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California Mosquito and Vector Control Association, Inc.

Volume 55 **January 25-28, 1987**

SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1986

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

This is the seventeenth (since 1969) in a series of annual reports to the California Mosquito and Vector Control Association, and it 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 other groups represented by the authorship or mentioned below. Weekly reports of the surveillance program were sent (27 issues) to a large mailing list, and an initial trial of an "electronic bulletin board" was participated in by some local mosquito abatement districts and health agencies.

As has been usual in the past two decades, natural conditions and comprehensive mosquito control efforts combined to limit epidemic mosquito-borne encephalitis. The recent emergence (or recognition) of arbovirus activity in urban and suburban southern California areas, which resulted in 26 confirmed St. Louis encephalitis (SLE) cases in 1984, again required special attention to this region in 1986, but only 3 cases were detected there. However, the year was marked by a moderate resurgence of western equine encephalomyelitis (WEE) virus activity in Northern California, where 2 human cases were confirmed.

As early detection of human cases is important for surveillance and control of arbovirus encephalitis, physicans and local health departments are encouraged to submit specimens for diagnostic testing as promptly and completely as possible. During 1986, 5 human cases were detected throughout the state and are briefly described as follows. Two presumptive-positive SLE cases were detected by screening tests at the Microbiology Reference Laboratory (MRL), Cypress, California, and at the State's Viral and Rickettsial Disease Laboratory (VRDL). Additional tests at these laboratories and by the Los Angeles County Public Health Laboratory confirmed the findings. The first SLE case was a 59 year old man from Covina, Los Angeles County--a truck driver with regular routes over a wide area of southern and central California and parts of Nevada, but no significant mosquito exposure could be recalled, either at his home or work places, so the probable source of infection remains unclear. He had an onset of illness July 3. An acute-phase serum sample had high IgG and IgM antibody titers for SLE, and convalescent serum showed rising titers of complement fixing (CF) antibody and of IgG and IgM antibodies by the indirect immunofluorescence (IIF) test. The second SLE case was a 66 year old man from Norwalk, Los Angeles County, who became ill on July 13. He had been exposed to numerous mosquito bites at his home, with no travel or other mosquito exposure history. An acute-phase serum showed high IgM antibody titers for SLE by the IIF test, and convalescent serum showed a significant rise in IIF and CF antibodies. Isolates of SLE virus were made from mosquito pools collected near his home. A third case of SLE was reported by the Los Angeles County Public Health Department which did the initial serologic tests. This case was also verified at the State VRDL. The patient was a 37 year old man from El Monte, Los Angeles County, who had an onset of illness September 20. The source of exposure was most likely at his home where he experienced extensive

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Table 1.-Number of mosquitoes and pools tested during 1986 by the Viral and Rickettsial Disease Laboratory by county and species.

	Mosquito Species											
	Aedes melanimon		Other Species		*Culex peus*		*Culex pipiens* complex		*Culex tarsalis*		Total	
	Mosq	Pools	Mosq	Pools	Mosq	Pools	Mosq	Pools	Mosq	Pools	Mosq	Pools
Butte	500	10			200	4			2740	55	3440	69
Imperial			245	15					13717	290	13962	305
Inyo	2230	46							429	15	2659	61
Kern	16691	358	31	1					42611	929	59333	1288
Kings			17	1			29	1	97	3	143	5
Lake									636	13	636	13
Los Angeles			3951	86	906	48	6793	182	8754	209	20404	525
Marin							30	1	85	2	115	3
Merced	50	1							298	7	348	8
Mojave,AZ									241	6	241	6
Monterey									33	2	33	2
Orange			4685	105	133	8	2243	75	11948	277	19009	465
Riverside			749	27	2361	61	7637	166	17355	369	28102	623
San Bernardino					124	3	4343	88	5461	115	9928	206
Sacramento					115	6	833	19	19665	406	20613	431
San Diego							18	1			18	1
Santa Barbara							850	17	1231	26	2081	43
Shasta									250	5	250	5
Sonoma					349	7	57	2	257	6	663	15
Stanislaus	22	1			202	6	419	14	426	16	1069	37
Sutter									1028	23	1028	23
Ventura					117	3	94	4	392	10	603	17
Yolo	439	9							17857	361	18296	370
Yuba									691	15	691	15
TOTAL	19932	425	9678	235	4507	146	23346	570	146202	3160	203665	4536

mosquito bites. Paired sera showed rising SLE antibody titers by the CF and IIF tests, and acute-phase serum also had an IIF-IgM titer. A significant encephalitis occurred with high fever and neurologic findings and the recovery was slow.

The first confirmed case of WEE was a Shasta County girl born May 12, 1986, who had an onset of febrile illness August 7, followed by signs of encephalitis and was hospitalized in Sacramento. The case was initially thought to be possibly related to poliomyelitis immunization and was not studied for arbovirus infection until paired sera could be collected and submitted to the VRDL. The sera showed significantly rising titers to WEE by the CF test and the IIF test, and WEE-specific IgM antibody was shown in both specimens. The source of mosquito exposure was apparently in the home environment in either Happy Valley or the Palo Cedro area of Shasta County. The second case of WEE was a 37 year old woman from Yolo County with an onset of aseptic meningitis-like illness on August 18. She had stationary CF antibody titers and IIF antibody titers for WEE in paired sera taken more than a month apart. The only recent travel had been a trip to Sierra County August 8-10, with some mosquito exposure, but it is not certain that this was the source of infection.

During 1986, a total of 18 clinically-suspected cases of WEE in equines was reported from 14 California Counties. Fourteen of the cases were tested serologically, 4 cases were tested by virus isolation attempts from brain specimens and one case was tested by other virus isolation attempts and serology. Only one case of WEE was confirmed in a 2 year old unvaccinated mare from an area in Tehama County near the Shasta County line. There was no history of the horse traveling elsewhere. The onset of illness was September 1, and paired blood serum samples taken on September 2 and 16 showed a 4-fold increase in CF antibody.

A suspected case of California encephalitis (CE) in a 17 year old girl in Inyo County, with onset of illness July 13, was not verified, and is presumed to have been due to an enterovirus infection. A screening test of IIF at the VRDL showed IgG antibody to CE in an initial serum sample, but no IgM antibody. No human cases of CE with the source of infection in California have been found since 1942/43, when CEV was first

Table 2.-Number of viral isolates from mosquitoes tested during 1986 by the Viral and Rickettsial Disease Laboratory by species, county and agent isolated.

Species	County	WEE	SLE	Turlock	California Group	Hart Park	Unident. Agent	Total
Aedes dorsalis	Imperial		2					2
Aedes melanimon	Inyo				5			5
	Kern				41			41
Culex erythrothorax	Imperial	1						1
	Los Angeles			1				1
Culex peus	Los Angeles	1	1					2
	Sacramento			1				1
Culex pipiens complex	Los Angeles		5	5		1		11
	Orange			1				1
Culex tarsalis	Butte			1				1
	Imperial	14	5	1				20
	Inyo			1				1
	Kern		3	39	1	17		60
	Lake			1				1
	Los Angeles	1	26	16		5		48
	Merced			2				2
	Orange		5	14		9		28
	Riverside	2	4	2		1		9
	San Bernardino	3		1				4
	Sacramento	2		32		4		38
	Sutter	1		1				2
	Ventura			1				1
	Yolo	10		13		1	1	25
TOTAL ISOLATES		35	51	133	47	38	1	305

discovered. However, CE group viruses are occasionally isolated from mosquitoes, and CEV antibody can be found in California residents, indicating that transmission of virus is still occurring even though clinical disease has not been recognized. Further epidemiologic and entomologic/virologic studies should be done.

There were 4,536 mosquito pools tested during the year, 119 more than in 1985. As usual for the past several years, the majority were from southern California areas--Imperial, Los Angeles, Orange, Riverside, San Bernardino, and Kern Counties; but Sacramento and Yolo counties were also well represented (Table 1). There were 305 virus isolates: 51 SLE, 35 WEE, 47 CEV group, 133 Turlock, 38 Hart Park, had 1 unidentified virus. All WEE and SLE virus isolates were from *Culex tarsalis*, except 1 WEE from *Cx. erythrothorax* (Imperial County); 1 WEE from *Cx. peus* (Los Angeles County); 2 SLE from *Aedes dorsalis* (Imperial County); 1 SLE from *Cx. peus* (Los Angeles County); and 5 SLE from *Cx. pipiens* complex (Los Angeles County) (Table 2). A complete listing of viruses isolated during the year is included in this report and summarizes the information sent on a weekly basis during the year (Table 3).

In the spring of 1986, 59 sentinel chicken flocks were placed throughout the state, 4 more than in 1985. Seroconversions for WEE were seen in August (Butte and Turlock Counties); then fairly high seroconversion rates occurred in September and October in northern and central regions (Tehama, Butte, Sutter-Yuba, and Solano Counties) and single chickens seroconverted in Stanislaus and Fresno counties. As usual, WEE activity also was detected in southern California, beginning in July (Coachella Valley, Imperial County, and at Havasu and Blythe along the Colorado River). Seroconversions for SLE were seen in Imperial County, the Coachella Valley, Orange County, and in Los Angeles County. Single chicken seroconversions for SLE were found in Kern County (Wasco and Westside/Maricopa Flocks) and in Sacramento County (Natomas flock) (Table 4).

We hope to continue this program in 1987 in a similar fashion, but close collaboration and help by all participating agencies will be more important than ever in this era of tight funding and many other competing needs and programs.

ERRATUM

Please correct 3 errors in the 1985 Summary: Proceedings and Papers of the 54th Annual Conference of the CMVCA, Inc. Volume 54, March 16-19, 1986, page 6 (Table 3). Under Kern County Mosquito Pools No. 5746, 5747, and 5749 should be *Aedes melanimon*, not *Culex tarsalis*, as listed.

ACKNOWLEDGMENTS

We thank Patricia Worrell, Kate Haber, Marcus Guerrero and Lau Nguyen for special assistance in the mosquito testing and chicken serosurvey; we also thank the many staff members of the Viral and Rickettsial Disease Laboratory; Vector Surveillance and Control Branch; Arbovirus Field Station in Bakersfield; Infectious Disease Branch; and others in the California Department of Health Services; all participating local Mosquito Abatement agencies and County Health Departments; the California Department of Food and Agriculture; private physicians and veterinarians who submitted samples for testing; the Microbiology Reference Laboratory; and all others who assisted in the surveillance program.

Table 3.-Viral isolates from mosquito pools tested during 1986 by the Viral and Rickettsial Disease Laboratory, compiled chronologically and by Mosquito Abatement District.

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	COLLECTION MONTH	DAY	PLACE	COUNTY
BUCO	66	CX TARS	50	TRLK	AUG	11	NORD	BUTTE
CHLV	1	CX TARS	50	TRLK	MAY	15	MECCA	RIVERS
	40	" "	50	TRLK	JUN	25	MECCA	RIVERS
	46	" "	39	SLE	JUL	23	MECCA	RIVERS
	54	" "	50	WEE	JUL	24	MECCA	RIVERS
	78	" "	50	SLE	AUG	19	MECCA	RIVERS
	98	" "	50	SLE	AUG	19	MECCA	RIVERS
COLO	6	CX TARS	50	WEE	JUL	22	BLYTHE	RIVERS
	14	" "	50	WEE	AUG	19	NEEDLES	SBERN
	17	" "	50	WEE	AUG	19	NEEDLES	SBERN
	19	" "	50	WEE	AUG	19	NEEDLES	SBERN
IMPR	51	CX TARS	50	TRLK	APR	24	BRAWLEY	IMPERL
	139	" "	50	WEE	MAY	21	BRAWLEY	IMPERL
	181	AE DORS	24	SLE	JUN	3	HEBER	IMPERL
	225	CX TARS	19	WEE	JUN	17	BRAWLEY	IMPERL
	226	" "	50	WEE	JUN	17	BRAWLEY	IMPERL
	228	" "	50	WEE	JUN	17	BRAWLEY	IMPERL
	229	" "	50	SLE	JUN	17	CALAPATRIA	IMPERL
	232	" "	50	SLE	JUN	19	HEBER	IMPERL
	247	" "	21	WEE	JUN	24	WINTERHAVEN	IMPERL
	248	" "	50	WEE	JUN	24	WINTERHAVEN	IMPERL
	249	" "	50	WEE	JUN	24	WINTERHAVEN	IMPERL
	251	" "	50	WEE	JUN	24	WINTERHAVEN	IMPERL
	264	" "	50	SLE	JUL	17	SEELY	IMPERL
	270	" "	50	WEE	AUG	5	SEELY	IMPERL
	271	" "	50	WEE	AUG	5	SEELY	IMPERL
	276	" "	50	WEE	AUG	5	SEELY	IMPERL
	277	" "	50	WEE	AUG	5	SEELY	IMPERL
	282	" "	50	SLE	AUG	5	SEELY	IMPERL
	284	" "	50	WEE	AUG	5	SEELY	IMPERL
	287	CX ERYT	10	WEE	AUG	5	SEELY	IMPERL
	291	AE DORS	10	SLE	AUG	5	SEELY	IMPERL
	312	CX TARS	50	WEE	SEPT	16	SEELY	IMPERL
	330	" "	50	SLE	SEPT	24	WINTERHAVEN	IMPERL
INYO	22	AE MELN	50	CEV	JUN	17	BIG PINE	INYO
	28	" "	50	CEV	JUL	29	BISHOP	INYO
	31	" "	50	CEV	JUL	29	BISHOP	INYO
	34	" "	50	CEV	JUL	29	BISHOP	INYO
	50	" "	50	CEV	SEPT	10	BISHOP	INYO
	59	CX TARS	32	TRLK	SEPT	10	INDEPENDENCE	INYO
KERN	5214	AE MELN	50	CEV	APR	30	LOST HILLS	KERN
	5216	" "	50	CEV	APR	30	LOST HILLS	KERN
	5219	" "	50	CEV	APR	30	DELANO	KERN
	5240	" "	50	CEV	MAY	7	LOST HILLS	KERN
	5241	" "	50	CEV	MAY	7	LOST HILLS	KERN
	5368	CX TARS	50	HART	MAY	27	BAKERSFIELD	KERN
	5373	" "	50	TRLK & HART	MAY	27	BAKERSFIELD	KERN

continued -

Table 3-continued

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	COLLECTION MONTH	DAY	PLACE	COUNTY
KERN	5429	CX TARS	50	HART	MAY	28	LOST HILLS	KERN
	5455	AE MELN	39	CEV	JUN	3	BAKERSFIELD	KERN
	5500	CX TARS	50	HART	JUN	4	DELANO	KERN
	5558	AE MELN	50	CEV	JUN	11	LOST HILLS	KERN
	5598	CX TARS	50	TRLK	JUN	16	BAKERSFIELD	KERN
	5609	AE MELN	50	CEV	JUN	19	DELANO	KERN
	5613	" "	50	CEV	JUN	19	DELANO	KERN
	5616	CX TARS	14	TRLK	JUN	19	BUTTONWILLOW	KERN
	5633	" "	50	HART	JUN	19	LOST HILLS	KERN
	5639	" "	50	TRLK	JUN	24	ARVIN	KERN
	5643	" "	50	TRLK	JUN	24	ARVIN	KERN
	5644	" "	50	TRLK & HART	JUN	24	ARVIN	KERN
	5649	" "	50	TRLK & HART	JUN	24	BAKERSFIELD	KERN
	5650	" "	50	TRLK	JUN	24	BAKERSFIELD	KERN
	5652	" "	50	TRLK	JUN	24	BAKERSFIELD	KERN
	5653	" "	50	TRLK	JUN	24	BAKERSFIELD	KERN
	5667	" "	29	TRLK	JUN	25	BUTTONWILLOW	KERN
	5668	AE MELN	50	CEV	JUN	25	DELANO	KERN
	5688	" "	50	CEV	JUN	25	LOST HILLS	KERN
	5692	" "	50	CEV	JUN	25	LOST HILLS	KERN
	5695	CX TARS	50	TRLK	JUN	25	LOST HILLS	KERN
	5703	" "	50	TRLK	JUN	30	BAKERSFIELD	KERN
	5705	" "	50	HART	JUN	30	BAKERSFIELD	KERN
	5710	" "	50	TRLK	JUN	30	BAKERSFIELD	KERN
	5712	" "	50	TRLK	JUN	30	BAKERSFIELD	KERN
	5722	" "	50	TRLK	JUN	30	ARVIN	KERN
	5723	" "	50	HART	JUN	30	ARVIN	KERN
	5743	" "	50	TRLK	JUL	2	LOST HILLS	KERN
	5752	AE MELN	50	CEV	JUL	2	LOST HILLS	KERN
	5757	" "	50	CEV	JUL	2	LOST HILLS	KERN
	5758	" "	50	CEV	JUL	2	DELANO	KERN
	5759	" "	50	CEV	JUL	2	DELANO	KERN
	5761	" "	50	CEV	JUL	2	DELANO	KERN
	5774	CX TARS	50	HART	JUL	8	ARVIN	KERN
	5780	" "	50	TRLK	JUL	8	BAKERSFIELD	KERN
	5781	" "	50	TRLK	JUL	8	BAKERSFIELD	KERN
	5782	" "	14	TRLK	JUL	8	BAKERSFIELD	KERN
	5783	" "	50	HART	JUL	8	BAKERSFIELD	KERN
	5785	" "	50	TRLK	JUL	8	BAKERSFIELD	KERN
	5786	" "	50	TRLK	JUL	8	BAKERSFIELD	KERN
	5797	" "	43	TRLK	JUL	9	BUTTONWILLOW	KERN
	5802	" "	50	HART	JUL	9	LOST HILLS	KERN
	5806	" "	50	TRLK	JUL	9	LOST HILLS	KERN
	5816	AE MELN	50	CEV	JUL	9	LOST HILLS	KERN
	5817	" "	50	CEV	JUL	9	LOST HILLS	KERN
	5822	" "	50	CEV	JUL	9	DELANO	KERN
	5831	CX TARS	50	TRLK	JUL	14	BAKERSFIELD	KERN
	5833	" "	50	HART	JUL	14	BAKERSFIELD	KERN
	5836	" "	50	TRLK	JUL	14	BAKERSFIELD	KERN
	5837	" "	50	HART	JUL	14	BAKERSFIELD	KERN
	5839	" "	50	TRLK	JUL	14	BAKERSFIELD	KERN
	5841	" "	50	TRLK	JUL	14	BAKERSFIELD	KERN
	5846	" "	50	TRLK	JUL	14	BAKERSFIELD	KERN
	5847	" "	50	TRLK	JUL	14	BAKERSFIELD	KERN
	5871	AE MELN	50	CEV	JUL	16	DELANO	KERN
	5872	" "	50	CEV	JUL	16	DELANO	KERN

continued -

Table 3-continued

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	COLLECTION MONTH	DAY	PLACE	COUNTY
KERN	5896	AE MELN	50	CEV	JUL	16	LOST HILLS	KERN
	5897	" "	50	CEV	JUL	16	LOST HILLS	KERN
	5900	CX TARS	50	TRLK	JUL	23	ARVIN	KERN
	5901	" "	50	HART	JUL	23	ARVIN	KERN
	5904	" "	50	TRLK	JUL	23	ARVIN	KERN
	5907	" "	50	TRLK	JUL	23	BAKERSFIELD	KERN
	5913	" "	50	TRLK	JUL	23	BAKERSFIELD	KERN
	5923	" "	50	HART	JUL	23	BAKERSFIELD	KERN
	5925	" "	50	TRLK	JUL	23	BAKERSFIELD	KERN
	5927	" "	50	CALGP	JUL	24	LOST HILLS	KERN
	5935	" "	50	TRLK	JUL	24	LOST HILLS	KERN
	5938	AE MELN	50	CEV	JUL	24	LOST HILLS	KERN
	5939	" "	50	CEV	JUL	24	LOST HILLS	KERN
	5940	" "	50	CEV	JUL	24	LOST HILLS	KERN
	5942	" "	50	CEV	JUL	24	LOST HILLS	KERN
	5943	" "	50	CEV	JUL	24	LOST HILLS	KERN
	5963	CX TARS	50	HART	JUL	30	LOST HILLS	KERN
	5976	AE MELN	50	CEV	JUL	30	LOST HILLS	KERN
	5978	" "	50	CEV	JUL	30	LOST HILLS	KERN
	5984	CX TARS	50	TRLK	AUG	4	BAKERSFIELD	KERN
	6017	" "	50	TRLK	AUG	11	ARVIN	KERN
	6068	" "	50	SLE	AUG	19	BAKERSFIELD	KERN
	6120	AE MELN	50	CEV	AUG	29	LOST HILLS	KERN
	6129	CX TARS	50	SLE	SEPT	3	ARVIN	KERN
	6183	" "	50	SLE	SEPT	9	ARVIN	KERN
	6209	AE MELN	50	CEV	SEPT	10	LOST HILLS	KERN
	6211	" "	50	CEV	SEPT	10	LOST HILLS	KERN
	6212	" "	50	CEV	SEPT	10	LOST HILLS	KERN
	6230	" "	50	CEV	SEPT	10	TAFT	KERN
	6255	" "	50	CEV	SEPT	17	LOST HILLS	KERN
	6275	CX TARS	50	TRLK	SEPT	23	ARVIN	KERN
	6295	AE MELN	50	CEV	SEPT	23	OLD RIVER	KERN
	6301	" "	50	CEV	SEPT	23	OLD RIVER	KERN
	6304	" "	50	CEV	SEPT	23	OLD RIVER	KERN
LAHD	46	CX PEUS	16	WEE	JUL	17	ENCINO	L A
	48	CX TARS	29	TRLK	JUL	30	GRIFFITH PARK	L A
	50	" "	50	SLE	JUL	30	NORWALK	L A
	52	" "	50	HART	JUL	30	NORWALK	L A
	53	" "	50	WEE	JUL	30	NORWALK	L A
	55	" "	50	TRLK	JUL	30	NORWALK	L A
	59	" "	50	TRLK	JUL	31	NORWALK	L A
	60	" "	50	SLE	JUL	31	NORWALK	L A
	62	" "	50	SLE	JUL	31	NORWALK	L A
	64	" "	50	SLE & TRLK	JUL	31	NORWALK	L A
	66	" "	50	SLE & TRLK	JUL	31	NORWALK	L A
	69	" "	50	SLE	JUL	31	NORWALK	L A
	70	" "	50	SLE	JUL	31	NORWALK	L A
	74	" "	50	SLE	JUL	31	NORWALK	L A
	75	" "	50	SLE & HART	AUG	5	NORWALK	L A
	76	" "	50	SLE	AUG	5	NORWALK	L A
	77	" "	42	SLE & HART	AUG	5	NORWALK	L A
	80	" "	50	SLE	AUG	7	NORWALK	L A
	82	" "	50	SLE & TRLK	AUG	7	NORWALK	L A
	83	" "	50	TRLK	AUG	7	NORWALK	L A

