temperature differences inside and outside the cages were minimal (<1°C) demonstrating that temperatures recorded by the probes adjacent to the cages were representative of temperatures inside the cages. Thus, differences in water temperature between the rice systems were small and apparently not the major factor causing the differential developmental rate in wild versus white rice.

Since wild and white rice water temperatures were similar and there were no major differences among the non-supplemental food treatments, the faster developmental rate of *Cx. tarsalis* larvae in the wild rice was apparently due to differences in the nutrient content of the water. Approximately 10-20% of the water surface in the uncovered wild rice field cages was covered with plant debris compared with <1% in the white rice cages. Thus wild rice plants shed more debris than white rice plants. This plant material, once broken down in the rice field water, is potentially a very important source of nutrition for the developing larvae and may explain the faster rate of development in wild rice. The lack of difference in developmental time between larvae in uncovered cages and those with covers (and without supplemental food) may be due to the decay process required before the nutrients are available to the larvae.

The earlier pupation in the wild rice of *Cx.*

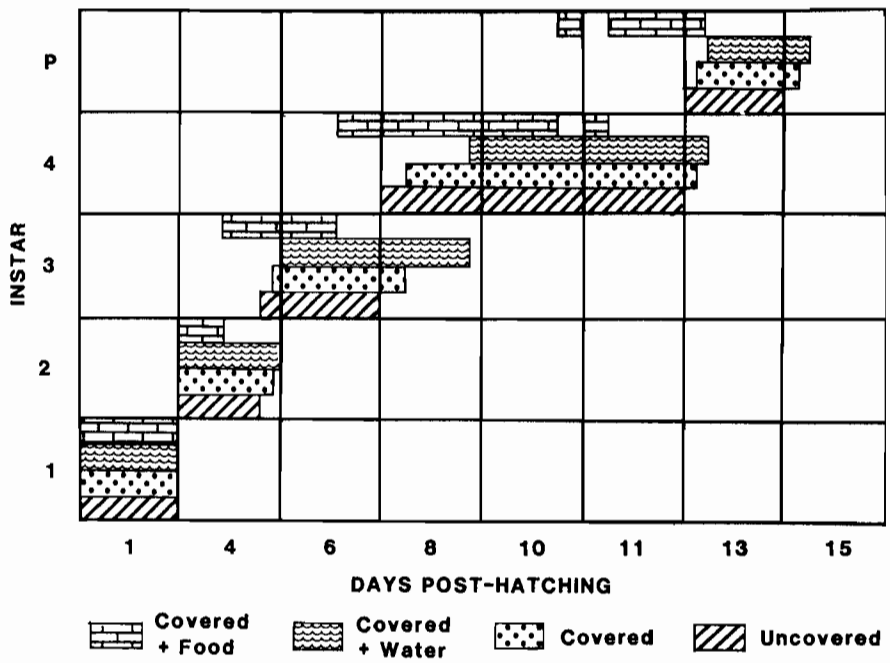

Figure 3.-Percentage of surviving *Cx. tarsalis* larvae at different stages of development in wild rice fields, site 1, Sutter County, 1987 (percentage indicated by the bar length on each day, a complete bar equals 100%; i.e. 4 days post-treatment, 85% of the larvae in uncovered cages were second and 15% third instar).