continued-

Table 3-continued

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	COLLECTION MONTH	DAY	PLACE	COUNTY
LAHD	84	CX TARS	41	TRLK	AUG	7	NORWALK	L A
	85	CX QUIN	20	SLE	AUG	7	NORWALK	L A
	105	CX TARS	50	SLE	AUG	13	NORWALK	L A
	106	" "	50	SLE	AUG	13	NORWALK	L A
	107	" "	45	SLE & TRLK	AUG	13	NORWALK	L A
	112	" "	50	SLE	AUG	26	NORWALK & PICO	L A
	113	" "	50	SLE	AUG	26	NORWALK & PICO	L A
	114	CX QUIN	40	SLE	AUG	26	NORWALK & PICO	L A
	117	CX TARS	41	SLE	AUG	26	NORWALK & PICO	L A
	123	CX QUIN	25	SLE	AUG	26	CITY/INDUSTRY	L A
	136	CX TARS	50	SLE	SEPT	16	MONTEBELLO	L A
	154	" "	37	SLE	SEPT	23	MONTEBELLO	L A
LAKE	8	CX TARS	50	TRLK	AUG	12	UPPER LAKE	LAKE
LONG	22	CX QUIN	49	TRLK	AUG	15	LONG BEACH	L A
	25	" "	57	TRLK	AUG	15	LONG BEACH	L A
	26	" "	50	TRLK	AUG	15	LONG BEACH	L A
	31	" "	50	TRLK	AUG	22	LONG BEACH	L A
MERC	2	CX TARS	50	TRLK	JUL	22	MERCED	MERCED
	3	" "	50	TRLK	JUL	22	MERCED	MERCED
NWST	215	CX TARS	50	SLE	JUL	28	PRADO BASIN	RIVERS
	222	" "	50	HART	JUL	28	PRADO BASIN	RIVERS
ORCO	94	CX TARS	30	TRLK	JUL	29	IRVINE	ORANGE
	97	" "	22	HART	JUL	31	IRVINE	ORANGE
	99	" "	44	SLE & TRLK	JUL	31	IRVINE	ORANGE
	101	" "	50	HART	JUL	31	IRVINE	ORANGE
	103	" "	37	TRLK	JUL	31	IRVINE	ORANGE
	105	" "	34	TRLK	AUG	5	MISSION VIEJO	ORANGE
	107	" "	18	HART	AUG	5		ORANGE
	109	" "	50	TRLK	AUG	6	IRVINE	ORANGE
	110	" "	47	HART & TRLK	AUG	6	IRVINE	ORANGE
	114	" "	26	TRLK	AUG	8	IRVINE	ORANGE
	115	" "	36	TRLK	AUG	8	IRVINE	ORANGE
	116	" "	49	TRLK	AUG	8	IRVINE	ORANGE
	120	" "	50	HART	AUG	12	IRVINE	ORANGE
	128	" "	50	TRLK	AUG	12	IRVINE	ORANGE
	130	" "	37	TRLK	AUG	12	IRVINE	ORANGE
	145	" "	50	HART	AUG	15	IRVINE	ORANGE
	160	" "	50	TRLK	AUG	19	IRVINE	ORANGE
	181	CX QUIN	18	TRLK	AUG	26	IRVINE	ORANGE
	193	CX TARS	13	SLE	AUG	26	IRVINE	ORANGE
	269	" "	50	SLE	SEPT	11	IRVINE	ORANGE
	275	" "	50	SLE	SEPT	11	IRVINE	ORANGE
	279	" "	17	TRLK	SEPT	11	IRVINE	ORANGE
	284	" "	50	SLE	SEPT	16	IRVINE	ORANGE
	295	" "	50	HART	SEPT	16	IRVINE	ORANGE
	318	" "	50	TRLK	SEPT	16	IRVINE	ORANGE
	333	" "	50	HART	SEPT	19	IRVINE	ORANGE
	365	" "	50	HART	SEPT	23	IRVINE	ORANGE
SACR	8	CX TARS	50	TRLK	JUN	9	RIO LINDA	SACRA

continued-

Table 3-contined

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	COLLECTION MONTH	DAY	PLACE	COUNTY
SACR	48	CX TARS	50	TRLK	JUN	16	ELK GROVE	SACRA
	79	" "	50	TRLK	JUN	23	DAVIS	YOLO
	84	" "	50	TRLK	JUN	23	GALT	SACRA
	131	" "	50	TRLK	JUN	30	GALT	SACRA
	134	" "	50	TRLK	JUN	30	WOODLAND	YOLO
	136	" "	50	TRLK	JUN	30	WOODLAND	YOLO
	145	" "	50	TRLK	JUN	30	WILTON	SACRA
	147	" "	50	TRLK	JUN	30	WILTON	SACRA
	148	" "	50	TRLK	JUN	30	WILTON	SACRA
	228	CX PEUS	12	TRLK	JUL	14	ELK GROVE	SACRA
	245	CX TARS	50	TRLK	JUL	14	BRYTE	YOLO
	261	" "	50	TRLK	JUL	14	WILTON	SACRA
	266	" "	50	HART	JUL	14	WILTON	SACRA
	326	" "	50	TRLK	JUL	28	ELK GROVE	SACRA
	341	" "	50	TRLK	JUL	28	BRYTE	YOLO
	372	" "	50	TRLK	AUG	4	DAVIS	YOLO
	391	" "	50	TRLK	AUG	4	FRANKLIN	SACRA
	394	" "	50	TRLK	AUG	4	ELK GROVE	SACRA
	397	" "	50	TRLK	AUG	4	ELK GROVE	SACRA
	398	" "	40	TRLK	AUG	4	ELK GROVE	SACRA
	415	" "	50	HART	AUG	4	WOODLAND	YOLO
	426	" "	50	TRLK	AUG	4	WILTON	SACRA
	432	" "	50	TRLK	AUG	11	WILTON	SACRA
	438	" "	50	TRLK	AUG	11	WILTON	SACRA
	451	" "	50	HART & TRLK	AUG	11	GALT	SACRA
	453	" "	50	TRLK	AUG	11	GALT	SACRA
	456	" "	50	TRLK	AUG	11	GALT	SACRA
	458	" "	50	TRLK	AUG	11	GALT	SACRA
	461	" "	50	TRLK	AUG	11	ELK GROVE	SACRA
	467	" "	50	TRLK	AUG	11	ELK GROVE	SACRA
	475	" "	50	TRLK	AUG	11	WILTON	SACRA
	477	" "	50	HART & TRLK	AUG	11	WILTON	SACRA
	492	" "	50	TRLK	AUG	18	HERALD	SACRA
	493	" "	50	HART	AUG	18	HERALD	SACRA
	510	" "	50	TRLK	AUG	18	DAVIS	YOLO
	511	" "	50	TRLK	AUG	18	DAVIS	YOLO
	516	" "	50	TRLK	AUG	18	FRANKLIN	SACRA
	536	" "	50	TRLK	AUG	18	ELK GROVE	SACRA
	550	" "	50	TRLK	AUG	25	ELK GROVE	SACRA
	561	" "	50	TRLK	AUG	25	ELK GROVE	SACRA
	568	" "	50	TRLK	AUG	25	GALT	SACRA
	579	" "	50	WEE	AUG	25	DAVIS	YOLO
	621	" "	50	TRLK	SEPT	2	FRANKLIN	SACRA
	626	" "	50	TRLK	SEPT	2	GALT	SACRA
	630	" "	50	TRLK	SEPT	3	HOOD	YOLO
	636	" "	50	TRLK	SEPT	3	HOOD	YOLO
	637	" "	50	TRLK	SEPT	3	HOOD	YOLO
	668	" "	47	TRLK	SEPT	5	DAVIS	YOLO
	674	" "	50	WEE	SEPT	5	DAVIS	YOLO
	678	" "	50	WEE	SEPT	5	DAVIS	YOLO
	681	" "	50	WEE	SEPT	5	DAVIS	YOLO
	696	" "	50	WEE	SEPT	5	DAVIS	YOLO
	698	" "	50	WEE	SEPT	5	DAVIS	YOLO
	704	" "	50	WEE	SEPT	5	DAVIS	YOLO
	732	" "	50	WEE	SEPT	9	DAVIS	YOLO
	733	" "	50	TRLK	SEPT	9	DAVIS	YOLO

continued-

Table 3-continued

DIST	POOLNO	SPECIES	NUMBER MOSQUITO	VIRUS	COLLECTION MONTH	DAY	PLACE	COUNTY
SACR	766	CX TARS	50	WEE	SEPT	22	WILTON	SACRA
	769	" "	30	WEE	SEPT	22	WILTON	SACRA
	779	" "	50	WEE	SEPT	29	DAVIS	YOLO
	780	" "	12	WEE	SEPT	29	DAVIS	YOLO
	785	" "	50	UNIDENTIF.	OCT	6	HOOD	YOLO
SANB	29	CX TARS	50	TRLK	JUL	31	NEEDLES	SBERN
SOUE	127	CX QUIN	15	HART	JUL	21	ENCINO	L A
	130	CX TARS	50	HART	JUL	21	WHITTIER	L A
	131	" "	31	SLE	JUL	21	WHITTIER	L A
	140	" "	50	TRLK	JUL	29	HARBOR CITY	L A
	145	" "	16	TRLK	JUL	29	ENCINO	L A
	161	" "	29	TRLK	AUG	11	ENCINO	L A
	177	CX QUIN	50	TRLK	AUG	11	HARBOR CITY	L A
	185	CX PEUS	42	SLE	AUG	13	GARDENA	L A
	197	CX TARS	50	TRLK	AUG	18	HARBOR CITY	L A
	200	CX ERYT	50	TRLK	AUG	18	HARBOR CITY	L A
	230	CX TARS	50	HART	AUG	26	LOS ANGELES	L A
	233	" "	32	TRLK	AUG	26	LOS ANGELES	L A
	244	" "	50	TRLK	AUG	27	DOWNEY	L A
	245	" "	50	SLE	AUG	27	DOWNEY	L A
	246	" "	50	SLE	AUG	27	DOWNEY	L A
	247	" "	60	SLE & TRLK	AUG	27	DOWNEY	L A
	248	CX QUIN	50	SLE	AUG	27	DOWNEY	L A
	270	CX TARS	30	SLE	SEPT	15	DOWNEY	L A
	271	CX QUIN	21	SLE	SEPT	15	DOWNEY	L A
SUYA	20	CX TARS	50	TRLK	JUN	30	LIVE OAK	SUTTER
	34	" "	32	WEE	AUG	19	SUTTER	SUTTER
VENT	7	CX TARS	20	TRLK	AUG	15	SIMI VALLEY	VENTURA

Table 4.-Serological conversions to WEE and SLE viruses in sentinel chickens, California, 1986.

Flock location	WEE positive/total (% positive)				SLE positive/total (% positive)			
	JULY	AUG	SEP	OCT	JULY	AUG	SEP	OCT
NORTHERN CALIFORNIA								
Shasta, MAD office	0/20	0/20	0/20	Dead	0/20	0/20	0/20	Dead
Tehama, MAD office	0/20	0/19	4/18(22)	5/20(25)	0/20	0/19	0/18	0/20
Corning, Martin Ranch	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Butte, Chico	0/20	0/20	2/20(10)	2/20(10)	0/20	0/20	0/20	0/20
Butte, Honcut	0/20	0/20	1/19(5)	1/20(5)	0/20	0/20	0/19	0/20
Butte, Gray Lodge	0/20	1/19(5)	8/13(62)	9/13(69)	0/20	0/19	0/13	0/13
SNBYuba, P. V. Ranch	0/20	0/17	0/20	0/20	0/20	0/17	0/20	0/20
SNBYuba, Dean's	0/20	0/20	6/20/(30)	6/20(30)	0/20	0/20	0/20	0/20
SNBYuba, Barker	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
SacNBYolo, Merritt	0/19	0/19	0/18	0/19	0/19	0/19	0/18	0/19
SacNMYolo, Natomas	0/20	0/20	0/20	0/20	0/20	0/20	1/20(5)	1/20(5)
SacNBYolo, Elk Grove	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Solano, Dixon	0/21	0/21	4/21(19)	11/21(52)	0/21	0/21	0/21	0/21
Santa Clara, Gilroy	0/19	0/21	0/22	NB	0/19	0/21	0/22	NB
SAN JOAQUIN VALLEY								
San Joaquin, Galt	0/19	0/18	0/18	0/18	0/19	0/18	0/18	0/18
Eastside, Oakdale	0/15	0/14	0/15	0/15	0/15	0/14	0/15	0/15
Turlock, Victoria	0/20	1/20(5)	0/20	1/20(5)	0/20	0/20	0/20	0/20
Merced, Borba Dairy	0/19	0/19	0/19	0/19	0/19	0/19	0/19	0/19
Fresno WS, Mendota Ref.	0/21	0/21	0/21	1/21(5)	0/21	0/21	0/21	0/21
Consolidated, Friant Rd.	0/18	0/19	0/19	0/18	0/18	0/19	0/19	0/18
Kings, Riverview Ranch	0/19	0/19	0/19	0/19	0/19	0/19	0/19	0/19
Delta, Kingsburg GC	0/19	0/18	0/19	0/19	0/19	0/18	0/19	0/19
Tulare, MAD office	0/18	0/10	0/10	0/10	0/18	0/10	0/10	0/10
West Side, Lost Hills	0/18	0/18	0/18	0/18	0/18	0/18	0/18	0/18
West Side, Maricopa	0/19	0/17	0/16	0/16	0/19	1/17(6)	1/16(6)	1/16(6)
Delano, Teviston	0/18	0/18	0/18	0/18	0/18	0/18	0/18	0/18
Kern, Wasco	0/19	0/18	0/19	0/19	0/19	0/18	1/19(5)	1/19(5)
Kern, F.C. Tracy	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25
Kern, Buttonwillow	0/20	0/17	0/17	0/17	0/20	0/17	0/17	0/17
Kern, Wildlife Refuge	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25
Kern, Oildale	0/17	0/17	0/17	0/17	0/17	0/17	0/17	0/17
Kern, John Dale	0/25	0/24	0/24	0/24	0/25	0/24	0/24	0/24
Kern, Poso West	0/23	0/23	0/23	0/23	0/23	0/23	0/23	0/23
Kern, River Bottom	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24
SOUTHERN CALIFORNIA								
Santa Barbara, Isla Vista	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21
Ventura, Pt. Mugu	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Ventura, Simi Valley	0/18	0/18	0/18	0/18	0/18	0/18	0/18	0/18
Los Angeles, La Brea	0/19	0/19	0/19	0/19	0/19	0/19	1/19(5)	1/19(5)
Southeast, Balboa Golf*	0/19	0/19	0/19	0/18	0/19	0/19	0/19	2/18(11)
Southeast, Harbor Lakes*	0/22	0/22	0/22	0/21	0/22	0/22	0/22	2/21(10)
Los Angeles, Cal Poly*	0/19	0/19	0/19	0/19	0/19	0/19	2/19(11)	2/19(11)
Southeast, El Dorado	0/21	0/35	0/22	NB	0/21	0/35	0/22	NB
Orange, Duck Club	0/19	0/19	0/18	0/17	0/19	0/19	1/18(6)	2/17(12)
Orange, San Mateo Pt.	0/20	0/20	0/19	0/19	0/20	0/20	0/19	0/19
Orange, Featherly Park	0/17	0/18	0/17	0/12	0/17	0/18	0/17	0/12
San Bernardino, San Brdo	0/18	0/14	0/17	0/17	0/18	0/14	0/17	0/17
Riverside, Lake Elsinor	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
Coachella Valley, Mecca	0/23	1/23(4)	1/23(4)	1/23(4)	0/23	3/23(13)	8/23(35)	11/23(48)
Northwest, Corona*	0/20	0/20	0/20	0/19	0/20	0/20	0/20	0/19
Imperial, Palo Verde	0/22	2/22(9)	2/22(9)	2/20(10)	0/22	0/22	2/22(9)	2/20(10)
Imperial, Bard**	18/19(95)	19/20(95)	NB	NB	1/19(5)	3/20(15)	NB	NB
Imperial, El Centro	0/17	0/17	2/13(15)	NB	6/17(35)	6/17(35)	7/13(54)	NB
San Diego, Vista	0/14	NB	0/15	0/16	0/14	NB	0/15	0/16
San Diego, Lakeside	0/18	NB	0/18	0/18	0/18	NB	0/18	0/18
San Diego, San Ysidro	0/19	NB	0/19	0/19	0/19	NB	0/19	0/19
Colorado River, Needles	0/6	Dead	Dead	Dead	0/6	Dead	Dead	Dead
Colorado River, Havasu Ref.	0/25	9/25(36)	15/25(60)	18/23(78)	0/25	0/25	0/25	0/23
Colorado River, Parker Dam	0/22	0/22	0/20	0/17	0/22	0/22	0/20	0/17
Colorado River, Blythe	0/24	16/24(67)	18/24(75)	20/21(95)	0/24	0/24	0/24	0/21

All sera WEE negative in May and June; SLE negative in May, 1/21 positive in June from Imperial, El Centro flock; NB = not bled.
*Bled in November; no new seroconversions.
**8 chickens bled in November; 6 new SLE seroconversions.

SELECTIVE DISTRIBUTION OF ARBOVIRAL INFECTIONS IN WILD VERTEBRATES IN THE CENTRAL VALLEY OF CALIFORNIA

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Serological and virological surveys of inapparent infection with arboviruses in wild vertebrate hosts are important to determine possible virus reservoirs and to understand the basic infection cycles of the viruses. The isolation of virus from, or the detection of antibody in, a single animal may or may not be a clue to its involvement in the infection chain. It is necessary to have repeated isolations and positive serological findings from a species over a period of years and in different geographical regions to establish the epidemiological importance of any individual species.

This paper summarizes serological data from tests on nearly 30,000 blood samples from wild birds and mammals collected primarily in Kern, Butte and Glenn Counties in the period 1952 through 1974. This is a preview of analyses done in preparation for the forthcoming monograph on "The Epidemiology and Control of Arthropod-Borne Viruses in California" by W.C. Reeves and co-workers.

Studies in the Yakima Valley of Washington in the early 1940's led to the establishment of 5 criteria that must be met if a vertebrate is to be an effective host for western equine encephalomyelitis (WEE) or St. Louis encephalitis (SLE) viruses (Hammon et al. 1943). These criteria are still valid and can be applied to other arboviruses in addition to WEE and SLE. To be an effective source of vector infection a vertebrate species must:

1. be abundant in the area.

2. show no apparent signs of infection.

3. have a high titer viremia for more than a fleeting period after acquiring infection from the bite of an infected vector.

4. not bestow a first season's protection to its offspring by maternal transmission of antibodies, thus losing the advantage of a high titer viremia in the young as a source of vector infection.

5. be a preferred host of the vector species.

Wild birds, especially nestlings and fledglings, best satisfied these criteria, and were considered to be the most likely primary hosts of WEE and SLE viruses.

Vertebrate studies in Kern County in the 1950's involved the year-round sampling of a wide range of wild bird species. Blood samples from these birds were tested for viruses and antibodies. Virus isolation efforts were discontinued after it became obvious that finding a viremic bird was like finding a needle in a haystack. Experimental infection studies showed that the period of detectable viremia in birds usually lasted for less than 1 week and thus viremic birds would rarely be found in field-collected samples.

The early bird studies failed to answer many questions about the overwintering of WEE and SLE viruses, so attention turned to rodents and other small animals. Mammalian surveys were carried out in Kern County in the spring and fall from 1959 through 1962. These were followed by a 5-year period of intensive sampling of a large trapping grid in the Jerry Slough area from 1963 through 1968. This grid study was expanded in 1965 to include trap lines in a nearby smaller area on the Valley floor and in a foothill area of Kern County. Birds were also sampled at these locations throughout this time period.

In 1968, we began vertebrate studies in 2 new areas. The first was on the West Side of the valley in Kern County. This area was opened to irrigated agriculture when the California Aqueduct was completed. The second, in Butte and Glenn Counties, was initiated after the Viral and Rickettsial Disease Laboratory, California Department of Public Health, isolated WEE virus from 8 tree squirrels from this region in 1965-67 (R. W. Emmons, pers. comm.). Lennette et al. (1956) made the initial isolations of WEE virus from Sacramento Valley squirrels that had been submitted to the Health Department for rabies tests. Studies in these 2 areas were completed in 1974, and we have done no further sampling of wild vertebrates.

The summary results presented here combine data from all these studies. They represent a considerable effort by many individuals over an extended period of time. We have tested sera from 14,713 wild birds and 14,951 wild mammals. All samples were not tested against all viral antigens. Initially, sera were tested for antibodies to WEE, SLE, Powassan (POW), Modoc (MOD), and Rio Bravo (RB) viruses. Buttonwillow (BUT) and California encephalitis (CE) viruses were added in 1962, and RB virus was discontinued except for samples from bats collected in the Sacramento Valley. Turlock (TUR), Lokern (LOK) and Main Drain (MD) viruses were added along the way, and some of these sera have recently been pulled out of the freezer and tested for antibodies to Hart Park (HP) and Llano

Table 1.-Antibody prevalence in wild birds vs. wild mammals.

		California Central Valley, 1952-1974			
Virus	Principal Calif. vector	Birds		Mammals	
		No. tested	% positive	No. tested	% positive
WEE	*Cx. tarsalis*	14,713	4	14,816	3
SLE	*Cx. tarsalis*	14,705	4	14,951	4
TUR	*Cx. tarsalis*	11,033	5	7,542	<1
HP	*Cx. tarsalis*	155	34	341	1
LLS	*Cx. tarsalis*	914	17	612	8
CE	*Ae. melanimon*	6,371	2	12,901	5
BUT	*Culicoides*	4,682	<1	11,881	7
LOK	*Culicoides*	4,682	<1	12,053	4
MD	*Culicoides*	0	.	6,096	9
POW	ticks	4,682	<1	14,648	3
MOD	none	4,682	1	14,545	3
RB	none	2,249	0	3,600	3

Seco (LLS) viruses. Because the blood samples were usually quite small, once we had a reasonably clear picture of the species least likely to be infected with a particular virus, sera from that species were no longer tested. For this reason, the number of samples tested varies from virus to virus.

Results of tests on the arboviruses that are transmitted primarily by *Culex tarsalis* in the Central Valley are interesting (Table 1). The similarity in WEE and SLE antibody prevalence between birds and mammals demonstrates the diverse feeding habits of this mosquito. On the other hand, the difference in prevalence between the 2 vertebrate groups for TUR, HP and LLS viruses probably indicates a difference in susceptibility, with mammals being less likely to become infected with or to develop antibodies to these 3 viruses (Ksiazek 1984; R. R. Graham, pers. comm.).

The remaining viruses for which we have summarized antibody prevalence data primarily infect mammals. This is assumed to reflect the feeding preference of the primary vectors. In the case of CE virus, mammals are a preferred host of *Aedes melanimon*, although some feedings on birds do occur, as shown by host preference studies (Nelson et al. 1976) and by the 2% of bird sera that were positive for CE virus antibodies (Table 1). The preference is more clear-cut for the viruses that are transmitted to mammalian hosts by *Culicoides* (BUT, LOK and MD) or by ticks (POW). MOD virus is mainly associated with *Peromyscus* mice and RB virus with bats. These 2 viruses are not classified as arboviruses as they have no known arthropod vectors and are spread mainly by contact from mammal to mammal (Karabatsos 1985).

If we separate out the samples from House Finches and House Sparrows, we see that House Finches are the most frequently infected with all 5 of the *Cx. tarsalis*-transmitted viruses (Table 2). This, coupled with their abundance throughout the Central Valley and their susceptibility to experimental infection, establish this species as a primary host for both WEE and SLE viruses. The prevalence of CE virus antibodies was low, but uniform for all bird species. Only an occasional individual bird was found infected with the remaining viruses.

A very different picture emerges for the mammals (Table 3). *Lagomorphs*, primarily Blacktail Jackrabbits, were frequently infected with a wide variety of arboviruses, especially those transmitted by mosquitoes and *Culicoides*. Jackrabbits were much more likely to be infected with WEE virus than *Sylvilagus audubonii*, the Desert Cottontail, even when collected in the same place at the same time (Hardy et al. 1974). Experimental studies showed that cottontails were sus-

Table 2.-Antibody prevalence in wild birds.

Virus	California Central Valley, 1952-1973 Percent Positive House Finches	House Sparrows	All other species
WEE	7	5	3
SLE	6	3	3
TUR	17	2	3
HP	70	0	NT*
LLS	34	17	14
CE	2	2	2
BUT	0	0	<1
LOK	0	0	<1
POW	1	1	<1
MOD	1	1	1
RB	0	0	0
Mean no. tested	912	1,615	4,076

*Not tested.

Table 3.-Antibody prevalence in wild mammals.

Virus	California Central Valley, 1959-1974 Percent positive Rodents	Lagomorphs	Carnivores
WEE	1	9	4
SLE	3	5	4
TUR	<1	1	2
HP	3	0	0
LLS	1	23	3
CE	2	13	12
BUT	2	20	3
LOK	1	13	2
MD	1	20	4
POW	4	2	2
MOD	3	2	5
RB	<1	0	2
Mean no. tested	7,349	2,547	103

ceptible to infection with WEE virus, so this result was unexpected and must reflect an escape of this host from vector feedings.

The antibody prevalence data for *Chiroptera* (bats) have been omitted. Although a number of samples were positive for SLE virus, these were considered to be cross-reactions to antigenically related RB virus, for which 38% of the bat sera were positive.

To summarize, birds -- especially House Finches -- are most commonly infected with the viruses that are transmitted by *Cx. tarsalis*: WEE, SLE, TUR, HP and LLS. Infection with the remaining arboviruses -- CE, BUT, LOK, MD and POW -- is found primarily in mammals, especially rabbits.

These vertebrate host findings from the Central Valley may or may not be relevant to the Southern California urban environment which has been the focus of SLE virus activity in California in recent years. Because of this uncertainty, we hope to initiate new studies in 1987 on the involvement of birds in the transmission cycle of SLE virus in the Los Angeles Basin.

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EVOLUTION OF MOSQUITO CONTROL IN THE DELTA VECTOR CONTROL DISTRICT

Wm. Donald Murray[1]

I. FORMATION OF DISTRICT AND PRE-WWII ERA.

Introduction

The Delta Mosquito Abatement District was formed in 1922. The original district was 16 square miles, which included all of the City of Visalia plus some adjacent suburban area. The Visalia Women's Club played a key part in its formation. Malaria was reported as a rather common disease at that time, and pest mosquitoes provided an added stimulus for the residents to obtain control.

Prior to 1900, the area of northern Tulare County which is presently included in the Delta Vector Control District was largely desert during the summer months. Several rivers or streams flowed through this area during winter and spring, but these usually dried back toward the foothills as the summer progressed. However, the water table was high near these streams, and seepage areas and swamps sometimes persisted throughout the year. One such area was the extension of the Kaweah River known as Mill Creek, which ran through Visalia.

Malaria and Pest Mosquitoes

The wooded seepage and swampy areas along Mill Creek provided a favorable larval habitat for two native mosquitoes, the malaria vector *Anopheles punctipennis* and the pest mosquito *Aedes vexans*. Mosquito control in the early 1900's, wherever it was carried out in the world, was rather unsophisticated. There was brush clearing, drainage, and spraying with oil. This was the program followed by the Delta Mosquito Abatement District (MAD) soon after its formation and results appear to have been quite satisfactory. There seemed to be no need for a highly technical program at that time. A common sense program carried out by an untrained Superintendent and several part-time workers could do a rather surprisingly good job against the mosquitoes of major concern, because the sources were limited and the basic control efforts of clearing and oiling were effective.

The House Mosquito (*Culex quinquefasciatus*) Era

If conditions had remained static, the original program might have sufficed for many years, but conditions changed. One of the first evidences of the inadequacy of the District's program occurred when the house mosquito, *Culex quinquefasciatus*, became the dominant species during the late 1920's and throughout much of the 1930's. During this period there was a great increase in available polluted water:

1) Sewage from Visalia was piped less than 2 miles west of the city to a "sewer farm", where it pooled under dense weeds and water grass. This sewer farm was located outside the original District boundary, but the house mosquitoes which were produced in tremendous numbers readily flew the several miles into the city.

2) Housing around the outskirts of the city began using water for individual sewage systems, replacing the outhouses so common up to then. Unfortunately, covering of these inexpensive "cess pools" was frequently inadequate, so that house mosquitoes could easily enter and leave such sources. Thus the city was ringed by heavy producing sources of these mosquitoes.

3) Several dairies began doing business inside or slightly outside the 16 square mile area of the District, within easy flight range of the entire city. In place of the old procedure of milking cows in the yard or pasture and using a minimum of water to wash the equipment and the cow's udders, dairies began milking cows in a concrete-floored milk barn, and wastes were washed out of the barn with large quantities of water. This water was directed via a small trench or pipeline to a ditch, a slough, or a small pond where it stimulated a heavy weed growth and large numbers of house mosquitoes.

4) In addition to the previous major developments, many types of sources of lesser importance increased greatly as the population of the city grew: street gutters and catch basins, backyard barrels and other containers, standing water in culverts under streets, fish ponds, and numerous other sources.

The Pasture Mosquito (*Aedes nigromaculis*) Era

The problems which provided for an explosion of house mosquitoes were not amenable to the simple "brush clear, drain and oil spray" program carried out by the District since its formation. An effort was made in 1935 to meet some of these new problems by expanding the District to 29 square miles. But even as this modest expansion was occurring, a major new problem appeared which rendered this minor adjustment futile. In

[1]Dr. Murray managed the Delta Vector Control District from 1947 to 1978.

Passage of Proposition 13 in 1978 was the single worst disaster to confront organized mosquito control since World War II. Local tax revenue for MADs was reduced to about half. The Source Reduction Units within the MADs were the first to be either eliminated or drastically reduced. Howard Mathews, Source Reduction Foreman of the Delta VCD said, "In the 1970's, because of insecticide resistance the airplane was grounded and subsequently sold and after Proposition 13 the District parked the Source Reduction equipment and I went back to spraying."

The Recovery-Adjustment Period (1980-1985)

During the first half of the 1980's, major adjustments had to be made due to loss of revenue, further environmental constraints, changes in pesticide regulations, land use permits, hazardous waste regulations and worker safety requirements. Five MADs held elections for a special tax rate to restore a major portion of revenue lost through passage of Proposition 13. A new concern emerged with the occurrence of St. Louis encephalitis cases in populated urban areas of southern California.

In past years, most of the attention devoted to mosquito prevention activities were focused on the Central Valley area. During that time, the coastal MADs continued to carry on the maintenance phase of mosquito prevention. A large portion of the salt marshes were filled and converted to industrial uses. However, with the upgrading of waste-water standards and the pressure to restore and develop new marsh habitats, new mosquito problem-areas began to occur adjacent to population centers. In response to this development, the Vector Biology and Control Branch, Department of Health Services, in cooperation with the CMVCA prepared criteria for mosquito prevention in waste-water reclamation projects. The coastal region MADs developed mosquito prevention standards for restoration of salt marshes. These standards and waste-water criteria continue to be used during the review of water quality discharge requirements, land use permits and environmental impact reports. During this period, the CMVCA Environmental Committee developed a computer program to track the occurrence and location of rare and endangered species to evaluate the impact of mosquito control activities that would need to be carried out in these critical areas.

In retrospect, the period since 1948 has been a time when mosquito control agencies have placed mosquito prevention in a secondary position. The control agencies have taken the pragmatic approach of using temporary control measures as the primary course of action. Mosquito prevention programs have been complex, difficult, and have required long-term efforts involving land use changes, cost to landowners, educational and engineering programs and legal action. They require astute negotiations with landowners, necessary engineering and entomological surveys, and cost analyses. The use of the legal abatement procedures can cause confrontations with landowners and place Boards of Trustees in the uncomfortable role of a judicial panel. The legal approach demands a strong commitment by a Board of Trustees, even under conditions where it is used only occasionally.

In contrast, providing a service to immediately get rid of mosquitoes by the use of pesticides and selected predators is much more dynamic, has a high visibility to the public, is much less complicated, and avoids the confrontational aspects of abatement procedures. Nevertheless, we must reaffirm the need to place primary emphasis on mosquito prevention through environmental management, public education, and code enforcement. History has taught us that a sound investment in policy and resources to implement mosquito prevention is the best method of mosquito control. Richard F. Peters in his 1973 talk on "Environmental Approaches to Vector Control" said, "the greatest hazard to a mosquito is dehydration."

prevention recommendations.

The Environmental Period (1970-1980)

The major event that seemed to have triggered the environmental protection movement was Earth Day--April 22, 1970. Demonstrations occurred on college campuses across the country, signalling the beginning of a national concern for conservation of natural resources and control of environmental pollution. The repair of a damaged environment had to start. This period of environmental constraints brought forward an array of federal and state laws and regulations. The Porter-Cologne Water Quality Control Act of 1971 provided for strict control of waste-water discharges, including agricultural waste-waters. The California Environmental Quality Act of 1970 established the ground rules for land use planning, setting into motion the environmental impact review process. The 1974 and 1976 amendments to the State Fish and Game Code concerning endangered species and the requirement for permits for any physical modification of riparian habitats impacted mosquito prevention efforts. Laws were enacted by the State Legislature to establish the San Francisco Conservation and Development Commission and Coastal Zone Conservation Commission, which required extensive reviews and permits for land development or alteration of habitats. The updated Federal Pesticide Control Act of 1972, the National Environmental Policy Act of 1970, the Federal Endangered Species Act, and the 1975 amended River and Harbors Act, which required public hearing and permits from the U.S. Army Corps of Engineers, were laws passed reflecting Congressional concern for the environment. This whole complex of State and federal environmental laws and regulations had a major adverse impact on mosquito prevention activities.

The requirements for permits from both the U.S. Army Corps of Engineers and the S.F. Bay Conservation and Development Commission (BCDC) made it virtually impossible to conduct mosquito prevention projects. Under this procedure a MAD had to obtain separate permits for BCDC and the Corps for each source reduction project including the maintenance operation on ditches and tide gates that were installed many years ago. The average time for this permit process was fourteen months. In order to eliminate this obstacle and to expedite the permit requirement, the Bureau of Vector Control and Solid Waste Management, California Department of Health Services negotiated, in 1976, on behalf of the mosquito control agencies in the San Francisco Bay and the North Coast a general five-year permit with both the U.S. Army Corps of Engineers and BCDC to carry out mosquito prevention projects. These two general permits were renewed in 1982 for a new five-year period. Subsequently, the general permit provisions became Part B of the Cooperative Agreement between the State Department of Health Services and local mosquito control agencies.

Continued reliance upon insecticides developed into a very grim situation. Dr. W. Donald Murray, manager of the Delta VCD, said "the string on broad spectrum economic pesticides ran out in 1970." Richard Frolli, manager of the Kings MAD, in his talk at the 1971 annual CMCA conference remarked, "the resistance phenomenon has matured, the pasture mosquito and the encephalitis mosquito have triumphed over sprays in many parts of California. So we have to change our approach, and de-emphasize spraying and re-emphasize other avenues of mosquito control".

In his 1970 paper, Dr. R.F. Smith of UCB introduced the concept of integrated control principles to mosquito control practices. More commonly referred to as Integrated Pest Management (IPM), it is defined in simple terms as the selection and application of control methods to achieve optimum results. IPM became a popular term in the 1970's, but derived from a concept practiced by entomologists for many years (i.e., the selection of chemical, biological or cultural control methods based on a thorough knowledge of the pest's biology).

In 1972, a group of MADs joined together to form the San Joaquin Valley Information Committee. This group hired a public information consultant and a writer-cartoonist to prepare educational material emphasizing the need for public participation in solving the mosquito problem for the press and radio.

James W. Bristow, trustee member of the Southeast MAD and Chairman of the 1972 Ad-Hoc CMCA Committee of the Future, reported that prescription insecticides would have to be used in the immediate future: the use of a specific chemical to control a specific species of mosquito in a particular source. Beyond this, subsequent strategies would include sterilants, growth regulators, bacteria, protozoa, viruses and aquatic predators. The cost would be significant and this type of prescription control would only be used as a supportive control measure. The report concluded that the primary objective of future mosquito control programs would have to be source prevention.

In response to a request from the CMCA, the BVC&SWM established in 1971 a Mosquito Surveillance and Prevention Unit, composed of teams of engineers and vector biologists to directly assist local mosquito control programs in performing mosquito source reduction demonstrations in irrigated agricultural areas.

During the mid-1970's, there were appeals by long-time leaders of mosquito prevention (Robert Peters, George Whitten, Richard DeWitt, and W. Donald Murray) for a restoration of source reduction programs. Discussions were also held on the use of legal approaches to abate mosquito sources.

One of the trends that took place in the decade of the 1970's, particularly in the San Joaquin Valley, was the shift away from irrigated pastures and reduction in the use of excessive water for reclamation of marginal alkali lands. The impact of the energy crisis, inflation and major changes in feeding dairy cattle made the growing of pastures uneconomical and caused growers to change over to tree and row crops with a higher monetary return.

At the 1952 annual meeting of the CMCA in Fresno, a major portion of the program was devoted to mosquito prevention. Richard F. Peters, in his paper on "The Scope of California Mosquito Control", called for a redefinition and re-direction of mosquito control programs emphasizing dedication to mosquito prevention efforts. This, he stated, was necessary because of chlorinated insecticide resistance, rapid population growth, and greatly expanded irrigation and water resource development.

Lloyd E. Meyers, a federal engineer assigned to the State Health Department and later manager of the Merced County MAD, was part of a symposium on the need for cooperative relationships with federal agencies, state water resources agencies, and the California Irrigation Districts Association. He emphasized their need of mosquito prevention in their planning, construction and operational activities. Lloyd Meyers later became Western Regional Manager of Agriculture Research Service (ARS) USDA and continued to focus attention on the need for federal agencies to cooperate with mosquito control interests.

In the early 1950's, Jack Kimball, then manager of the Orange County VCD, pioneered the use of the permit system as a primary tool to incorporate mosquito prevention requirements in the disposal of industrial and liquid dairy wastes. A Waste Water Advisory Committee was organized to set policy and review waste-water discharge permits. Agricultural waste-water forums were held periodically with county agencies, flood control districts, agricultural commissioners, the U.C. Extension Office and dairy industry representatives to initiate cooperative efforts to solve mosquito problems. In 1954, the U.C. Extension bulletin "Mosquito Control on the Farm" was published.

One of the most comprehensive outlines of a source reduction program was presented by Dr. Ernest Tinkham, of the Coachella Valley MAD at the 22nd Annual Conference of the CMCA.

Harold Gray, former manager of the Alameda County MAD, continued to speak out during this period, emphasizing the need to eliminate mosquito sources instead of repetitive insecticide spraying. He asked the question: "Why produce them wholesale and try to kill them retail?"

In 1955, Richard C. Husbands of the Bureau of Vector Control and Sterling Davis of ARS, USDA, reported on a study of the relationship between irrigation practices and mosquito production. This study made a major contribution to the better understanding of this complex problem and stimulated agricultural and water resource agencies to include mosquito prevention recommendations in their reports and publications.

Howard Greenfield, manager of the North Salinas Valley MAD and 1955 Chairman of the Water Resources and Practices Committee of the CMCA, reported on a 1955 survey of source reduction activities. The summary indicated that on average, mosquito abatement districts devoted 25% of their control effort to mosquito prevention.

The North Salinas Valley MAD's source reduction program developed a somewhat different format than other districts. In 1952, the District took over the operation of a reclamation district that had ceased to function after the initial construction of a drainage ditch system in 1916. The North Salinas Valley MAD obtained the necessary equipment to rehabilitate and maintain the drainage channels, which had become a major mosquito source.

In the late 1950's, the CMCA Source Reduction Committee was very active in sponsoring water management seminars at the U.C. Davis campus and conducted field trips throughout the Central Valley to observe different types of source reduction equipment, return flow systems and field drainage projects.

The Maximum Control Period (1960-1970)

The decade of the 1960's was highlighted by the extensive use of low-cost organophosphorous insecticides. Aircraft application had reached a high level of efficiency. The 1967 CMCA Yearbook recorded a total 1,832,868 acres treated by aircraft in 1966. In 1977, the total treated by aircraft was 602,729 acres.