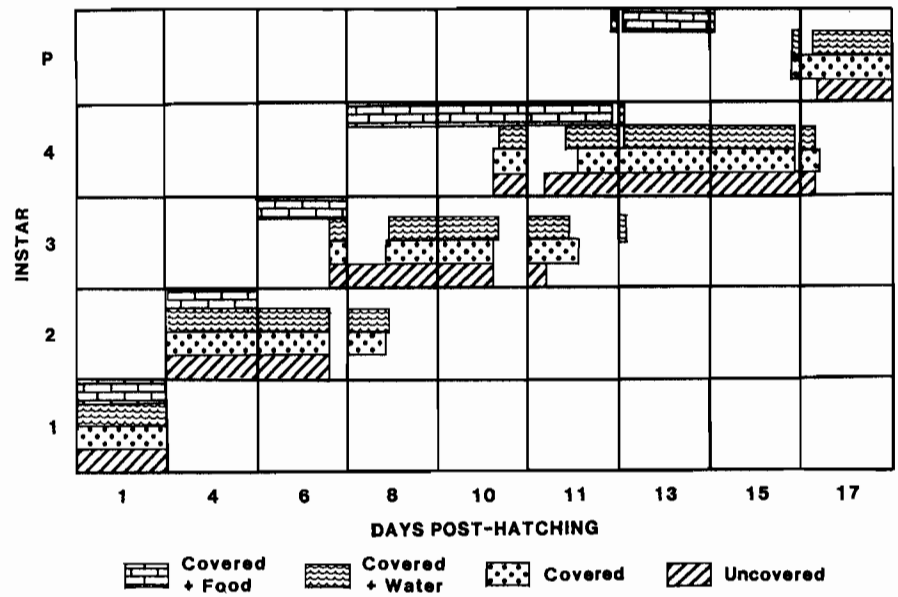

Figure 4.-Percentage of surviving *Cx. tarsalis* larvae at different stages of development in white rice fields, site 1, Sutter County, 1987.

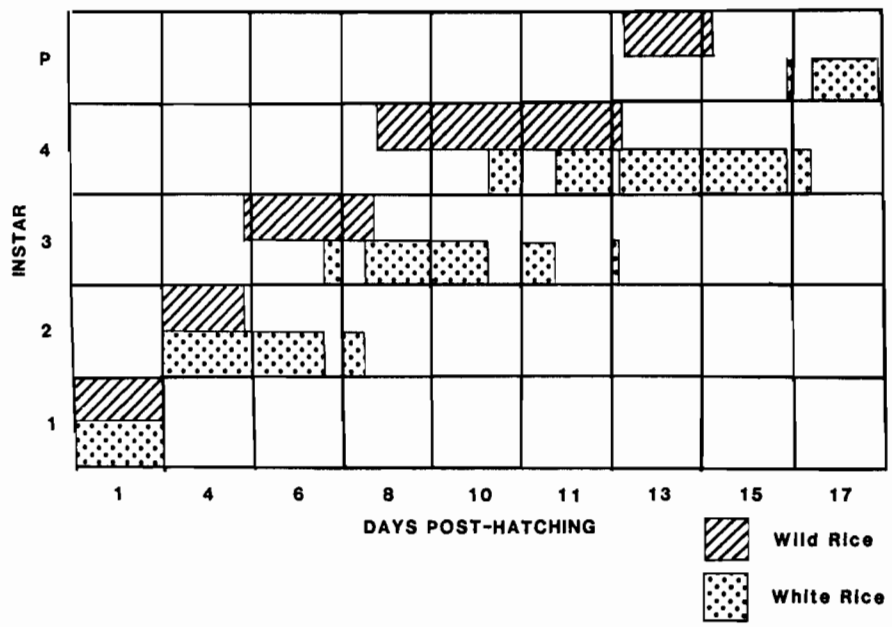

Figure 5.-Percentage of surviving *Cx. tarsalis* larvae at different stages of development in wild versus white rice fields, site 1, Sutter County, 1987.

tarsalis larvae given supplemental food over those in the white rice suggests that some nutrient not supplied by the supplemental food was sufficiently available in the wild rice system and not in the white. The shorter developmental time among larvae given supplemental food compared with the other treatment groups indicates that neither rice system had optimal nutrients and further emphasizes the importance of nutrition on *Cx. tarsalis* development.

Water samples were analyzed to detect differences in nitrate and phosphate content, alkalinity, conductivity, turbidity and pH between the two rice systems (Table 1). At site 1, the phosphate concentration in the wild rice water was greater than in the white (0.45 and 0.20 mg/l, respectively). Other components measured were similar. At site 2, nitrate and phosphate contents were higher in the wild rice water than in the white (0.93 vs. 0.35 mg/l and 1.64 vs. 0.56 mg/l, respectively). However, it is not evident from this study whether these differences had any effect on the rate of larval development in the two rice systems.

Laboratory colonized *Cx. tarsalis* females oviposited 42 egg rafts over a three week period; 12, 16 and 14 egg rafts were laid in the wild rice, white rice and tap water respectively. Apparently the females did not have an ovipositional preference as almost equal numbers of egg rafts were laid in each water type. The ovipositional rate was, however, very low. In 1986, 79 egg rafts were laid within a week, 71 of them in the wild rice water (Kramer and Garcia 1987). This preliminary data was not substantiated in 1987.