However, by the late 1960's, organophosphorous insecticide resistance had developed to a critical level in the Central Valley. The use of light oils for larvaciding and ultra low-volume adulticiding were initiated toward the end of the decade.

The CMCA Source Reduction Committee continued to sponsor training seminars, panel discussions at the annual conferences and started to develop recommendations for improving the construction and management of dairy drains.

Sterling Davis, Engineer with ARS, USDA, indicated in his paper at the 1966 Annual Conference of the CMCA that the studies conducted by Richard Husbands on the relationship of irrigation efficiency and mosquito production showed that major mosquito production occurred at the 25% irrigation efficiency level. However, if the efficiency level reached 66%, then mosquitoes would not be produced. He further indicated that these studies provided guidelines that agricultural interests were beginning to include in publications on irrigation practices.

Source reduction programs in the lower San Joaquin Valley had by the mid-1960's been responsible for the promotion and installation of a large number of return flow systems at the low end of irrigated fields to recycle excess water. Richard DeWitt, Source Reduction Specialist with the Kern MAD, used this system extensively throughout the District and made a major impact in reducing mosquito breeding areas.

A panel discussion on source reduction policies at the 1968 CMCA Annual Conference provided a comprehensive review of legal approaches, interagency cooperation, planning permits and source reduction equipment ownership. The Merced County MAD had established a practice of reviewing land use permits of any property that was to be land-leveled for irrigation. The District's Source Reduction Specialist routinely met with County Planning and Public Works representatives to review applications for land leveling permits and provided mosquito

A HISTORICAL REVIEW OF MOSQUITO PREVENTION IN CALIFORNIA - PART II (1946-1985)

Earl W. Mortenson[1]

INTRODUCTION

At the 1985 Annual Conference of the CMVCA, Part I of "A Historical Review of Mosquito Prevention" was presented. It covered the early years, beginning in 1904, and concluded at World War II. It was indicated that mosquito prevention consists of two main parts. The first is the initial actions taken to prevent environmental conditions from occurring that might be responsible for propagating mosquitoes. These actions would be classified as "primary prevention". The second part is the physical modification of an existing mosquito source to eliminate or reduce mosquito populations, commonly referred to as source reduction. This latter part would be "secondary prevention".

The Post War Period (1946 - 1960)

The post war period was characterized by an unprecedented growth in population, completion of major elements of the Central Valley Project, rapid expansion of irrigated agriculture, and the urbanization of California. The State's population increased from seven million in 1940 to sixteen million by 1960. In a four-year period (1946-1950) irrigated agriculture increased by one million acres. Irrigated pasture acreage alone, increased from 440,000 acres in 1946 to over one million acres by the 1970's. By 1969, California had 1,076 major dams and impoundments with a storage capacity of 20 million acre feet. In Orange County, a thousand subdivisions were under construction during 1954.

In response to this dynamic growth in both population and irrigated agriculture and due to the inherent serious threat of mosquito-borne diseases, the number of mosquito control agencies increased from twenty-five agencies covering 2,500 square miles in 1946 to fifty-three local agencies controlling 25,000 square miles in 1953. Richard F. Peters, former Chief of Bureau of Vector Control, California Department of Public Health, was the leader of this expansion. It was largely due to his untiring efforts, traveling extensively throughout the State during the late 1940's and early-1950's, meeting with local community groups, city councils, and boards of supervisors to promote organized area-wide mosquito control programs. This control effort led to the formation of new mosquito abatement districts and major annexations of uncontrolled areas adjacent to existing districts.

The key element in advancing the cause of mosquito prevention in the post war period was enactment of legislation in 1946 that provided a $400,000 State subvention fund to local mosquito control agencies. In April of 1949, the California State Board of Health adopted Standards and Recommendations for Local Mosquito Control Agencies as requirements for State aid, and the standards were codified under the California Administrative Code, Title 17, Public Health. In Article 4, under Program Standards, one of the basic principles was "a primary program shall be continuously carried out based on the incorporation of measures aimed at progressive reduction of known mosquito sources". The Bureau of Vector Control, organized in July 1947, was given the responsibility of administering the mosquito control subvention fund.

A special 1950 California Mosquito Control Association (CMCA) committee on State subvention for mosquito control recommended that 50% of the fund be allocated to the implementation of source correction and prevention control measures.

The assignment of USPHS engineers William Warner, William Buchanan, and Mr. Wysong resulted in establishment of an engineering section within the Bureau of Vector Control to investigate mosquito problems and recommended preventive measures associated with construction, operation and maintenance of various elements of the Central Valley Water Project. Also, there was the responsibility of developing and negotiating contracts with the U.S. Bureau of Reclamation and the U.S. Army Corps of Engineers to reimburse MAD's in carrying out inspections and control activities in project areas. This engineering section was instrumental in developing statewide training programs for MADs on the fundamentals of land surveying and drainage ditch construction.

The mosquito control liaison activities with federal and State Water Resources agencies were continued by Marvin C. Kramer upon his joining the staff of the Bureau of Vector Control in 1955 as a Water Projects Consultant.

The CMCA's Water Resources and Irrigation Practices Committee, precursor to the Source Reduction Committee, had a major influence upon the incorporation of mosquito prevention concepts in local programs. In 1950, four of the six "Regional Forums" conducted in the San Joaquin Valley were devoted to the subject of mosquito source elimination. A meeting of MADs, held at the Hotel Johnson in Visalia in 1949, introduced the concept of section surveys to document and evaluate mosquito sources. Richard F. Peters, W. Donald Murray, and Jack Fowler developed and were early advocates of the section survey mapping system.

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RESEARCH NEEDS ON TOXIC WASTEWATER RECYCLING

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ABSTRACT

At the request of the CMVCA, the Mosquito Control Research Laboratory has initiated a service project to assist mosquito abatement districts in determining a means of recycling waste waters generated from mosquito abatement equipment. First it is essential to positively identify the type and amount of each pesticide that is present. Once this is done, using analytical chemical procedures, pesticide levels into and out of a prototype system and the quality and quantities of pesticides entering the system as well as those exiting into spray diluent will be studied. Hopefully such a system will allow for the elimination of wastewater holding pits or the discharge into sewage lines.

STUDIES ON ALTERNATIVE METHODS FOR THE COLLECTION AND DISPOSAL OF WASTEWATER

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BACKGROUND

Through the Alternative Technology Section of the Department of Health Services there are a variety of program supporting studies of alternative technology. The most prominent of these is the Hazardous Waste Technology, Research, Development and Demonstration Program which was created by AB 685 of 1985 (Farr). Under this program the Department has approximately $1,000,000 to fund projects including feasibility studies, design and permitting, construction and operation, and performance evaluation. The Department is also administering a $1.3 million cooperative agreement with EPA. Under this agreement, $300,000 in contract monies is available. Finally the Alternative Technology Section has a baseline contracts budget of $250,000 for studies related to improved management of waste. Under this latter program element the Department has conducted a study of solvent waste management alternatives, conducted a symposium on solvent waste and is contracting for a series of industry specific waste audits. The waste audits are to result in self audit checklists which businessmen can use to evaluate their waste management practices and options.

CURRENT RESEARCH

Through the various programs described above, the Department has sponsored five projects related to pesticide rinse waters. These include the following:

1. <u>Composting as a Hazardous Waste Treatment Process</u>.-This grant funded project entails a detailed literature search on the biological processes of composting as they relate to the degradation of hazardous materials. The project proponent is an independent consultant, Samuel Hart.

2. <u>Composting for Treatment of Toxic Wastes</u>.-This project is to be funded through the EPA research monies. It involves an actual field demonstration of composting as a waste management technique. The project is to be conducted at the Chemical Waste Management facility at Kettleman Hills. Rinse waters will be used to maintain moisture levels in the compost. From degradation curves generated in this pilot study, conclusions on the economic and technical feasibility of composting as an alternate waste disposal method will be made. California Agricultural Research, Inc. is the project proponent.

3. <u>Reduction of Pesticide Wastes Through a Value Engineering Analysis of Application Equipment</u>.-This project involves an analysis of pesticide application equipment in terms of waste generation and methods which can be implemented to reduce the amount of waste resulting from the equipment cleaning. The project proponent is Norman Akesson.

4. <u>UV/O3 Treatment of Pesticide Wastewater</u>.-This grant funded project will demonstrate the effectiveness of the Ultrox UV/O3 process for the on-site destruction of pesticide wastewaters. This is a transportable treatment system. The technology has already been demonstrated to have applicability in the cleanup of groundwater. The project proponent is Ultrox International.

5. <u>UV/Hydrogen Peroxide Treatment for Destruction of Pesticide</u>.-This project is similar to the Ultrox project. The major difference is the method used to generate peroxide. The Ultrox system uses an ozone generator while this project will simply add peroxide. This study proposes to evaluate the system for its ability to react with a broad range of pesticides, study variables related to the treatment process, and determine if the system is sufficient in itself for degradation or if it should be incorporated into a further treatment system.

In addition to these state sponsored projects, there are a couple of interesting private projects underway. These include the following:

1. <u>Canonie Engineers</u>.-This project is using above ground tanks with carbon adsorption. There are currently two demonstrations in progress; one involves a ground application rig in Ventura County and the other an aerial applicator in King City. These are both full scale demonstrations and have been operating for over one year. The results have been excellent with essentially 100 percent removal of pesticides.

2. <u>U.C. Davis</u>.-U.C. Davis is proposing a biological treatment at their Kearney Field Station. They have proposed a series of above ground tanks which would be packed with soil then washwaters would be introduced by gravity flow. Biological degradation would occur as a result of soil bacteria.

CONCLUSION

There is a tremendous need to develop cost effective alternatives for the management of pesticide washwaters. We have begun the research but it is no where near enough.

preventing problems in the future, others will be a burden and their reasonableness questioned again and again. Keep aware of these processes and your neighbor's problems because it could strike you without notice.

THE FUTURE

The future is also going to depend on an information sharing process. For example, you better be prepared to be informed about Proposition 65 and its potential impact. The first thing the responsible agencies are doing with "Prop. 65" is making lists. Lists of people handling or potentially releasing hazardous materials into the environment. I can guarantee you that vector control agencies will be on the lists! Also, be prepared to deal with the eventuality that those lists will go to the public because that's what "65" says to do.

A question then arises as to what this all will mean? To understand that, you have to look at the recent history of the laws we have had passed on toxics, especially like the ones in the above "present" discussion. The laws have been craftily drafted to be very specific. Previous laws were goal-oriented with specifics left to rule-makers and regulators. The new laws, while they are very specific, are not perfect and sometimes subject to interpretation. The recent resulting impact is the rules or the interpretations have tended to be expansive and give the rule-making agency more authority than the original legislation. This had happened with the Pits Act and most likely will happen with Prop. 65, so look out!

What can be done about this? For one thing, I believe that vector control agencies are among the premier environmental managers in our State. Therefore, you must collectively point out to everyone that they need to compare the risks to the environment of you losing say, all chemical tools, so that informed people know the difference between their potential exposure to vector induced diseases against the risk of an incremental increase in cancer from an economic poison. You must improve your communication on these issues or the right questions will not be asked. The future can and will be managed if we get over the current toxics "hump". We have to dig in and sort out the real problems, use the right tools, and get on with other pressing problems.

CONCLUSIONS

I'm convinced that our only real big water quality or toxics environmental problems are coming from our past and that those events have molded a mind-set that our environment cannot have anything toxic in it at all. That mind-set has moved the toxics issue beyond resource problem-solving into a societal crusade. You need to keep a sense of balanced environmental perspective in your business and communicate that sense to the rest of the environmental community to bring reasonableness and true problem solving back into practice.

TOXICS AND WATER QUALITY CONCERNS AND THEIR IMPACTS ON LOCAL AGENCIES

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INTRODUCTION

We have three major problems with toxics facing us today. They are the past, the present, and the future. I will discuss all of these from a water quality perspective, but more importantly from an institutional or societal viewpoint. Each problem area has a strong influence on how all vector control agencies will be able to meet the mission of their organization. I will also offer some suggestions on how to ensure the organizations' survivability in what I see as an ongoing "toxics crisis".

THE PAST

Past wastewater disposal activities are the real culprits that ushered us into the current toxic control frenzy. Long held concepts about economic poisons being rendered innocuous prior to reaching our groundwater resources went out the window with the discovery of supposedly "immobile" organic compounds such as DBCP in area-wide groundwater and numerous individual sites scattered throughout the State of California that have a myriad of materials in groundwater. These "problems" led to the development of a new generation of laws and rules that we are just now beginning to feel the brunt of. Several vector control agencies are amongst the regulated community feeling the impacts of those new forces. It means two things; 1) a protracted evaluation process at the site of the alleged disposal problems, which in itself is a very expensive activity, and 2) a potentially very expensive cleanup, which may exceed the resources of the involved agency.

How should vector control agencies deal with past problems? I have two suggestions. One is that the laws currently in place are very comprehensive and expensive to comply with, they have to be exercised with an element of reasonableness. That is a very important tool and right that you can exercise. Does the effort you might be asked to expend pass the test of reasonableness? Are the benefits to be obtained in returning a particular environment to a certain level of cleanliness measurably greater than the cost and impacts to the responsible agency? In the case of vector control agencies, certainly the total loss of the agency and the resulting impact to the environment is far worse than say, protracting a clean-up program longer than what an inconsiderate law or agency asks for. Become familiar with the use of administrative remedies and ask for reasonable responses wherever you go. The second suggestion I have is for more of the collective approach. Collectively, I understand you are working on solutions to some ongoing waste disposal problems, and self-inspection/evaluation programs, I suggest collectively you should also potentially pool resources for assisting fellow agencies with real cleanup problems.

THE PRESENT

In the present, we must discuss the current rules and regulations themselves and how to comply.

Again, however, the present is linked to the past and the future. For example, you're linked to your past if you're under the "Toxics Pit Act", and we are all linked to the future under "Proposition 65".

The main issue with the present rules and laws is information sharing. You need to learn what the rules and laws are, how they apply, know their inconsistencies, complain about them where it is appropriate and get the job done when that is appropriate. I can't go into details here, but some of the more significant programs include the following:

1. The Toxics Pit Act.

2. The Waters Amendment (AB 4325).

3. Subchapter 15 (water board land disposal regulations).

4. Proposition 65.

5. The Health and Safety Laws and regulations (toxics, tanks, air, etc.).

All these things have an influence on past, present and future disposal of wastewaters. As of this moment for you, the most significant could be two (2) above, the Waters amendment to the Toxics Pit Act. It is a special relief provision to the very strict and expensive reporting requirements of the "Pits" Act. It was geared for agencies like the vector control districts. The application deadline is February 1, 1987 so if you think you have a "pits" problem, get a "Waters" amendment request in to your regional water board right away.

The rest of the laws and rules need to be tested and re-tested. Some will work as tools for and shows no problem, that the full report will not be required. The mini-assessment is estimated to cost up to $25,000 and requires a certified geologist to prepare it.

It is hard to find a politician today that wants to do anything except increase the restrictions on "toxics". Reasonable alternatives don't seem to count.

This brings us to the present time where we continue to wait for an answer to our petition on a variance to the classification of our dirt as "hazardous". This petition was filed 5 months ago. We have just paid the $3,000 fee required under the amended Katz Act, with the understanding that if we can show that we did not come under Katz, our money would be refunded. If our petition is granted, and our dirt declared not to be "hazardous waste" we would not have had a "hazardous waste pit", so a refund would be due and we could move our dirt to a less costly disposal site. The delay in the answer to our petition is also causing concerns over EPA regulations that are constantly being amended. This may change the ways in which our dirt may be legally disposed. Things seem to be getting worse and not better.

The cost to take our pond dirt to a class 1 landfill is about $25,000. Far more important, we are informed that we would have perpetual liability for it at such a disposal site, in case the rules on disposal get changed again.

All of this hassle has resulted because our pond would not percolate so it could be dried up and closed by the January 1, 1985 deadline.

A SEARCH FOR REASONABLE ANSWERS TO OLD WASTE WATER PIT PROBLEMS

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HISTORY

Twenty years ago, the District's then new facility had a drain which connected the steam cleaning area, the pesticide building drain, and the area where our equipment was filled with water. This drain discharged into a ditch at the side of the property, and during the rainy season, the ditch water appeared to drain into the Feather River. In order to correct this potential problem, we dug an open pit on the District property to collect this waste water, so it could be treated and controlled.

Over the years, we ran steam cleaner wastes and oxidized organophosphate pesticide rinse water into the pond. Before "triple rinse" of barrels was proposed, we killed the residues remaining in our pesticide drums in order to protect the workers at recycling facilities. Even after triple rinse was made a requirement, we continued to oxidize any residues remaining in the rinsed barrels and put that liquid in the pit.

Our pit was on the same clay soil which was used to make the impervious core of the Oroville Dam, so we felt sure no significant residues would get into the ground water. Water loss was only what we could attribute to evaporation from the pond.

This was how it was until late 1984, when it became apparent that something should be done about the pit. Winter rains delayed the dry down of it until the summer of 1985. If our pit had been percolating water into the soil, with its possible risk of water contamination, we could have filled it prior to the magic date of January 1, 1985, and we would not have been subject to all of the problems we have faced in the past year and a half.

In August of 1985, we filled the now almost dry pit with dirt, after an analysis of the remaining water for organophosphate and carbamate pesticides showed only small residues. These residues seemed insignificant because they compared favorably to residues we could create when we sprayed for mosquito control. We had no reason to think there would be any DDT present in the pond, so we did not analyze for it.

PRESENT PROBLEM

Jumping ahead to February of 1986, we were told that a complaint had been filed by a "concerned citizen", alleging that our pond had not been closed properly. Samples were taken over the next year, with subsequent samples taken, depending on what each earlier analysis showed. The chemists usually took at least a month for a sample to be analyzed, so we spent a lot of time waiting for laboratory results. There were also large variations in the residue levels in the same jar of dirt analyzed at 2 different laboratories. For some analogues of DDT, the results were over 10 times different from one chemist to another. The permitted analytical practice allows the chemist to pick out the rocks before a sample is weighed, and it also allows up to 40% variation on replicates of the same sample. Thus it turns out that 1 ppm of residues could actually be reported as 2 ppm from a sample with some rocky content. This means that when an action level is set at 1 ppm, there can be a penalty assessed, but the actual residues may actually only be one-half that amount in the whole sample.

Interpreting soil sample data requires an understanding of how those numbers were obtained, and what they mean.

At the present time, we have removed that dirt from the pond site, and have had it repeatedly analyzed. The dirt is stored on a black top area, under water proof covering. We have even put a roof over the hole which used to be the waste pit, for good measure. Any residues remaining in the bottom of the old pit are below the "hazardous" level, as defined by the Department of Health Services regulations, so there is no remaining real risk to underground water quality. However, the law does not always allow logical solutions to real world problems.

Space does not permit a detailed explanation of what options we may have in disposing of our dirt, except to say that if we had been in Nevada, under the Federal EPA standards, we could have taken the pond dirt to a garbage dump, and used it as cover for domestic garbage. What little residue it contains might have even helped control the flies which traditionally breed in garbage disposal areas.

What is pertinent to today's discussion is the way a legislative solution to protect water quality was apparently manipulated. The Hazardous Pits Closure Act of 1984, also known as the Katz Act, said that any hazardous waste pit closed after January 1, 1985 must have a hydrogeological assessment report, which is estimated to cost about $50,000. The Association and the Agricultural applicators asked for an amendment to this law, which would allow us to simply remove any dirt which had "hazardous waste", as defined by the DHS regulations, and haul it to suitable disposal sites. We argued that if there were no hazardous waste left under the pond site, there could be no risk of water contamination from it. The State Water Board lawyers and others would not accept our method and logic, and instead they insisted on the amendments to the Katz Act to provide for higher fees and penalties and other requirements. The final amended Katz Act therefore provides that if a mini-assessment is done

In San Mateo County, a resumption of surveillance of tire casings at Thompson Aircraft indicated to the authors that the potential for the entry of mosquitoes into California was far greater than had been supposed. Not only did the recapper accept casings from major domestic air carriers, but it also accepted casings from the military. Origin of the casings is determined by bills of lading attached to each casing. Overseas bases in addition to bases within the United States ship tires to Thompson Aircraft Tire Company for recapping. One overseas shipment of casings was identified to have originated in Atsugi, Japan, shipped to the United States via McCord Air Force Base, Washington to Alameda Naval Air Station, California, then to the recapper, Thompson Aircraft in South San Francisco.

Another concern is the interstate movement of tire casings. The authors inspected a shipment of 132 military aircraft tire casings that arrived at Thompson Aircraft Tire Company from Miami, Florida on January 13, 1987. Water was found in 129 tires and mosquito larvae were found in 10% of the water-filled tires. Lucia Hui[3] identified a dead 2nd instar larvae to be *Ae. aegypti* and live specimens were determined to be 3rd and 4th instar *Culex quinquefasciatus* Say. Follow-up inspection was impossible as the tires had been dispersed and restacked leaving only the end tires available for reinspection.

[3]Vector Surveillance and Control Branch, California Department of Health Services, Berkeley, California.

The importation of mosquito eggs or larvae from overseas military bases, or military bases within the United States exists as a real and ongoing threat. It is the opinion of the authors that immediate attention should be directed toward the development of a policy for the inspection, shipment and storage of tire casings, so as to avoid possible introduction or distribution of exotic mosquito species within or into the United States.

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AEDES ALBOPICTUS - THE SEARCH GOES ON FOR NEW INTRODUCTIONS

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In May 1979, a single 4th instar *Aedes aegypti* L. larva was collected from a water impoundment adjacent to the San Francisco International Airport, San Mateo County, California, reported by Jewell and Grodhaus (1984). Because of that find, an intensive sampling and trapping program was initiated by both the Vector Surveillance and Control Branch of the California Department of Health Services and the San Mateo County Mosquito Abatement District.

Of the various trapping sites selected in and around the Airport property, Thompson Aircraft Tire Corporation[1], a major recapper of aircraft tires, was considered to be a prime location for sampling. Dip counts and black jar ovitraps (Fay and Eliason 1966) were employed. Weekly sampling continued at that site through 1979. Thereafter, for three years, periodic inspections revealed only *Culex pipiens* L. larvae.

During May 1986, after reported introductions of *Ae. albopictus* (Skuse) in Texas (Sprenger and Wuithiranyagool 1986) and Louisiana (Chapman and Johnson 1986), this District reinstituted its trapping program in the vicinity of the San Francisco International Airport. Because *Ae. albopictus* has an affinity for tires as an egg deposition site, we considered that the inspection of tire casings should be resumed at the Thompson Aircraft Corporation. On October 8, 1986, two of nine sea cargo containers that arrived in Seattle, Washington, from Japan with tire casings, were found to have *Ae. albopictus* eggs and larvae (Walsh 1986). Although no *Ae. albopictus* have been found in California, the collections in Seattle indicated the importance for surveillance of tire casings imported into the United States from the Far East.

In mid-October, Dr. Bruce Francy[2] requested help from Bay Area mosquito abatement districts to assist the Communicable Disease Center in the inspection of a shipment of truck tire casings imported by Pacific Coast Retreaders, Oakland, California from Kobe, Japan. Kobe, a highly industrialized city and Japan's principal seaport, is situated on the island of Honshu at a latitude similar to that of Santa Barbara, California.

On October 29, 1986, 16 inspectors from mosquito abatement districts of San Mateo, Alameda, and Marin-Sonoma Counties, in addition to representatives from California Vector Surveillance and Control, U.S. Public Health Service and U.S. Custom Service, responded to Dr. Bruce Francy's request for assistance to inspect truck tire casings to be off-loaded at the Port of Oakland.

Numerous sampling techniques were utilized by the inspectors in an effort to recover any mosquito stages that may have been present. Before the seals were broken on the doors of the cargo containers, a D-Vac, gasoline powered portable vacuum was utilized to collect adults, if present, when the container doors were opened.

The truck tire casings, mostly 10.00 x 20, were removed with a fork lift and stacked adjacent to each cargo container to await inspection.

Tires that contained water accounted for 8% of the shipment. When water-filled tires were encountered, the entire volume of water was withdrawn with a "turkey baster" and the contents placed in individual plastic ziploc bags. Multiple bags were combined into larger bags in order to protect loss of samples.

The inner walls of casings without water were thoroughly cleaned with a stiff bristle brush. The contents of each casing were removed and pooled into lots of 25 tires. The contents were placed into plastic ziploc bags. A dead beetle removed from one casing was submitted to the California Department of Agriculture for identification. The specimen, determined to be Scarabaeidae, *Rhomborrhina spp.*, was considered to be of no economic importance.

After all casings were removed, the D-Vac portable vacuum was used again to vacuum walls, ceilings and corners of each cargo container. The collection was negative for adult mosquitoes.

Dry samples were shipped to the Vector-Borne Disease Laboratory, Ft. Collins, Colorado to be processed. All dry samples were negative for mosquitoes.

Water samples were taken to the Vector Surveillance and Control Branch, California State Health Department in Berkeley. Individual samples were placed into separate containers and maintained in the laboratory at 70°F for two weeks. All water samples were negative for mosquitoes.

While Pacific Coast Retreaders, Oakland, California is a recapper of casings, other importers wholesale casings from the Far East to tire recappers throughout the United States. A strong potential for the further spread of *Ae. albopictus* as well as other exotic pests exists unless specific control measures are adopted to curtail the importation of truck tire casings.

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[1] 160 Beacon Street, South San Francisco, California.

[2] Division of Vector-Borne Viral Diseases, Communicable Disease Center, Ft. Collins, Colorado.

tive cover is available to adult mosquitoes in the form of vegetation and a variety of man-made and natural structures. Studies are needed on movements of mosquitoes after exposure to toxicants.

Studies done at the CDC Laboratory at Fort Collins, Colorado, have shown that female anophelines differ in their susceptibility to insecticides depending upon their physiological status. Specifically, females that are actively digesting blood meals tend to be less susceptible to insecticides than at other times. This may be because of the induction of relatively large amounts of detoxifying enzymes such as the mixed function oxidases at the time blood is being digested. More studies along these lines are needed.

We know little enough about the specific status of susceptibility to insecticides in encephalitis vectors such as *Culex tarsalis* and *Cx. quinquefasciatus,* and nearly nothing on *Anopheles* susceptibility.

Formulation technology has only partially solved the problem of penetration of heavy vegetation for control of mosquito larvae. Granular formulations require specialized application equipment, and are not available for all pesticides. Also, options with granular formulations are much more limited in terms of application techniques. In some situations, vegetation may intercept granular formulations as much or more than liquid formulations. At best, this area needs further research to establish appropriate dosages for anophelines in various aquatic habitats containing vegetation. At worst, entire new approaches need to be developed.

Routine testing of adult and larval populations of mosquitoes for susceptibility to insecticides is not a popular area for research, and may not even fall under accepted definitions of research. Nevertheless, we lack susceptibility profiles for populations of anophelines in California. Such information is vital to strategic planning for malaria control, and must be developed within the near future.

VECTOR ECOLOGY OF MALARIA

Vector incrimination.-Vector incrimination in malaria requires, among other things, evidence that a given population of a species of *Anopheles* harbors the parasite causing the disease outbreak. In the past, this has meant dissection of individual female mosquitoes. However, conventional technology for detection of malarial parasites in anopheline mosquitoes is so cumbersome and tedious as to render it unfeasible except for specialized research studies. Even in epidemic malaria situations, the number of infected anophelines usually does not exceed 5-10%, and in endemic situations the percentages are far lower. Another drawback to existing methods is that the species or strain of parasite detected can be identified only through extremely laborious methods. New methods using immunological techniques have been developed, and reagents using the ELISA test are available at the Walter Reed Army Institute of Research for several strains of malarial parasite. Adopting, and if necessary modifying, this technology for California conditions should be given a high priority. It is difficult to imagine conducting epidemiological studies in California using conventional techniques of dissection for parasite detection.

Malaria forecasting.-Given the present status of knowledge of malaria and malaria vectors in California, it is nearly impossible to forecast what the future holds for malaria in the state. It should be possible, however, to identify ecological situations in California which are conducive to malaria transmission. Studies using remote sensing of vegetation, such as those being conducted by Dr. Robert Washino at UC Davis would seem to hold considerable promise for this. There are still many biological questions which remain, however, such as correlations between adult mosquito density and malarial transmission which must be answered before we can predict future threats from introduced malaria.

Finally, it should be remembered that malaria is a global problem. The pattern of introduced malaria in California is closely related to the world-wide malaria situation, and especially to the epidemiology of malaria in the New World tropics. It goes without saying, therefore, that the future of malaria in California cannot be predicted without detailed knowledge of malaria conditions elsewhere.

SUMMARY

The sudden appearance of 30 or more cases of malaria in California in 1986 found us ill-prepared in many areas of research to predict future trends in malaria in California and to combat further occurrences. Research in a number of areas of vector biology and control must be conducted, including basic systematic studies, as well as applied control studies.

RESEARCH IMPERATIVES FOR MALARIA IN CALIFORNIA

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INTRODUCTION

Since the virtual disappearance of malaria in the continental United States as an endemic disease after World War II, research priorities for mosquitoes shifted in this country from anopheline mosquitoes to culicine vectors of human disease pathogens. Research has continued on the biology and control of anophelines on a limited basis, but not on their role as vectors of malaria, and certainly not on the epidemiology of the disease in the U.S. Patterns of research in California have followed this trend. With the outbreak of 30 or so cases of introduced malaria in California in 1986, the status of research on *Anopheles* and its role as a malaria vector was reevaluated. It became obvious that we faced numerous unanswered questions in this area. As a result of a meeting held at the Headquarters of CMVCA on July 10, 1986, a number of research priorities were identified. I will briefly review the priorities and provide a few remarks concerning the rationale behind the importance placed on them.

BIOLOGY OF ANOPHELINE MOSQUITOES

Geographic and ecological distribution.-At present, five species of *Anopheles* are recognized as occurring in California: *An. freeborni, An. punctipennis, An. occidentalis, An. franciscanus,* and an undescribed species commonly referred to as *An. hermsi*. General, but not specific, information on the geographic ranges of these species is available. In terms of biological attributes of the five species, and the factors which determine their distribution, we know much less. From a practical standpoint, it means that we cannot, in every instance, predict which of the species might pose a threat in a given location. Much of the problem stems from the present poor status of biosystematic information available for North American anophelines. Much of the available biological and ecological information was gathered before modern tools of biosystematic investigation were available. When these tools have been used, they have shown that traditional species such as *An. quadrimaculatus* are actually complexes of species. For California, more research is needed such as that done at UCLA which validated the occurrence of an undescribed species of *Anopheles* in southern California.

Daily activity patterns of adults.-Nearly all mosquitoes show daily patterns of activity which are fairly consistent within a given species. These patterns include bloodfeeding, mating, ovipositing, sugar feeding, and others. Specific information on activity patterns is very important in designing control strategies using chemical adulticides. Atmospheric conditions must be taken into consideration in planning adulticiding, and these conditions vary according to the time of day. Daily activity patterns of adults must also be considered, however, to insure optimum contact between insecticide and mosquito. Unfortunately, sufficient information is seldom available to permit this consideration.

Flight ranges.-Information on flight ranges is needed to design control strategies as well as for conducting epidemiological studies. Yet there have been few reliable flight range studies conducted over the years for any species of mosquito. Most problems in conducting such studies on mosquitoes have arisen from the lack of adequate experimental methods. Recapture rates have been low, requiring the marking and release of enormous numbers of mosquitoes so as to obtain a statistically valid recapture sample. There is much that can be learned from such studies, however, including estimates of absolute population sizes of adults. The techniques are also applicable to studies of immature stages, but have been seldom used.

Improved sampling methods for larvae and adults.-In spite of years of effort to design valid sampling procedures for adult and larval mosquitoes, present methods still suffer from inadequacies. This is also true for anophelines. Adult sampling procedures are biased, sometimes strongly, for one species of mosquito over another, and for components of populations in certain physiological states. We lack satisfactory methods of sampling the blood-fed component of populations. For larvae, we have never come to grips with the problem of their clumped distribution in aquatic habitats, nor of the variable amount of dispersion among life cycle stages. For both adults and larvae we need better sampling methods and better methods of analysis of sampling data.

Longevity studies of adults.-We lack satisfactory methods of determining parity, yet parity determinations are extremely important in many types of studies. Both epidemiological studies and control studies require data on adult age. For anophelines, we need comparative data for populations within species as well as data between species even using the present available methods.

CONTROL OF ANOPHELINE MOSQUITOES

Chemical control of adults.-The behavior of adult mosquitoes in nature in response to adulticiding is poorly understood. Many studies on the efficacy of adulticides have been based on the observation of caged females. In nature, protec-

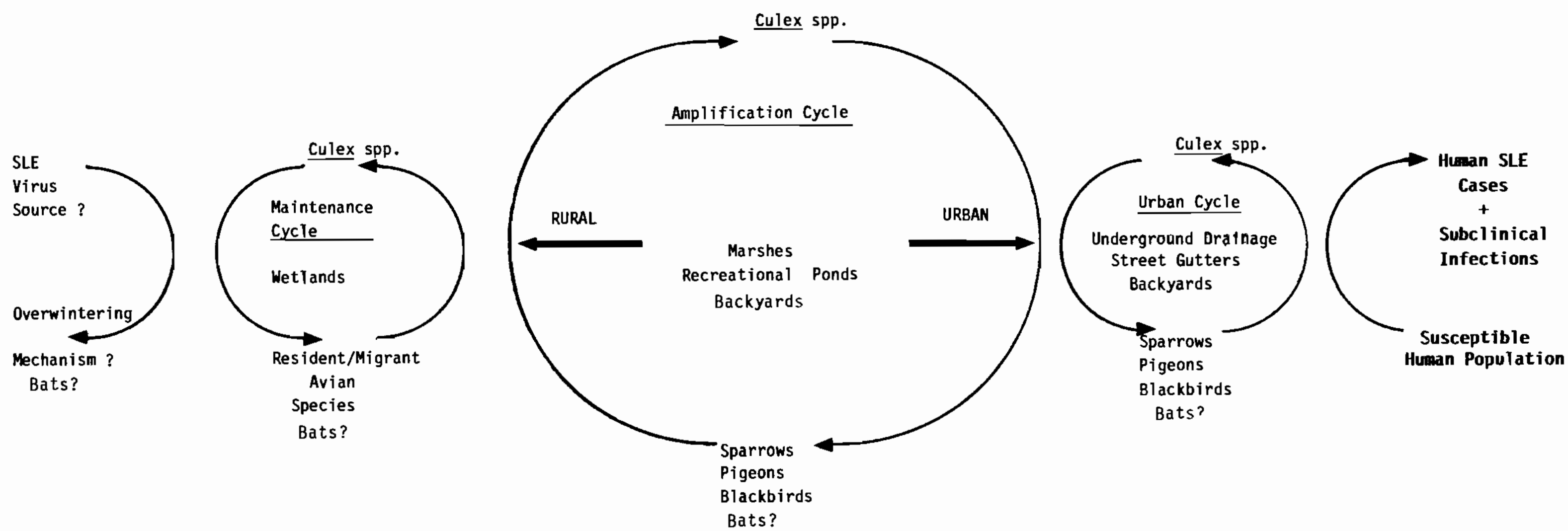

Figure 4.-Hypothetical flow system illustrating mosquito/host/virus interrelationships that depict a possible pathway for SLE infections in humans.

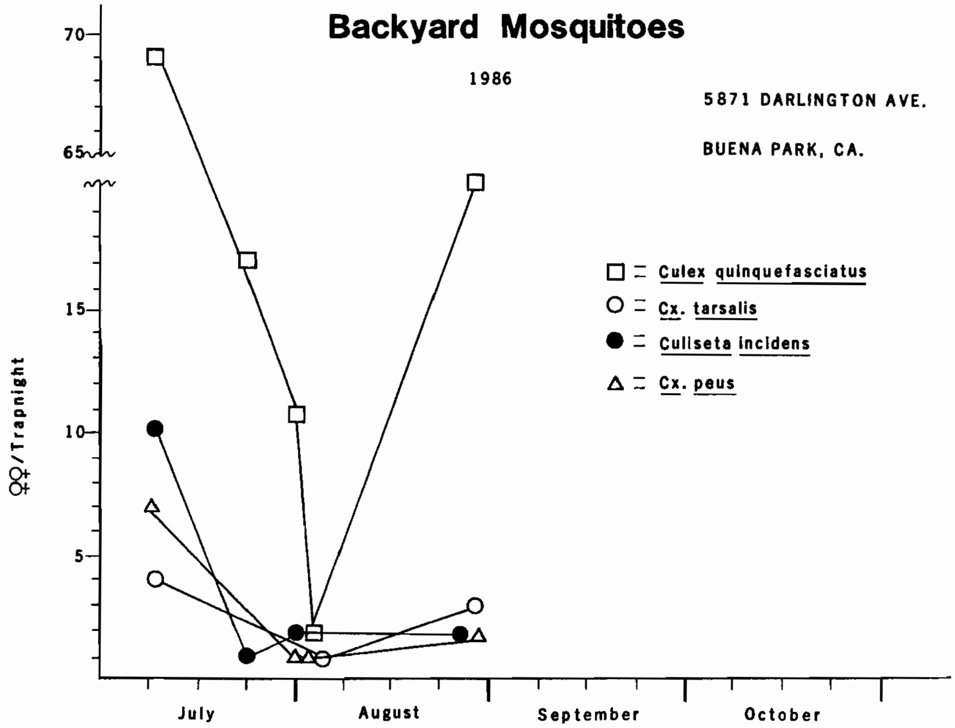

Figure 2.-Female mosquitoes (per trap night) collected (1986) in the back yard of a residence in Buena Park; CDC CO_2 trap.

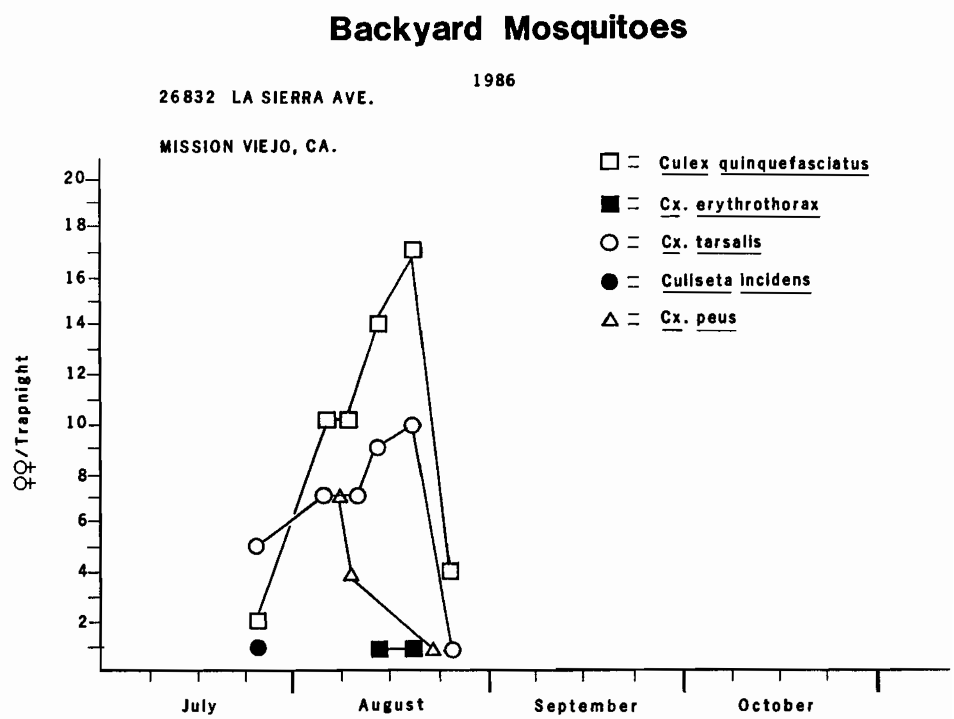

Figure 3.-Female mosquitoes (per trap night) collected (1986) in the back yard of a residence in Mission Viejo; CDC CO_2 trap.

Control District personnel for their assistance in trapping mosquitoes: James C. Barnes, B. Fred Beams, Stephen G. Bennett, Viki L. Blaylock, James M. Campbell, Gilbert L. Challet, Ronald H. Elliott, Kathryn S. Fine, Rudy Geck, Justine Keller, William Lane, Robert Lucas, Douglas A. Mathews, Robert Merryman, Jon E. Miller, Steve Nippert, John O'Loughlin, John E. Parsons, John U. Pett, Larry H. Shaw, and Ronald Ung.

Table 2.-*Culex tarsalis* (females) collected (1985) from back yards of 1984 SLE victims; CDC CO_2 traps.

	Culex tarsalis (Females/Trap Night)			
	Westminster	Tustin	Orange	Los Alamitos
6/11/85	-	-	0	-
6/28/85	0	-	-	0
7/28/85	-	53	-	-
8/14/85	-	14	-	-
8/17/85	0	-	-	0
8/20/85	-	-	0	-
8/22/85	-	0	-	-
8/28/85	-	11	-	-

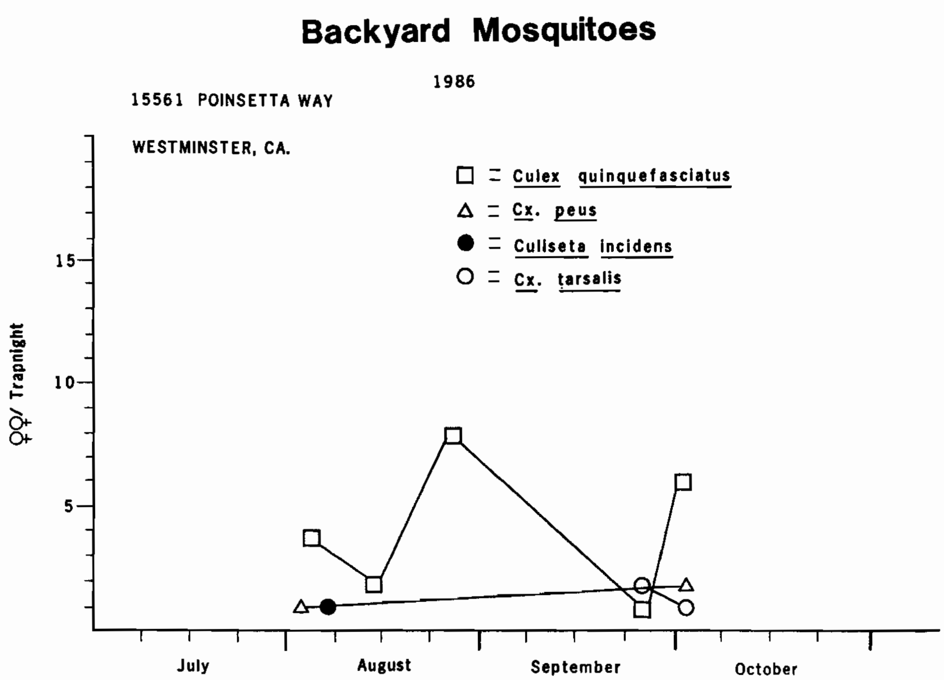

Figure 1.-Female mosquitoes (per trap night) collected (1986) in the back yard of a residence in Westminster; CDC CO_2 trap.