In conclusion, there were significantly more mosquito larvae in the wild than in white rice fields during the last few weeks of the wild rice growing season. Furthermore, *Cx. tarsalis* developed more rapidly in the wild rice, pupating ca. 4 days earlier than in the white rice. The faster developmental rate was probably due to greater nutrient availability in the wild rice system. *Culex tarsalis* females did not seem to prefer a particular type of rice water for oviposition.

These differences between the rice systems may have broad management implications. For instance, since wild rice is often planted earlier than white rice, has a shorter growing season and a relatively high mosquito population during the last few weeks of its growing season, it is potentially generating a larger adult mosquito population than would be present if just white rice were grown, particularly during the early part of the white rice growing season when the wild rice plants are reaching maturity. Thus, planting white rice adjacent to wild rice may increase mosquito populations breeding in white rice fields. Data collected

Table 1.-Water quality analysis for wild and white rice fields, Sutter County, 1987.

	Nitrate (ppm)	Phosphate (ppm)	Alkalinity (ppm)	Conductivity (mhos/cm)	Turbidity JTU	pH
Site #1						
Wild	0.32	0.45	187	367	3.2	7.16
White	0.34	0.20	197	423	4.7	7.27
Site #2						
Wild	0.93	1.64	180	470	5.4	7.26
White	0.35	0.56	187	453	5.6	7.29

from the white rice field at site 1 in June support this hypothesis.

In addition, the difference in larval development rate between the rice systems may influence predation rate and, consequently, mosquito abundance. The longer developmental time for larvae in white rice would increase their exposure to co-inhabiting predators and thus result in a greater natural mortality. The slower developmental rate coupled with the extensive predator complex (Miura et al. 1981) in white rice fields may explain, in part, the relatively low mosquito densities found in white rice systems. The aquatic predator populations in white and wild rice are reported to be similar (Kramer and Garcia 1987). The potential increase in nutrients to wild rice water when the plants shed debris during the final stages of growth may accelerate larval development, thereby reducing exposure to predation and resulting in a higher mosquito population. It therefore appears that the last 3 to 4 weeks of the wild rice growing season are the most critical for mosquito control. Mosquito control measures, such as the application of *Bacillus thuringiensis* var. *israelensis* (H14), would be most effective if applied during this period.

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PRELIMINARY GENETIC STUDIES OF ESTERASE ACTIVITY VARIATION ASSOCIATED WITH OP RESISTANCE IN *CULEX QUINQUEFASCIATUS*

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Introduction.

Insecticide resistance is a major obstacle in the effort to control insect vectors of disease. Resistance has long been recognized as an evolutionary phenomenon yet we know very little about the genetic and population processes that influence how resistance develops.

One of the most thoroughly examined cases of resistance, from a genetic standpoint, is resistance to certain organophosphate (OP) insecticides conferred by esterases in *Culex quinquefasciatus* Say. Georghiou and Pasteur first demonstrated an association between OP resistance and a highly active esterase allozyme, which they designated (Esterase B1) (Georghiou and Pasteur 1980, Georghiou, Pasteur and Hawley 1980).

Recent work has established that the molecular basis of high esterase activity in some populations is amplification of the gene coding for Est B1 (Mouches et al. 1987). Individuals from a highly resistant laboratory strain carry about 250 times as many copies of the Est B1 gene as susceptible individuals. This results in far higher esterase enzyme titers in resistant vs. susceptible individuals (Mouches et al. 1987). If esterase titer is correlated with the number of copies of the Est B1 gene that an individual carries, a new technique for estimating esterase activity in individual insects (Dary and Georghiou, in preparation) may prove useful in studying the quantitative genetic variation in esterase activity associated with organophosphate resistance.

The present study is a preliminary evaluation of the utility of this technique in genetic studies. If this technique proves useful in identifying genetic variants for esterase activity it could be a powerful tool in studying the dynamics of esterase genes in populations and how they contribute to the evolution of resistance.

Materials and Methods.

A susceptible laboratory strain without highly active esterase (S-lab) and a strain highly resistant to OP's and possessing the highly active B1 esterase (Tem-R) were used in this study.