PRELIMINARY STUDIES OF URBAN/SUBURBAN MOSQUITO SPECIES IN RELATION TO SLE VIRUS TRANSMISSION IN ORANGE COUNTY

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In the summer of 1984, five human cases of St. Louis encephalitis were diagnosed in Orange County. Four of these infections were determined by Orange County Health Department epidemiologists to have been acquired at or in the nearby vicinity of each victims' home site. The victims lived in the cities of Anaheim, Los Alamitos, Orange, Tustin, or Westminster.

The following summer (1985) modified CDC CO_2 traps were placed in the back yards, side yards, or front yards of the 1984 SLE victims' homes. *Culex quinquefasciatus* was the most frequently trapped mosquito species (Table 1). Significant numbers of *Culex tarsalis* were also taken at one of the localities (Table 2). The living female mosquitoes trapped at this time were submitted for SLE virus analysis. All the pools tested negative.

During the late summer of 1986, modified CDC CO_2 traps were sent home with volunteer Orange County Vector Control District personnel. The traps were placed in the back yard of each volunteer's home in the early evening and retrieved the next morning. The results (Figures 1, 2, and 3) indicated that *Cx. quinquefasciatus* was the most numerous species collected and also the most frequent. *Culex tarsalis* and *Culex peus* were also trapped with regularity at some sites but were present at much lower levels. *Culiseta incidens* was occasionally taken in relatively large numbers and *Culex erythrothorax* was found only infrequently. Female mosquitoes trapped in the back yards were submitted for SLE/WEE virus testing and all tested negative.

In addition to the five 1984 SLE cases occurring in Orange County, 21 more were recorded from Los Angeles and Riverside Counties. Approximately one-half of the 26 total cases were associated with virus transmission in or near the home of the victims. In light of the fact that no coordinated studies of mosquitoes in the urban and suburban habitats have been conducted in southern California, it seems that urban/suburban sites, particularly back yard habitats, should be intensively and extensively investigated. Results from these endeavors may provide the key for the development of an effective mosquito control system directed toward peridomestic mosquitoes.

Studies of the urban and suburban mosquito habitats should not however preclude in-depth coordinated evaluations of other links in the hypothetical mosquito/virus flow system (Figure 4). Specific ecological parameters characteristic of vector mosquito species are of particular interest, especially in wetland habitats.

ACKNOWLEDGMENTS

We thank the following Orange County Vector

Table 1.-*Culex quinquefasciatus* (females) collected (1985) from back yards of 1984 SLE victims; CDC CO_2 traps.

	Culex quinquefasciatus (Females/Trap Night)			
	Westminster	Tustin	Orange	Los Alamitos
6/11/85	-	-	10	-
6/28/85	17	-	-	23
7/28/85	-	10	-	-
8/14/85	-	0	-	-
8/17/85	29	-	-	38
8/20/85	-	-	23	-
8/22/85	-	10	-	-
8/28/85	-	48	-	-

[1]Orange County Vector Control District, P.O. Box 87, Santa Ana, CA 92702. Present Address: Manager, Moorpark MAD, P.O. Drawer 62, Moorpark, CA 93020.

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the late 1930's the pasture mosquito was introduced into the San Joaquin Valley. Tied in with this introduction was a concept by much of agriculture that alkali lands could be developed and made fit for quality farming by planting them in irrigated pastures on which large quantities of water could be applied to wash away - or leach - the alkali. Care in preparation of these pastures was of little concern.

Much of the land put in pasturage was only roughly graded, and pockets of water remained after each irrigation throughout much of the fields but especially at the low ends. Little thought was given to drainage of excess water, except where there was adjacent undeveloped hog-wallow land, on which any drainage simply compounded the pasture mosquito problem. During the 1940's the Delta District became inundated with swarms of mosquitoes - pasture mosquitoes by day and house mosquitoes by night. Control efforts by the District were essentially useless. Certainly there was great dissatisfaction among the residents of Visalia. The problems of malaria and of the flood-water pest mosquito were of the past.

II. THE POST-WWII PROFESSIONAL ERA

Introduction

Several factors precipitated a tremendous expansion in mosquito control in California immediately after WWII. Probably the most important was the knowledge by the public that the American military forces had controlled mosquitoes and prevented much mosquito-borne illness in the areas of the world they had occupied. What could be accomplished for military forces should be available for all citizens. Another major factor in the expansion of mosquito control was the development and use of the "miracle" insecticide, DDT, by the military. At the close of the war this became available to civilians. Tied to these military items was the aforementioned introduction of the pasture mosquito into California shortly before the war. The numbers of this species in many populated areas of the San Joaquin Valley were almost unbelievable -- hundreds of hungry female mosquitoes swarming on and around people walking across city lawns and school yards in broad daylight. Without being fully aware of how the control should be accomplished, but generally believing that insecticides would be THE means of control, the public worked quickly to expand existing districts or to form new ones. The Delta District expanded from 29 square miles to 712 square miles, and now included the Valley floor of the northern part of Tulare County.

A new agency of the State Department of Public Health, called the Bureau of Vector Control, was created immediately after the war and served to help guide the mosquito control program in California, without at the the same time taking away the local autonomy of the new and expanded districts. One item, however, was mandated: "There must be technical guidance in all programs which participated in state subvention assistance." It was recognized by the state as well as by most local districts that effective mosquito control was complex and required professional, primarily entomological guidance. The primitive "clear - drain - spray" program of the past would never again be effective. Specifically, the Delta MAD hired myself, a professionally trained entomologist who had also served in the Navy malaria and epidemic disease control program during the war. It was believed that the professional, technical, and especially environmental approach to mosquito and related vector control could make possible a program that would be able to adapt successfully to the many changing problems. Various features of this professional era will be presented in the following sections. Programs were established and followed based on what seemed best for the Delta MAD, later to be known as the Delta Vector Control District (DVCD).

Insecticides and Their Application

1) Aerosol. Two developments during WWII played a major part in public mosquito control programs immediately after the war: DDT and aerosol applications of insecticides. The earliest aerosol dispensers were developed during the war. They were small, hand held "bombs", and contained pyrethrum under pressure. They were meant to be used inside buildings against night-flying mosquitoes. Later, light weight dispensers containing DDT under less pressure were used. The Navy also developed a large fog dispenser, not for insecticide dispersal but to hide a ship at sea from the enemy. One type of unit was known as the TIFA. When DDT was added to the oil fog dispensed by a TIFA, mosquitoes immersed in the fog were killed, and this action under favorable conditions might extend for a half mile or more.

Certain mechanically minded persons in California believed that a far cheaper device than the commercial TIFA was a fog dispenser operating off the exhaust manifold of a car or Jeep, and this "Plumber's Nightmare" was built on many Jeeps and even a few pickups used by MADs in California. When I arrived at the Delta MAD in June, 1947, the staff was installing these exhaust manifold units on a pickup truck and on every new Jeep the District had just purchased. The concept that adulticiding was one of the preferred methods of mosquito control was common among California MADs.

Several mechanical and operational problems developed from the use of these units. They created excessive back pressure on the engines, destroying valves and other engine parts which required excessive repairs. When a vehicle was down for repairs, it was not available for inspections or for larval control. The DDT aerosol under certain conditions did kill mosquitoes, but under other conditions it was not effective and resulted in a severe waste of manpower. Operators who worked much of the night on fogging operations were not able to perform their jobs well the following day.

The public, with no technical "savvy", sometimes believed they were "cleansed" after the fogging Jeep passed by, and made many claims of the programs great value, even when technical surveys indicated poor mosquito control. On the other hand, people who were up-wind from the fog felt they were not receiving their money's worth and began dictating how the District should run its program. One District Trustee tried to force the staff to take all its vehicles and, starting at one edge of the District, fog back and forth until all the District had been covered by fog. Many people phoned in justifiable mosquito complaints, but almost invariably requested that the District fog around their premises. When the District's Inspector-Operators bowed to the appeals of the public for personal service, they neglected the basic program that the District was attempting to establish - control at the source.

A small but important part of the citizenry claimed to be asthmatic or otherwise adversely affected by the fog and urged us to discontinue it. In view of all the problems created by this aerosol program, I decided that fogging off the exhaust manifold of the vehicles must stop so that these vehicles could be operated reliably to carry out the basic daytime program of the district. One TIFA unit was purchased in 1948 and it was used extensively for a few years, especially during the encephalitis year of 1952. Sometimes we kept the public placated while we focused our attention upon mosquito sources. In all our aerosol operations, we were highly concerned about what we might be doing to the environment. DDT was an insecticide and as such was capable of killing many other insects, including beneficial ones. About 1955, long before "Silent Spring", we ceased all use of aerosols.

2) <u>Mist Blowers</u>. A new method of insecticide dispersal, with a unit called a "mist blower" or "cold fogger", was developed by commercial and governmental agencies in the 1960's. Although we were encouraged by other districts and by governmental and commercial agencies to obtain these units, we never believed they were appropriate for the conditions existing in the Delta MAD.

3) <u>Airplane</u>. One other method of area-wide insecticide dispersal was an airplane. Insecticides were applied primarily on open areas such as irrigated pastures where there was no significant environmental conflict, since the pastures themselves were artificial crops. None of the insecticides we used were harmful to pasture animals at the dosages used. From 1947 through 1958 we contracted with private agencies to apply our insecticides by air. In 1959, we purchased a plane of our own and during subsequent years we replaced this plane several times. In 1973, we ceased aerial spraying and sold the plane. Contract aerial spraying at a very minor level has continued since then.

4) <u>Hand and power spraying</u>. Basic spraying by the Delta MAD has been with hand sprayers and Jeep or pickup-mounted power sprayers. Our goal over the years has been to obtain sufficient reduction of source areas that hand spraying would be feasible.

5) <u>Vehicles</u>. The District gradually phased out the Jeep as the major vehicle for Inspector-Operators and replaced most of them with small pickups. The 4-wheel drive Jeep was not needed for most spray work as spray vehicles were prohibited from driving up and down wet fields. When such driving had been done in the early years of our program, the vehicles made ruts which sometimes increased the sources, and needless to say the growers were not anxious to cooperate with us when we damaged their fields. The purpose of the vehicles was to drive on roads or lanes to get to the fields for inspection and hand spraying, or for calling for the airplane.

6) <u>Specific insecticides</u>.

(a). Oils. These have been used from the beginning of the District's control program up to the present time. Diesel oil was one of the best early oils. However, as it was cleaned up for better performance in diesel engines, it became less effective as a larvicide. Weed oils have been used for larviciding and for weed control, especially on dairy drains. More recently, special oils have been formulated to provide better larvicidal action, and these have been used extensively.

(b). DDT. This "miracle" insecticide had a very short life in the Delta District. While there was concern as early as 1948 that our spraying of DDT on pastures and alfalfa might be putting DDT in the milk of cows, this problem became academic because by 1949 the pasture mosquito had developed so much resistance to it that we ceased using it.

(c). Toxaphene. This proved effective as a larvicide on DDT-resistant mosquito larvae. It was claimed that it did not get into the milk of cows. However, by 1951 it failed because of mosquito resistance.

(d). EPN. This organophosphate insecticide was recommended by governmental agencies to replace the chlorinated hydrocarbon (DDT and toxaphene) insecticides. It provided fairly good control, but required much more careful handling then did the chlorinated hydrocarbons. It was also more expensive. In 1955, its production was stopped and we had to look for another material.

(e). Parathion. This organophosphate had been available for several years. It was much cheaper than any of the insecticides used previously, but we had avoided it because of its much greater mammalian toxicity, especially the hazard to the applicator. When it became the only available material, we developed safety precautions and began using it for hand, power and airplane applications. It proved to be very effective and no evidence of toxicity ever developed in any of our employees. Unfortunately, the mosquitoes

became resistant to it by 1959.
(f). Methyl parathion. This material had a slightly different molecular structure than Ethyl parathion and was used until 1961 when resistance developed.
(g). Baytex. This organophosphate insecticide was sufficiently different from the parathions that there appeared to be no cross-resistance. It was very effective on both larvae and adults, and continued thus year after year. It had a much lower mammalian toxicity than the parathions and it appeared that we had finally found the ideal and permanent insecticide. Unfortunately, in 1971 it began failing and in 1972 it was essentially useless for pasture mosquito control.
(h). Altosid. This is an insect growth hormone regulator, a completely different chemical from any we had used previously. When sprayed on 4th instar larvae, usually these larvae were not killed but went into pupation, where the physiological reorganization towards adult formation was disrupted, and the pupae died or the emerging adults did not survive. This material had no effect when sprayed on adults or pupae, nor did it prevent young larvae from molting to subsequent instars. It had a very short life after application in open sunny fields, perhaps only a few hours to a day. The question we faced was whether it could be used effectively in a major control program. Precision application was essential - we had to know exactly where the larvae were located and when they were in the 4th instar. The material was far too expensive to apply over wide-spread non-producing areas, as had been done when air spraying with other insecticides. With our system of mapping and trained Inspector-Operators, the transition was made smoothly, and Altosid proved to be very effective for the District-wide program. Within several irrigations, larvae of the pasture mosquito once again became scarce in the fields. According to the manufacturer, the Delta MAD was the first in the world to go "all out" with Altosid. Although laboratory studies at Riverside indicated that *Culex* mosquitoes could develop some resistance, no resistance has been encountered in the field after 14 years. Altosid is non-toxic to mammals and its short life after application assures that there will be no residue. A jug of this material can be carried in the Operator's vehicle and a measured quantity can be mixed with water as needed.
(i). Dursban. This organophosphate gave promise of being a very effective insecticide in polluted water, primarily against the house mosquito. A measured amount could be added and mosquito breeding prevented for several months or more. Two things prevented a continuation of this use: (1) mosquito larvae became resistant to it, and (2) many new dairies in the District constructed such large ponds that the cost of the Dursban was prohibitive. Dursban, an emulsified material, distributed throughout the depth of the ponds, sometimes a depth of 20 or 30 feet, whereas larvae were only on the surface. Larvicide oils alone would not spread evenly over the surface, which was usally covered with manure, organic oils and weeds. Dursban was added to the larvicide oil and sprayed uniformly over the surface, usually with good results.
(j). Herbicides. The philosophy of this District relative to dairy drain wastewater ponds was that weed control, especially on the banks, was essential if larval control was to be achieved. Soil sterilants were applied during the winter and contact chemicals were applied during the summer. Dairymen were generally very cooperative by providing money for the cost of the chemicals.

Inspection Procedures

The program of the Delta MAD has stressed "species control", not just mosquito control. By far the most important species from the beginning of the 1940's up through the 1970's has been the pasture mosquito. It breeds almost entirely in open fields -- pastures, alfalfa and a miscellany of less important areas. These fields are dry until irrigated. Eggs have been laid during or just after preceding irrigations and most will hatch when covered with the next irrigation. Development is very rapid, larvae progressing from 1st through 4th instars within 4 days in the summer, with one day for the pupa and one day for the adult to begin searching for a blood meal. For the Inspector-Operator there is no such thing as covering a part of his zone each day on a weekly basis. Rather, he must cover his entire zone on a daily basis, with priority given to fields which had been or were undergoing irrigation. The Inspector-Operator needed maps which showed all actual and potential producing fields in each section of his zone and he needed charts of the irrigation status of all producing fields. He could not rely on memory.

When I became Manager of the Delta MAD in June, 1947, the personnel were using county road maps and road numbers. However, mosquito sources are rarely found on roads. Rather they occur in irrigated or flooded fields, sometimes a mile or more from any road. We needed maps on which field sources could be identified. In this District, the San Joaquin Valley floor is very neatly divided into square miles or sections. The section became the unit which was adopted for recording source areas of the pasture mosquito.

At first we made our own maps, but soon aerial photographs were taken by the ASC office and by the County, enabling us to trace the roads and field lines onto our own maps. During the year, we recorded the precise areas where mosquito larvae were found. Changes in crops and field boundaries were easily made, primarily during the winter months. It was because the District had developed and established this map program that it was able to use Altosid so effectively.

A very important part of the inspection procedure was to employ trained Inspector-Operators. From my first year as District Manager, I insisted that these be permanent employees, not temporary ones to be hired in the spring and released in the fall. To my knowledge, the Delta MAD was one of the first districts in California to adopt this policy.

It should be noted that house mosquito control followed radically different inspection procedures, and the District effectively used high school and college students for this program. Even here, however, most students returned for several years, so could be classified as trained and experienced operators.

Source Reduction

Mosquito sources in the Delta MAD have been mostly man-made, the result of irrigation use -- and misuse. It has been held by the Agricultural Extension Service that water which remained on ANY of the crops grown in the Delta MAD long enough to raise mosquitoes was actually harmful to such crops. Why, then, was there so much mosquito production? In 1953, the District hired a graduate agronomy student to work with growers to reduce mosquito production. Over the years, the District purchased several crawler tractors (D4 size), a motor grader, a Ford tractor with backhoe and loader, and dump trucks.

Most growers who were producing mosquitoes did not fully comprehend how they were responsible. After all, textbooks which presented information on mosquitoes stated that mosquito eggs were laid on water and that the larvae lived in ponds. But the pasture mosquito does not lay its eggs on water but on vegetation or detritus; these eggs hatch very quickly after water covers them; and larval development and pupation can occur within 4 or 5 days, after which time the field may be quite dry. This is not the textbook story of the mosquito life cycle, and it is easy to understand why the growers, as well as the public at large, do not understand exactly how these mosquitoes are produced, especially in such tremendous numbers when control is inadequate. Therefore, a major effort of the Source Reduction Staff was general education of the public and specific education of the mosquito producers. Our heavy equipment was made available to growers, especially to assist in drainage projects. Unfortunately, there were two groups of mosquito producers for which the source reduction program was ineffective: 1) growers who wanted to develop land and who did not follow quality procedures to do so -- that is, poor grading and little or no provision for drainage, and 2) hobby farmers who did not understand or carry out good irrigation management. In 1972, when all insecticides had failed due to resistance, the District adopted a policy of citing these mosquito producers to meet with the District Board of Trustees to review the specific problems and to consider steps to prevent further mosquito production. The results were outstanding, and the major reduction of sources contributed to the successful precision control with the new insecticide Altosid.

There is a 1950 report of a Superior Court Judge in San Joaquin County who declared that a farmer has a right to run excess irrigation water onto his neighbor's land, even though it might cause damage to such adjacent property. The public, environmental groups, and governmental agencies have gone a long way towards reversing this judge's concept. For example, dairies are now prohibited by the state from running any dairy wastes off their property, whether into a canal, a ditch, or over land. Water users must be fully responsible not to create environmental problems.

Vector Control

In 1962, the District was requested by a dairyman to investigate the possibility of fly control. The district made studies for several years and then adopted a program to control flies. At this time the District changed its name so as to cover other insect pests (vectors) as well as mosquitoes and we officially became the Delta Vector Control District. Control of urban flies, primarily the result of fly breeding in garbage containers was virtually complete, with full appreciation by the public. Poultry ranches and fruit waste sites were similarly controlled. Control of flies on dairies was studied; however, the program never developed sufficiently to accomplish its goal. Proposition 13 was passed, the District funds were cut, and the fly control program was dropped.

Summary and Conclusions

Proposition 13 was a severe blow to the finances of mosquito control in California. The Delta VCD in 1978 was forced to drop one third of its staff. However, because of a number of factors, mosquito control continued at a very effective level.

1). Water availability. Concern had long been expressed that, as more and more water was made available in the San Joaquin Valley, mosquito problems would increase. The reverse has been true -- water management has become so much better that mosquito problems have actually become much fewer. Dams which hold water in storage have prevented much of the flooding of river drainage areas which had occurred in wet years in the past. Releases have been well planned and managed so that needed irrigation water was made available, with less waste. The reservoirs themselves have not been significant mosquito sources.

2). Land Development. Section after section of new land has been graded and put into crops. It was assumed that this would greatly increase the mosquito problem. Actually, mosquito problems became much fewer. In recent years, almost all commercial land grading is carried out with laser beam control of the grading equipment. Low areas over the old fields did not occur in the newly graded fields because of the greater precision of the laser beam. Also, when old fields were taken out of a crop, especially of alfalfa and pasture, laser beam grading was usually used to improve the grade.

3). Crop Changes. At one time, pastures were a major crop for dairy and beef cattle. In recent years, dairy cattle have been maintained in corrals, and no milking cows in the Delta VCD feed in open pastures. Also, the poor economy of pastures for beef cattle production has resulted in much reduction in irrigated pasturage for them. Alfalfa production has continued high, but better water management has resulted in far less mosquito production in this crop than in former years. Other crops which are far less of a mosquito source have replaced the pastures; good corn can be produced on some of the alkali land that never produced good pasture. Winter barley does not produce significant numbers of mosquitoes, and it, too, has replaced much of the "permanent" pastures.

4). Mosquito Ecology. Some have claimed that, if a mosquito cannot find its favorite site for egg laying, it will go to less favored sources. This District has ample evidence that the reverse is true. We have always made an effort to control the most important sources first. As these sources were eliminated or brought under control, mosquito production in less favored sources decreased or disappeared. For example, if a dairy drain was producing tremendous numbers of mosquitoes which scattered for a distance of 4 to 5 miles, production would occur in such minor sources as street gutters, catch basins, back yard containers, etc. When production at the dairy drain was stopped, most of the minor sources ceased producing. In other words, the minor sources may not be self supporting, but serve as sources only when large numbers of mosquitoes boil over from major sources. While this concept may not be 100% valid, it never- theless enables the District to set priorities and use its funds most effectively.

One final problem the District faces -- its program has been so successful in recent years that most of the public seldom see a mosquito. The importance of the District in maintaining a relatively mosquito free environment may not be appreciated, especially by the newcomers to the District. One quarter of the population did not live here 20 years ago. A continuing public education program is essential to assure the required public funding and support.

CONTINUING EDUCATION PROGRAM FOR CALIFORNIA MOSQUITO AND VECTOR CONTROL PERSONNEL

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INTRODUCTION

The California Administrative Code, Title 17, Section 30055, requires that any mosquito or vector control agency employee who applies or supervises the application of restricted use pesticides must be certified as a mosquito and/or vector control technician. This certification is gained through the passage of a series of vector control examinations administered by the California State Department of Health Services, as authorized by the California State Department of Food and Agriculture and the Federal Environmental Protection Agency.

Once certification is granted, no formal continuing education is required other than remaining actively employed by a mosquito/vector control agency. Until recently the only provision for expiration of a certificate was if a certified vector control employee discontinued active employment in the practice of vector control for one year or more. This regulation was repealed in 1985.

The California Mosquito and Vector Control Association was the first such association in the United States to establish a certification program and for several years has, in conjunction with the University of California and the Vector Surveillance and Control Branch, State Department of Health Services, worked toward implementation of a standardized continuing education program aimed at assuring that vector control technicians maintain a high standard of competency in the field of vector biology, ecology, and control. It has also been recognized that technicians should regularly review all aspects of pesticide storage, application, and safety procedures. Hazardous waste handling must be similarly addressed.

Mosquito and vector control agencies in California have traditionally embraced the concept of comprehensive vector control. This concept integrates and utilizes a variety of scientifically planned preventive, correctional, management, biological and chemical application measures, designed to control vector populations with the least possible effects on other living things. These measures are applied selectively either singly or in combination to obtain sustained vector control in the most effective and economical way.

OBJECTIVES

To maintain a high standard of competence in certified mosquito and vector control technicians in California by requiring certificate holders, including managers and technical staff, to successfully fulfill the biennial continuing education requirements to retain certification.

PROGRAM DESCRIPTION

There are two types of vector control agencies in California. Many agencies perform only mosquito control in their area of jurisdiction, while others carry out more expanded control activities including flies, midges, rats, rodent fleas, yellow jackets and other potential public health pests. The program curriculum is therefore designed to maintain currency in the three vector control certificate categories now issued by the California State Department of Health Services. They are Mosquito Control, which also includes training in pesticide use, safety, application, and equipment calibration; Terrestrial Invertebrate Vector Control; and Vertebrate Vector Control. This program would require that holders of Mosquito Control Certificates complete a minimum of 26 hours every 24 calendar months. Vector control workers who also hold Terrestrial Invertebrate Vector Control Certificates would be required to complete an additional six hours of training within a 24 calendar month period in that specialty. Those who hold Vertebrate Vector Control Certificates would have an additional eight hours requirement of training in vertebrate vector biology and control techniques. Finally, there is an eight hour allotment for In-Service Training. In-house instruction in any relevant subject will qualify for inclusion of the total hourly requirement for the 24 calendar month period.

It should be noted that certificate holders may elect to use any sections not listed as a requirement for this certificate for optional training to total the required number of hours of instruction. As an example, mosquito control certificate holders may choose hours of instruction from invertebrate vector or vertebrate vector curricula to satisfy their biennial training requirement.

There is no annual requirement for hours logged. The biennial requirement can be offered at one time or spread out with frequent shorter offerings of material over the two year period.

PROGRAM COORDINATION

The continuing education program will be overseen by the California Mosquito and Vector Control Association Continuing Education Committee.

This committee is composed of a member appointed by each CMVCA region for a minimum of a one year term. The Vice-President of CMVCA will also sit on the committee as a voting member. The chairman of the committee will be appointed by the President of CMVCA from among the regional committee members. The committee will also utilize consultants from colleges, universities and the State Department of Health Services as necessary.

It will be the committee's responsibility to approve curricula from each region, document training, and forward records of training to the CMVCA Executive Director. Each regional committee member will be responsible for planning and executing the training programs within his or her region, as well as coordinating any cooperative arrangements with California community colleges. Regional committee members are expected to work closely with management of all agencies within the region to assure convenient and timely training for each mosquito/vector control worker in their area of responsibility.

Training deficiencies of mosquito/vector control workers will be noted and recorded by the regional committee members in each region.

Regional Curriculum Approval Procedure

It will be the responsibility of the regional member of the Continuing Education Committee to review and submit the course plan for each offering to the CMVCA Continuing Education Committee for approval. This can be done by submitting the unit number, instructor name, location of training, and number of hours to be devoted to the unit. In the case of the In-Service Training section, the title of the offering and brief description of the information to be presented, along with a statement of how training applies to mosquito/vector control, should be submitted for approval.

Biennial Training Requirement and Documentation

Each employee will have training hours recorded on his or her training logs. These logs will be completed and forwarded to the CMVCA office no later than December 15 of each year. A copy of each log should be kept on file by the agency who employs the technician. Beginning July 1, 1988, these logs will be audited by the Continuing Education Committee and any deficiencies in training requirements noted. The agency employing the technician will then be notified of the training deficiencies.

Community College Affiliation

Community colleges in each region may be utilized for presentation of some of the curriculum offered. These colleges are usually willing to offer these courses, on or off campus and grant college credit for successful completion of the courses. The usual formula is one unit of credit granted for 16 hours of course work successfully completed. Of the 40 hours total biennial requirement for holders of all three certificates, 32 hours may be used to earn two college units. CMVCA Continuing Education Committee regional members will be responsible for making contact with and coordinating community college affiliation in each region. It is also recommended that contacts with each community college coordinate with each other to insure standardized registration requirements, fees, and other administrative and procedural detail. To date, three California community colleges are participating in this program. They are the College of the Sequoias in Visalia, Fullerton College in Fullerton, and Yuba College in Yuba City. Representatives of these three colleges have agreed to serve as consultants to this committee.

Requirements to be Added to the State Health and Safety Code

Legislative sponsorship is being utilized to include continuing education requirement language in the California State Health and Safety Code. Similar language may be used to cover pest abatement district personnel. The CMVCA Legislative Committee is working in cooperation with the California Department of Health Services, Vector Surveillance and Control Branch in this legislative inclusion endeavor.

Reference Material and Texts

It is recommended that the primary reference material for this program be the following:

1. A Training Manual for California Mosquito Control Agencies, revised 1975. Prepared for the California Mosquito and Vector Control Association by the Vector Control Section, California State Department of Health.

2. A Field Guide to Common Mosquitoes of California, revised 1967. Entomology Committee of the California Mosquito Control Association.

3. Community Pest and Related Vector Control, 1975. Edited by Don J. Womeldorf and Thomas D. Peck, Vector Control Section, California Department of Health.

Implementation

Survey letters from each region are being sent to those persons who are qualified to conduct classes, seminars, and/or demonstrations relating to comprehensive vector control subjects. Once a list of resource persons who have agreed to participate has been established, training schedules will be finalized and distributed to the districts in each region. A master list of these resources is maintained by the Continuing Education Committee Chairman.

The Continuing Education Committee regional member is responsible for planning and coordination of the Continuing Education Program for the region.

Each program will be conducted at a facility as centrally located at all of the agencies within the region as possible. It is also recommended that regions combine their training program if possible for efficiency. A modest per person registration fee is charged to cover the costs of lunches, coffee, and other refreshments, and costs associated with the program. If affiliated with a community college, a small registration fee is also assessed by the College.

With the completion of the required number of hours biennially, each participant will be awarded a certificate of completion. It is also the responsibility of the regional Continuing Education Committee member to accurately record this training on each individual's training log, and distribute them to the appropriate agencies.

It is recommended that the Vector Surveillance and Control Branch of the California State Department of Health Services be asked to monitor and evaluate this continuing education program as it develops.

EPA Involvement

This committee has been in informal contact with Robert Denny, Regional Pesticide Certification and Training Advisor with the Environmental Protection Agency in Washington, D.C. Mr. Denny has reviewed the program as well as the study material to be used. His conclusion is that the program is a "very good combination of training and evaluation, without overkill." He recommends that we proceed with the program in view of the fact that stronger continuing education requirements will most likely take effect in the next several months. He also indicated that the EPA was in the process of strengthening its emphasis in the area of endangered species and that he felt some training time should be devoted to this subject in the future. He further recommended that this training be delayed until EPA guidelines on the subject were more clearly defined. This committee will be researching the subject of existing endangered species laws in the coming months in preparation for the requirements.

In view of the opinions expressed by the EPA, the Continuing Education Committee recommends that the program be adopted by the CMVCA Executive Board.

PROGRESS IN THE DEVELOPMENT OF THE FUNGUS *LAGENIDIUM GIGANTEUM* AS A MOSQUITO CONTROL AGENT

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ABSTRACT

Research on various factors in the development of *Lagenidium giganteum* as an operational biological control agent of mosquito larvae has resulted in significant advances in several areas: registration, fermentation production and oospore activation.

Following the completion of several years of safety tests designed on the basis of U.S. Environmental Protection Agency guidelines for the registration of microbial agents, a petition for federal registration of *L. giganteum* has been submitted to the EPA by the California State Department of Health Services. Overwhelming evidence of the safety of the fungus to mammalian and nonmammalian nontarget organisms greatly reduced the number of tests usually required for registration of a chemical or biological control agent. Although several additional tests may be required for final federal registration, it is anticipated that these tests will not delay registration by more than several months.

Protocols for fermentation production of the sexual and asexual stages of *L. giganteum* continue to be modified to improve yields and stability. During 1986, several multihectare aerial trials using the asexual stage demonstrated that mycelium from 20 - 30 liters of fermentation beer is sufficient to control *Anopheles freeborni* and *Culex tarsalis* breeding in areas associated with rice culture in the Central Valley of California. Subsequent improvements in media formulation have reduced these volume requirements by a factor of two. These production yields for the asexual stage approach those for the commercially available microbial insecticide *Bacillus thuringiensis* strain H-14.

The dormant sexual stage of the fungus, the oospore, has the necessary qualities of stability and ease of handling for large scale production; however, recent efforts to evaluate this stage of the fungus for field control of mosquitoes have been hampered due to the inability to predictably break spore dormancy. Recently, a two stage approach to oospore germination has resulted in increased control of this process. The first step involves conversion of the mature dormant spores by dissolution of the thick endospore wall and dispersion of the (sub)central vacuole to form a uniform distribution of cytoplasm throughout the spore. The best results in obtaining converted forms at present involve use of dilute aqueous solutions of dimethylsulfoxide and calcium. Converted spores can then be induced to complete the germination process by dilution of the spore suspension and addition of an appropriate carbon source.

These recent advances in registration, production, activation and application of the sexual stage of *L. giganteum* will encourage commercial use of this fungus as an alternative to chemical control measures extensively used in operational mosquito control.

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A PRELIMINARY EVALUATION OF THE MOSQUITOFISH AND THE INLAND SILVERSIDE AS MOSQUITO CONTROL AGENTS IN WILD RICE FIELDS

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ABSTRACT

Wild rice is grown in Lake County, California from May through October, providing a breeding habitat for *Culex tarsalis, Anopheles freeborni* and *Anopheles franciscanus*. The mosquitofish, *Gambusia affinis*, has been shown to be an effective mosquito control agent in white rice fields (Hoy and Reed, 1971), but little is known about the effectiveness of *Gambusia* in wild rice. Besides *Gambusia*, another fish common to the area is the inland silverside, *Menidia beryllina*. This fish effectively controlled mosquito larvae in laboratory and small field trials in Florida (Middaugh et al., 1985). This study was designed to evaluate the mosquito control potential of *G. affinis* and *M. beryllina* in wild rice fields.

In 1986, the Lake County Mosquito Abatement District constructed 18 quarter-acre rice plots, each with a separate inflow valve and outlet box to prevent the mixing of water among fields. Fields were randomly assigned one of four treatments: no fish, 0.5 or 1.5 lbs/acre of *Gambusia*, or approximately 0.8 lbs/acre of silversides. There were five replicates of the first three treatments and three silverside replicates. Mosquitoes were monitored by a weekly dipping regime and minnow traps were set bi-weekly to monitor the fish population. We were unable to evaluate the mosquito control potential of *M. beryllina* because this fish did not survive well in the rice field system. *G. affinis* thrived in the Lake County wild rice fields but did not substantially affect mosquito populations under the conditions of this study.

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AUGMENTATION OF *NOTONECTA UNIFASCIATA* EGGS FOR SUPPRESSING *CULEX TARSALIS* LARVAL POPULATION DENSITIES IN RICE FIELDS

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ABSTRACT

Notonecta unifasciata Guerin females readily oviposited their eggs on the artificial substrates in nature. Thus, harvesting masses of eggs from wild colonies was relatively easy and inexpensive. An experimental rice field at the Kearney Agricultural Center and a commercial rice field were used to demonstrate the ability of *N. unifasciata* to suppress field populations of *Culex tarsalis* Coquillett larvae. The experimental plots received one release of 1,000, 5,000, and 10,000 eggs per plot and the commercial field received several releases of 546,000 eggs. Larval densities of *Cx. tarsalis* was greatly reduced in the released fields.

INTRODUCTION

Mosquito control operations in California are predominantly oriented towards using larvicides even though biological and physical approaches are practiced as well. In the past decades, however, increasing attention has been focused on the use of biological agents (Bay 1974, Chapman 1974) because of growing concern over chemical related environmental hazards and because mosquito resistance to pesticides have become so severe and widespread (Schaefer and Wilder 1970, Womeldorf et al. 1972, Gutierrez et al. 1976).

There are many studies concerning rice field mosquito control using mosquitofish (Hoy and Reed 1970, Hoy et al. 1972) but few studies show the efficacy of invertebrate predators in the fields. During 1986, we conducted experiments on harvesting *Notonecta unifasciata* Guerin eggs from wild populations and suppressing *Culex tarsalis* Coquillett larval populations by augmentation of notonectid eggs.

MATERIALS AND METHODS

Naturally occurring *N. unifasciata* populations at the Parlier sewage ponds were used as the colony. Eggs were used because of the ease of collecting and handling large numbers of them. Our prior field observations indicated that *N. unifasicata* females oviposited readily on an assortment of submerged substrate such as vegetation, artificial material and even gravel. We have collected a large number of eggs on both living and dead plant stems hanging into the water.

To examine ovipositional substrate preference, we used five substrates: cotton/polyester sewing thread (0.35 mm diam.), cotton string (1 mm diam.), acetone-washed hemp twine (2.5 mm diam.), polypropylene tree twine (5.5 mm diam.) and black surveyor's plastic tape (10 mm wide). The test substrates were stretched clothes-line fashion horizontally 5 to 10 cm below the water surface for 7 days after which the substrates were collected and brought into the laboratory for examination.

In order to demonstrate the efficiency of augmentation of *N. unifasciata* eggs to control mosquito larval densities, a small-scale test was conducted on an experimental rice field at the Kearney Agricultural Center. The field contained twelve plots in four rows; each plot measured 20 x 20 ft. Water was supplied to each plot from a reservoir through an underground pipeline and water depth was maintained at ca. 10 inches by float valves. We used three plots (4A, 4B, 4C) and a sump (20 x 20 ft) in which 0, 1,000, 5,000 and 10,000 eggs were planted, respectively. To monitor population densities, twelve dip samples for mosquito larvae and five drop-cage (40 cm^3) samples for notonectids were taken weekly from each plot. To show the predation effects, all notonectids captured from the control plot during the population census study were eliminated. Ten egg rafts of *Cx. tarsalis* obtained from the laboratory colonies were released into each test plot on four occasions (July 14, 28, August 5 and 11) to ensure adequate numbers of larvae to sample.

To test the method in actual rice fields, two commercial rice paddies (ca. 1 acre each) of the Koda Farm, South Dos Palso, were used. A total of 546,000 eggs were collected and augmented in one paddy on four separate occasions (July 24, 31, August 7 and 14) and the other paddy was left as the control. Densities of mosquito larvae and notonectid nymphs were checked weekly during the test period.

The eggs used for field augmentation studies were harvested by using acetone-washed hemp twine; twenty 10 ft. lengths were stretched clothes-line fashion 5 to 10 cm below the water surface and kept for 7 days. The twine sections (ca. 3 cm) were collected and brought into the laboratory for examination and counts.

Table 1.-Substrate preference of *N. unifasciata* females for oviposition in natural breeding sites.

Substrate	Surface area	Eggs per cm^2 (mean ± SE)
Thread, sewing	0.110	< 1
String, cotton	0.314	< 1
Twine, hemp	.785	53.13 (0.73)
Twine, plastic	1.728	35.52 (0.72)
Tape, surveyor's	2.000	87.80 (0.75)

Table 2.-Viability test of *N. unifasciata* eggs.

Date	No. eggs tested	No. hatched (%)	No. not hatched (%)
July 14	1161	991 (85.4)	170 (14.6)
August 21	382	313 (81.9)	69 (18.1)
Substrate			
Tape	861	736 (85.5)	125 (14.5)
Tree twine	715	622 (87.0)	93 (13.0)
Twine	503	430 (85.5)	73 (14.5)

Table 3.-Results of releasing *N. unifasciata* eggs for control of *Cx. tarsalis* larvae on rice fields.

No. wks after 1st release	No. eggs released	No. larvae/60 dips		% reduction
		Released	Control	
-1	0	47	45	0
0	26,000	52	31	0
1	50,000	24	9	0
2	300,000	15	9	0
3	200,000	6	8	42
4	0	2	6	74
5	0	0	4	100

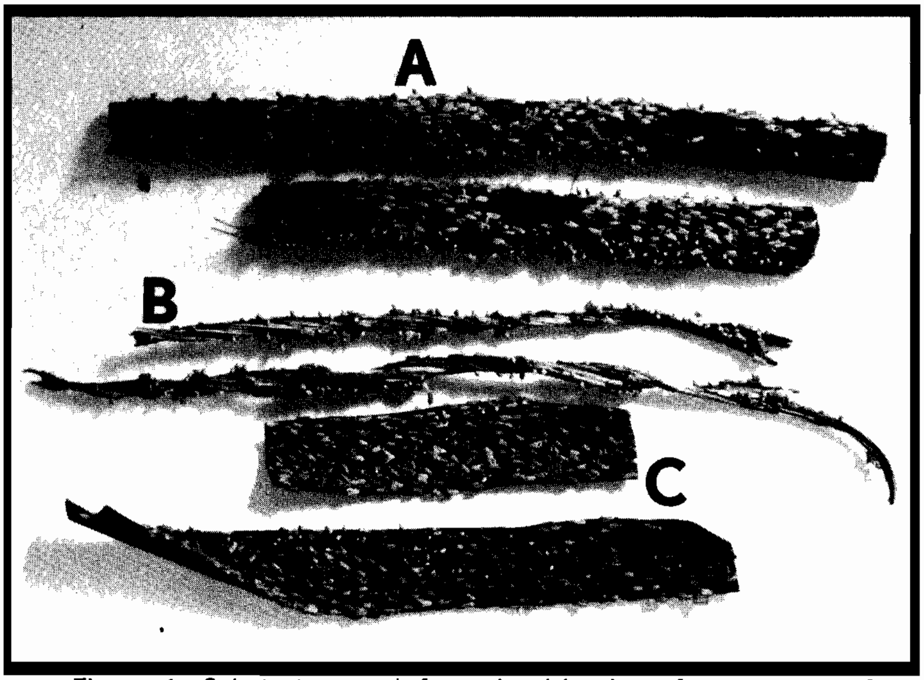

Figure 1.-Substrates used for ovipositional preference tests of *N. unifasciata* showing eggs on the substrates: A. Tree twine; B. Hemp twine; C. Tape.