Four initial crosses were carried out to establish esterase activity levels in the S-lab and Tem-R strains and in their reciprocal F1's; 1) Tem-R: Tem-R females X Tem-R males, 2) S-lab: S-lab females X S-lab males, 3) F1(TR): Tem-R females X S-lab males, 4) F1(SL): S-lab females X Tem-R males.

The segregational properties of the multiple copies of the Est B1 gene were determined in a backcross of F1(SL) females X S-lab males.

Larvae from all crosses were reared in uncrowded conditions on standard diets. Adults were collected daily, aged for three days and frozen in liquid nitrogen for esterase activity measurements and protein content determinations.

Esterase activity and protein content were measured for individuals of the crosses listed above. Assays were carried out in individual wells of microtiter plates according to the methods of Dary and Georghiou (in preparation).

Prior to carrying out the study outlined above, an analysis of the influence of various potential sources of error in the microtiter plate esterase and protein assays was designed as a nested analysis of variance. Twenty-four Tem-R females were homogenized individually. Each homogenate was divided into two parts, labelled day 1 and day 2, and frozen at -70 degrees C. One set of homogenates (day 1 or day 2) was assayed for esterase activity and protein content on successive days. On each day, each homogenate was used in two individual wells of each of two microtiter plates. This produced four different estimates of esterase activity and protein content for each homogenate on each day. This design provided estimates of the proportion of the variance in the readings of esterase activity and protein content attributable to well to well differences within microtiter plates, plate to plate variation on a given day, day to day variation and variation among individuals. These data were analyzed by the PRO NESTED procedure of the SAS statistical analysis system (SAS Institute, 1985).

Results.

Error analysis: The error analysis of the esterase microtiter plate assay (Table 1) showed that

Table 1.-Nested analysis of variance design showing the percentage of the variance attributable to different sources in the esterase and protein microtiter plate assays.

Source	df	Esterase Assay % of Variance	Protein Assay % of Variance
Female	23	90.3	60.0
Day	24	4.5	30.7
Plate	48	2.9	0.0
Well	96	2.3	9.6

Table 2.-Mean esterase activity (nmoles alpha-naphthol/μg protein) and standard errors (S.E.) in the pure strains and reciprocal F1's.

	Males			Females		
	N	Mean	S.E.	N	Mean	S.E.
Tem-R	45	111.5	3.2	44	89.6	1.9
S-lab	47	0.77	0.01	47	0.93	0.01
F1(SL)	48	36.4	1.9	48	44.8	1.3
F1(TR)	47	46.0	1.3	48	41.1	1.0

about 90% of the total variation among esterase activity readings was attributable to differences among females, with less than 10% attributable to potential sources of error. Error analysis of the protein content assay (Table 1) indicated that 60% of the variation was attributable to differences among females.

Pure strains and reciprocal F1's: Esterase activity of Tem-R females was about 100X that of S-lab females (Table 2). Reciprocal F1 females exhibited about half the esterase activity of Tem-R females and did not differ significantly from one another. Tem-R males had about 140X the activity of S-lab males. Reciprocal F1 males had less than half the activity of Tem-R males and ANOVA indicated that F1(TR) males had significantly higher activity than F1(SL) males.

The distribution of esterase activity among Tem-R progeny (Figure 1) was strongly skewed toward higher esterase activity values. The distribution of esterase activity among S-lab progeny was more uniform. The reciprocal F1's had distributions which exhibited the same skewness seen in the Tem-R individuals. The distribution of the Tem-R and reciprocal F1 individuals showed very little overlap.

Backcross: If amplified esterase genes are organized as a block on a single chromosome, or are closely linked, then backcrossing F1 females to S-lab males should result in one-half of the progeny exhibiting esterase activity in the S-lab range and one-half exhibiting esterase activity in the F1 range. Alternatively, a high frequency of progeny showing intermediate esterase activity would suggest independent assortment of esterase genes located in different regions of the genome.

Among 519 backcross progeny examined, 263 (50.7%) exhibited esterase activity in the F1 range while 255 (49.1%) exhibited activity in the S-lab range. One backcross individual showed an activity reading intermediate to the S-lab and F1 ranges. This was most likely the result of a rare recombination event in an F1 female.

Discussion.

The error analysis indicated that the esterase microtiter assay discriminated very well among Tem-R females. The protein assay also had discriminating power, however a substantial portion of the variation could be attributed to day to day differences in estimates of the protein content of individuals. This could be cause for concern, however, examination of the overall variation in protein content estimates indicated that there was very little variation present. The Tem-R females used in the analysis were reared together in uncrowded

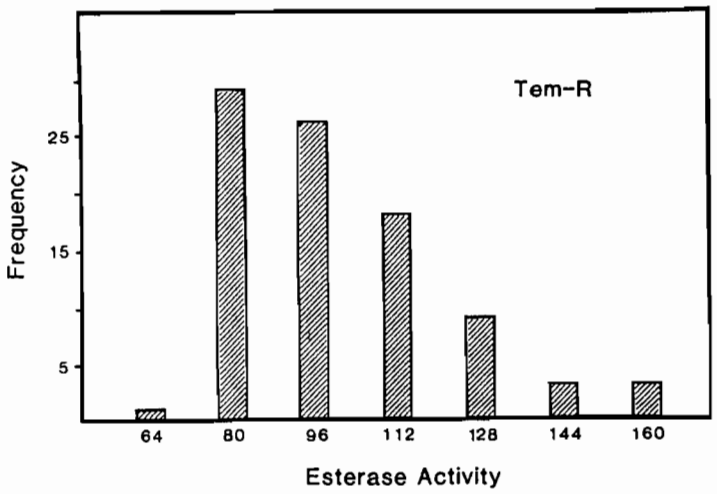

Figure 1.-Distribution of esterase activity (nmoles alpha-naphthol/ug protein) among Tem-R progeny.

conditions and given adequate food, resulting in a group of individuals that were very similar in size. The coefficient of variation (cv) in protein content estimates was only 4%. In contrast, the cv of esterase activity estimates was 19%. In spite of the small variation in protein content, the protein assay still indicated that a substantial portion of the variation was attributable to differences among females. For most purposes the protein estimate should be an adequate correction for esterase activity measurements, however, day to day variation in measures of protein content and esterase activity should be taken into account in experimental design. Correction of esterase activity by protein content is necessary to discriminate between differences in activity due to differences in the size of individuals from differences in activity that are due to genetic differences among individuals.

If esterase activity is an additive trait, F1 individuals should exhibit about half the activity of Tem-R individuals, since they possess half the number of copies of the esterase B1 gene. F1 females followed this pattern fairly well, however reciprocal F1 males had substantially less than half the activity of Tem-R males and differed substantially from another. This was initially interpreted as suggesting a maternal effect on esterase activity in males. Subsequent studies, to be published elsewhere, were done using an experimental design in which sampling variation and day to day variation in the assays were taken into account. This work indicated that maternal effects are not an important influence on esterase activity. The values obtained in the present study only give a rough estimate of the relationship among the strains.

The distribution of esterase activity in the Tem-R strain is likely due to the temephos selection imposed on this strain each generation. Low esterase activity variants are probably eliminated during this process, resulting in a distribution skewed toward higher values.

The esterase activity distributions of the Tem-R individuals and the reciprocal F1 individuals overlapped very little. The assay is thus capable of discriminating the three genotypic classes examined in this study; homozygous susceptible, homozygous resistant and heterozygous. The backcross analysis showed that the amplified esterase genes segregate almost exclusively as a block in F1 individuals, and that recombination in F1 females is very low. However, this result provides no information about the frequency of recombination between homologous chromosomes which both carry amplified blocks of esterase genes.

These preliminary results are encouraging. Further work is under way to determine if the esterase assay is capable of identifying genetic variants for esterase gene copy number within the Tem-R strain. If this is possible, this assay will permit us to examine the genetics of resistance in detail never before possible.

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THE POPULATION BIOLOGY OF *AEDES MELANIMON* IN THE SACRAMENTO VALLEY OF CALIFORNIA

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ABSTRACT

In 1987, a project on the population biology of *Aedes melanimon* was initiated in the Sacramento Valley of California in order to determine the possible role of this mosquito in the epidemiology of arboviruses. Studies conducted or begun in 1987 include: 1) a trap evaluation to determine which type of trap would be more effective in monitoring changes in *Ae. melanimon* populations, 2) a study of the daily host feeding rhythm using a sequential sampling trap, 3) a mark-release-recapture study to estimate daily survivorship, dispersal, absolute abundance and the length of the gonotrophic cycle, 4) a larval survivorship and development study to monitor survivorship and developmental time in the field and 5) blood engorged females were collected for determining the host feeding pattern using bloodmeal analysis techniques. All studies were conducted on the Colusa National Wildlife Refuge in Colusa County.

Conventional CDC traps baited with CO_2 were tested against inverted CDC, lard can, and malaise traps for their effectiveness in capturing *Ae. melanimon* females. Trapping was done once per week for 5 weeks. In total, 530 of 680 females (78%) were caught in the conventional CDC trap while 150 (22%) were caught in the inverted CDC trap. No females were caught in the lard can or malaise traps. We concluded that the conventional CDC trap was the most effective of the traps tested.

Sequential sampling trap studies conducted on 9-3-87 and 9-18-87 indicate that host seeking activity in *Ae. melanimon* is nocturnal at that time of year with peak activity noted between 2300 and 0100 hours.

Approximately 5500 female and 5900 male *Ae. melanimon* collected from the field as larvae were marked with a fluorescent dust and released on 9-17-87. Conventional CDC traps and sweep net collections were used to collect mosquitoes from the study site from 9-19-87 to 10-4-87, at which time no marked mosquitoes had been recaptured for 3 consecutive days. In total, 58 females (1.04%) and 6 males (0.1%) were recaptured. Marked females were caught in CDC traps every night but one, from 9-19-87 to 10-1-87. No marked mosquitoes were recaptured in sweep net collections. Based on a decrease in the number of recaptures over time, the daily survivorship rate of the females was estimated at .92 per day using linear regression. Marked males were recaptured up to 13 days after release indicating that the males survive longer in the field than previously thought.

Absolute abundance of the females was estimated using the modified Lincoln Index. Daily estimates of abundance ranged from 8.3 - 33 million *Ae. melanimon* females in a 2 hectare area during the study period.

The length of the gonotrophic cycle was estimated in the marked females from peaks in the recovery ratio which occurred at 5 day intervals. The recovery ratio is the ratio of the number of recaptured females over the total number of *Ae. melanimon* females caught in the CDC traps per day. We interpreted these peaks to indicate that the length of the gonotrophic cycle in the marked mosquitoes was approximately 5 days.

Over 600 blood engorged females were collected in sweep net collections during the mark-release-recapture study and these will be used to determine the host feeding pattern using blood meal analysis.

Larval survivorship and developmental rates were monitored in 5 flooded fields on the Colusa refuge from 8-12-87 to 9-17-87. Daily dipping of transects in each field was used to monitor changes in the abundance and age composition of the larvae. Sentinel buckets containing field collected larvae were set up at each of the study sites on the first day after the transect was flooded.

At site 1, larval counts went from 2982 to 129 larvae from the first to the eight day post-flooding. Approximately 5% of the larvae initially sampled at the site survived to pupation. Assuming constant mortality, the daily survivorship rate was estimated at .64. In the four other sites, larvae disappeared from the study site prior to pupation and no estimates of survivorship could be obtained.

Development time in sentinel buckets at each of the study sites was approximately 8 days, from

the time of flooding to pupation. There was no difference in developmental times or mortality between the caged populations in sentinel buckets at different study sites. Time from pupation to adult emergence was estimated at approximately 2 days.

The preliminary findings from the 1987 field season support the hypothesis that *Ae. melanimon* may play a more important role in the epidemiology of arboviruses than previously thought. The high daily survivorship, high local abundance and relatively short gonotrophic cycle length seen in the marked population at the Colusa Refuge are characteristics which are consistent with being an efficient horizontal vector. Determination of the host feeding pattern will be important in determining the frequency of feedings on vertebrate hosts for arboviruses and will further extend our understanding of the role of *Ae. melanimon* in arbovirus transmission.

The preliminary findings from the larval survivorship studies indicate that survivorship varies substantially between fields but that in some cases larval survivorship of *Ae. melanimon* is comparable to that of *Culex tarsalis* and *Anopheles freeborni* larvae in Sacramento Valley rice fields. Identification of those factors which enhance mortality may be important in developing future control strategies against *Ae. melanimon* larvae.

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