RESULTS AND DISCUSSION

The results of substrate preference of *N. unifasciata* females for oviposition in natural breeding sites are shown in Table 1. Sewing thread and cotton string attracted very few females to oviposit, therefore, only three substrates, hemp twine, tree twine and tape were used for subsequent tests. The surveyor's tape yielded the most eggs with an average of 87.80 eggs per 1 cm^2, the hemp twine was next with 53.13 eggs, followed by the tree twine with 35.53 eggs. An interesting ovipositional behavior of notonectids was observed during this study, e.g., *N. unifasciata* deposited eggs only on the underside of the substrates, particularly on the tape, where nearly all of the eggs were deposited on one side. This behavior was also observed on both twines but it seemed less pronounced (Figure 1).

Table 2 shows the results of viability tests of eggs collected for the field augmentation studies and from the substrate preference studies. There were no significant differences in viability of field collected eggs, although both mated and unmated females can deposit eggs; embryos can develop only from eggs deposited by mated females (Hazelrigg 1973).

The results of the small-scale augmentation studies are shown in Figure 2. Very few mosquito larvae were collected from the plots planted with *N. unifasciata* eggs; even the lowest rate (1,000 eggs/plot) eliminated almost all mosquito larvae. Some difficulties encountered in the study was the tendency of notonectid adults to fly into the control plot as the number of mosquito larvae there increased, although we removed some notonectids (Figure 2) at each sampling occasion, we were unable to exclude notonectids completely in the control plots.

The results of the large-scale augmentation study are shown in Figure 3 and Table 3. At the beginning, the mean number of mosquito larvae per dip was greater in the treated paddy, however, on the 5th sampling day (three weeks post augmentation) and thereafter, the mean number became smaller than the mean of the control paddy. No larvae were collected on the last sampling date. The means of the control paddy were lower most of the time during the study period, but never reached zero. The shaded area in Figure 3 indicates the estimated larval reduction by the augmentation of eggs. The reduction occurred late in the study period; the cumulative effect of the reduction might be affected substantially over the subsequent mosquito production from the paddy. Figure 3 also shows the recovery of nymphal notonectid populations captured by 20 bucket (2 gal. capacity) samples. The first nymphal collection was made on the 7th day postplacement of the eggs. Surprisingly enough, very few nymphs were recovered during the study period, indicating high nymphal mortality. Since the test rice fields we used were stocked with mosquitofish by the Fresno Westside MAD in early May and the fish were well-established in the fields, there is a possibility that the nymphs were preyed upon by the fish.

In summary, naturally occurring *N. unifasciata* readily oviposited on the hemp twine, tree twine and surveyor's tape. The surveyor's tape was easiest to manipulate and yielded the largest number of eggs per unit.

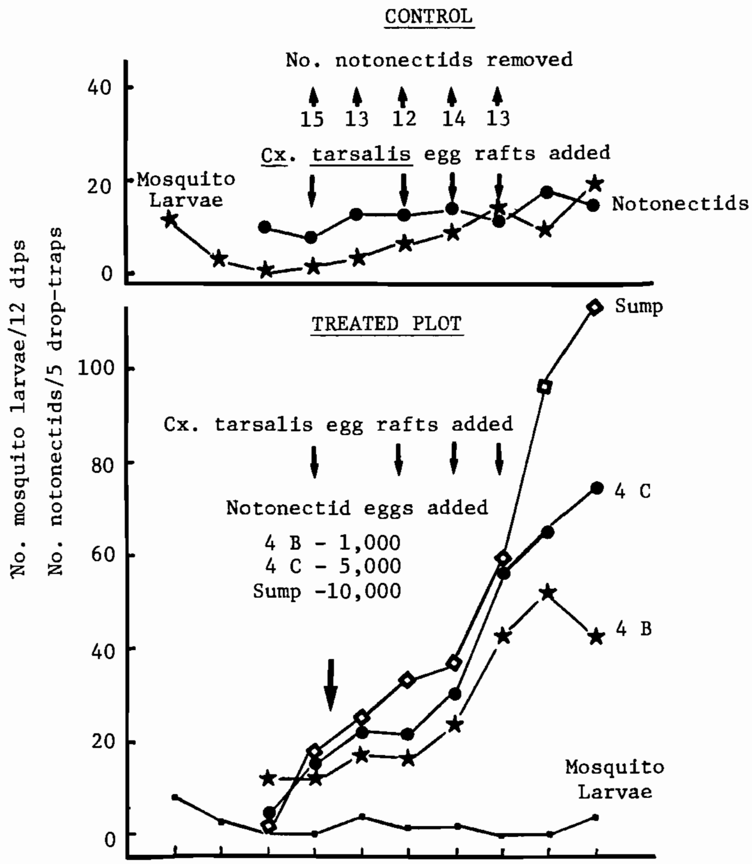

Figure 2.-Population densities of *Cx. tarsalis* larvae and *N. unifasciata* in the control and treated plots. Arrows: downward indicates additions and upward removals.

Field augmentation studies of *N. unifasciata* eggs to suppress mosquito larval densities were conducted on experimental plots and commercial rice fields. In both studies, mosquito densities were greatly reduced in treated fields. *N. unifasciata* might be used as a biological agent to control mosquitoes in rice fields by seeding the eggs to promote the early rapid buildup of predator populations.

ACKNOWLEDGMENTS

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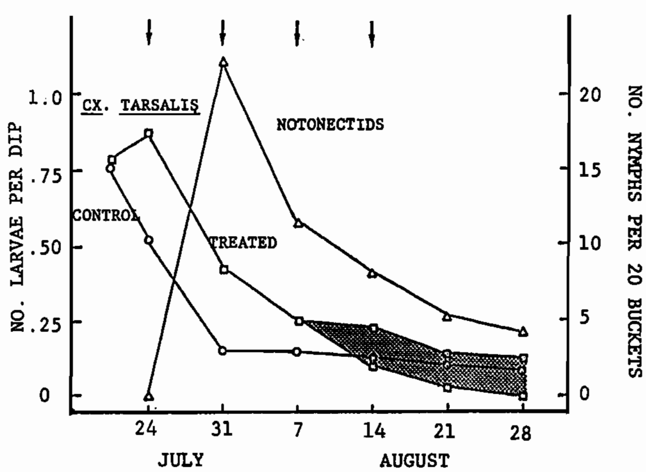

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BACILLUS SPHAERICUS 2362 FORMULATIONS FOR INITIAL AND PERSISTENT CONTROL OF STAGNANT WATER MOSQUITOES[1]

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ABSTRACT

Several corn cob granules of *Bacillus sphaericus* 2362 having similar spore contents ($5x10^9$ spores/gram), but varying in mesh size, exhibited excellent activity against 4th-instar larvae of *Culex quinquefasciatus* Say in the laboratory. All formulations produced high level of mortality 4 h after treatment at the rates of 0.125, 0.25, and 0.5 mg of the formulation/liter. At these rates, the smaller size granules (1 mm) remained active 24 h after treatment, but the larger size granules (3 mm) were only active at the high rate of 0.5 mg of formulation/liter. Activity of both formulations declined markedly 2, 6, and 12 days after treatment when water from treated jars was aspirated without agitation. However, when contents of the jars were stirred thoroughly prior to sampling, both formulations displayed high level of activity, similar to the results obtained initially 4 h after treatment.

Against a natural population of *Culex tarsalis* Coquillett and *Cx. peus* Speiser, the primary powder formulation of *B. sphaericus* 2362 (ABG-6184) caused excellent control for more than 2 weeks at the rates of 0.05 and 0.1 lb/acre. The liquid formulation (BSP-2) produced 95, 99 and 100% control of *Cx. peus* at the rates of 0.25, 0.5 and 1.0 lb of the preparation/acre, respectively. This formulation, however, was less active against *Cx. tarsalis*, causing 4 and 83% reduction at the rates of 0.25 and 0.5 lb/acre.

Corn cob granules of *B. sphaericus* 2362 produced excellent initial control of *Cx. tarsalis* and *Cx. peus* at all rates applied (0.5-4.0 lb of the formulation/acre). At the low rates (0.5-1.0 lb/acre) the population recovered within 7 days after treatment, while the high rates (2.0-4.0 lb/acre) in general suppressed the population for more than 2 weeks.

INTRODUCTION

Bacillus sphaericus 2362 yielded excellent initial control of *Culex tarsalis* Coquillett in organically enriched habitats at the rate of 0.2 lb/acre, but the population began to recover within 4 days after treatment. In non-enriched habitats, however, excellent control was obtained for more than five weeks. Extended control in the freshwater ponds, however, was attributed to joint action of the microbial larvicide and predators which were not affected in the treated ponds (Mulla et al. 1984). In recent field studies, several new formulations of *B. sphaericus* 2362 were reported to exhibit excellent biological activity against *Cx. tarsalis, Cx. peus* Speiser, *Psorophora columbiae* (Dyar and Knab), *Aedes nigromaculis* Ludlow and *Ae. melanimon* Dyar at rates ranging from 0.1-1.0 lb/acre (Mulla et al. 1985, 1987).

In addition to temperature, water quality, larval density and nutrient availability in the breeding sources, efficacy of microbial larvicides was shown to be influenced by the toxin availability in the larval feeding zone and by the feeding behavior of the target mosquito species (Aly 1983, Ramoska and Hopkins 1981). Therefore, in order to enchance activity and persistence of *B. sphaericus* 2362 against a wide range of mosquito species in various habitats, these factors were taken into consideration in the development of several new formulations. The following studies were carried out to determine the activity and persistence of new formulations of *B. sphaericus* 2362 in the laboratory and under field conditions.

METHODS AND MATERIALS

The new formulations evaluated included ABG-6184 primary powder, BSP-2 liquid, high-spore and low-spore concentrates (prepared by Yousten), and four corn cob granules having similar potency, but of different mesh sizes. Potency, lot number and type of each of these formulations are shown in Table 1.

Laboratory Evaluations:-The primary powder (ABG-6184), BSP-2 liquid, and high-spore and low-spore concentrate formulations were evaluated in the laboratory as previously described by Mulla et al. (1985). One-percent stock suspensions were prepared in distilled water via a sonicator (Fisher Sonic Dismembrator Model 300) at 35% speed for 15 s, after cooling in crushed ice for 5 min, and serial dilutions in distilled water were prepared as needed, without the use of sonicator.

[1]These studies were conducted with the cooperation of Coachella Valley Mosquito Abatement District, Thermal, California, and supported by funds from the State of California Special Mosquito Research Funds.

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Table 1.-*Bacillus sphaericus* 2362 formulations evaluated in the laboratory and field.

Formulation	Type	Size (mm)	Potency spores/g or ml[a]	Lot no.
ABG-6185	Corn cob G	3.0	5.0×10^9	87/009 BR
ABG-6185	Corn cob G	1.0	5.0×10^9	87/010 BR
ABG-6185	Corn cob G	1.0	5.0×10^9	88/034 BR
ABG-6185	Corn cob G	1.0	5.0×10^9	88/039 BR
ABG-6184	Primary powder	---	7.6×10^{10}	86/958 BD
2362	High-spore liquid	---	1.4×10^{10}	---
2362	Low-spore liquid	---	3.9×10^6	---
BSP-2 (10%)	Liquid	---	2.0×10^7	---

[a] Spore count is based on the dry wt or liquid volume of the various preparations.

The required amount for the desired strength concentration was added to 4-oz disposable ice cream cups containing 20 early 4th-instar larvae of *Cx. quinquefasciatus* in 100 ml of distilled water. Each material was tested at 4-5 different concentrations in triplicate on 3-4 different occasions. Test organisms were held in a holding room where temperature was maintained at 78°F, and mortality readings were taken 48 h after treatment. Along with each test, 3 cups were left untreated as checks.

Values obtained for each concentration tested were subjected to log probit regression analysis by using a Compucorp 145 E desk top computer to obtain the LC_{50}-LC_{90} estimates in mg/liter.

Procedures utilized in the evaluation of corn cob granules in the laboratory were the same as described by Mulla and Chaudhury (1968). Since weighing smaller quantities of large-size granules induces a greater margin of error, higher concentrations were used in the jar which on further dilution yielded concentrations in the activity range of the formulation when aliquots were added to bioassay cups. To determine release and activity, 350 mg of each formulation containing 5×10^9 spores/gram were added to 1-gal glass mayonnaise jars containing 3500 ml of distilled water, yielding a final concentration of 100 mg of formulation/liter. Two jars were used to study release of each formulation, and 2 jars were left untreated as checks. Most of the granules sank to the bottom 2-3 h after addition. Four and 24 h after addition, 10 ml of water were aspirated from each jar (5 ml from the top and 5 ml from a zone 1.5 cm above the bottom). This aspirated aliquot contained released toxin particles and not the granules. The 10 ml obtained from the treated jars were mixed with 10 ml of untreated distilled water to yield 50 mg of the formulation/liter. For bioassay, 0.25, 0.5 and 1.0 ml of the water mix was added to 4-oz disposable ice cream cups containing 20 early 4th-instar larvae of *Cx. quinquefasciatus* in 100 ml of distilled water, yielding final theoretical concentrations of 0.125, 0.25 and 0.5 mg of formulation/liter, utilizing 4 replicates per concentration. Four cups were treated with 1 ml (the maximum amount used in the treatments) from each of the check jars, and a total of 8 cups were utilized as checks. Treated organisms were kept in a holding room where temperature was maintained at 78°F, and mortality readings were taken after 48 h of exposure.

To compare activity and persistence of 1 and 3 mm mesh corn cob granules, water samples from treated jars and the check, in a second test, were taken 4 h and 1, 2, 6, and 12 days after treatment. Six and 12 days after treatment, 2 water samples were obtained from each jar. The first sample was taken as described above, while the second sample was taken after stirring and mixing the contents of each jar thoroughly.

Field Evaluations:-The new formulations of *B. sphaericus* 2362 were evaluated in experimental ponds at the Aquatic and Vector Control Research Facilities of the University of California, Riverside. These facilities are located in Riverside and in the Coachella Valley of southern California, and were fully described elsewhere by Mulla et al. (1982).

The Coachella Valley ponds measure 18 x 18 ft ($30 m^2$), fully vegetated, and with a water pH of 9.4. Ponds at Riverside measure 12 x 24 ft ($27 m^2$), free of vegetation and with a pH of 8.2. Water level in the ponds at both locations was kept constant (30 cm) by the use of float valves.

To ensure good coverage, the required amount for the low rates of the corn cob granules

Table 2.-Evaluation of *B. sphaericus* 2362 formulations against 4th-instar larvae of *Cx. quinquefasciatus* in the laboratory.

Formulations	Potency spores/gram	mg/liter LC_{50} - LC_{90}	Slope
ABG-6184 primary powder	7.6×10^{10}	0.002 - 0.004	3.89
High-spore concentrate liquid	1.4×10^{10}	0.012 - 0.024	4.75
Low-spore concentrate liquid	3.9×10^{6}	0.020 - 0.040	3.37
BSP-2 liquid	2.0×10^{7}	0.023 - 0.054	3.44

(0.5 - 1.0 lb/acre) was mixed with a blank corn cob of similar size, and a total of 7 grams of the mix was applied per pond. The granules were applied with a 150-ml size salt shaker provided with an adjustable opening. The required amount for each rate of application of the primary powder (ABG-6184), BSP-2, high-spore and low-spore concentrate formulations were mixed with 120 ml of water and applied with a polyethelene squeeze bottle.

To determine the activity and longevity of these formulations on mosquito larvae prevailing in the ponds at time of treatment, 5 dips/pond were taken prior to treatment and 2, 7, and 14 days after treatment. One hundred larvae from the pretreatment samples were identified under a dissecting microscope in the laboratory to determine the mosquito species prevailing during each test.

In all field studies, 2 replicates for each rate of application were used, and 2 ponds were left untreated during each test as checks. To account for natural fluctuation in the number of larvae in the ponds, percent reduction was calculated according to Mulla's formula (Mulla et al. 1971):

$$(\%)\ \text{Reduction} = 100 - \left(\frac{C1}{T1} \times \frac{T2}{C2}\right) 100$$

where C1 = no. larvae in check pretreatment, C2 = no. larvae in check posttreatment, T1 = no. larvae in treated pretreatment, T2 = no. larvae in treated posttreatment.

RESULTS AND DISCUSSION

The primary powder formulation (ABG-6184) was the most active of all *B. sphaericus* 2362 formulations tested to date, causing 90% mortality at the low concentration of 0.004 mg/liter. The high spore concentrate formulation was 6-fold less active than this primary powder with an LC_{90} of 0.024 mg/liter. The low-spore concentrate and BSP-2 formulations were 5- and 13-fold less active than ABG-6184 primary powder, respectively, and about 2-fold less active than the high-spore concentrate requiring 0.04 and 0.054 mg/liter to induce 90% mortality, respectively (Table 2).

The primary powder (ABG-6184) contains about 5X more spores than the high spore concentrate and was 6-fold more active. The low-spore concentrate formulation, which contains the least amount of spores/gram, yielded better results than BSP-2 and was slightly less active than the high spore concentrate formulation. This data clearly shows that an increase in spore count in the formulation does not necessarily result in an increase in activity. Type of formulation or preparation, however, could improve the activity of *B. sphaericus* 2362 against mosquito larvae.

All corn cob granule formulations tested in the laboratory produced similar results at all rates applied 4 h after treatment, however, the smaller size formulation remained active for 24 h at the two high rates of 0.25 and 0.50 mg/liter (Table 3). Activity of the larger particle formulation (3 mm mesh) began to decline drastically at the low rates of 0.125 and 0.25 mg/liter 24 h after treatment, but produced 91% mortality at the high rate of 0.50 mg/liter.

In the second test, the smaller size corn cob granules produced excellent results at all rates applied 4 h after treatment, while the larger size formulation was active only at the high rate of 0.50 mg/liter. The smaller size formulations showed a similar trend as in test 1 (Table 3), causing 73 and 94% mortality at the two higher rates of 0.25 and 0.50 mg/liter 24 h after treatment, and poor results were obtained at all rates applied with the larger size formulations. Drastic reduction in activity of both formulations was observed 2, 6, and 12 days after treatment. However, when treated water was obtained after thorough stirring with a pipette, both formulations exhibited excellent activity, and results were somewhat similar to those obtained at the start, 4 h after treatment (Table 4). This clearly demonstrates that *B. sphaericus* granules and toxin particles settle, and remain stable in water. Mixing of aliquots from the top and bottom of the jar without stirring results in lower activity levels.

These findings indicate that *B. sphaericus* 2362 toxin particles, after their release from corn cob granules, have the tendency to sink within 1-2 days after application, and remain potent in water for more than 2 weeks. Additional studies are needed to determine length of time these bioactive agents remain viable in water. Similar trends and relationships established in the laboratory may not hold true under field conditions because of many biotic and abiotic factors which may influence availability of the toxin to target species. However, these studies clearly indicate that the toxin is quite stable in water under controlled laboratory conditions.

In the field, the corn cob granules, having similar potency (5.0×10^9 spores/gram), produced excellent results against *Cx. tarsalis* larvae in the Coachella Valley facility 2 days after treatment at the rates of 1, 2, and 4 lb of the formulation/acre. The larger size granules (lot 87/009 BR) and one of the smaller size granules (lot 88/034 BR) lost their activity at the low rate of 1.0 lb.acre within 7 days after treatment. At the rate of 2.0 lb/acre, the larger size granules produced poor results 14 days after treatment, but excellent control was obtained at the high rate of 4.0 lb/acre for more than 14 days. The smaller size granules (lot 88/039 BR) were the most active, producing excellent control for more than 2 weeks at the rates of 2.0 and 4.0 lb/acre (Table 5). As found in the laboratory, the larger particle size formulation probably sinks faster and remains at the bottom of the pond, away from the larval feeding zone.

When the three most active granules were evaluated in field ponds at Riverside at the low rates of 0.5 and 1.0 lb/acre, excellent control of a mixed population of *Cx. peus*, *Cx. tarsalis*, and *Cx. quinquefasciatus* was obtained 2 days after treatment, but the larval population recovered within 7 days after treatment (Table 5). Longevity was limited at these low dosages.

According to the data presented here, activity and longevity of granular formulations were primarily dependent on rates of application and secondarily influenced by mesh size of the granules. Higher rates (2 and 4 lb/acre) yielded extended control of mosquito larvae for more that 2 weeks, while the low rates (0.5 and 1.0 lb/acre) produced excellent initial control, but the population recovered 7 days after treatment.

The liquid formulation BSP-2 produced 95,

Table 3.-Evaluation of granular formulations of *B. sphaericus* 2362 against 4th-instar larvae of *Cx. quinquefasciatus* in the laboratory.

Material and formulations	Theoretical amount of granules mg/liter	(%) Mortality after application (h) [a/] 4	24
ABG-6185	0.125	80	56
(3 mm)	0.25	91	75
lot 87/009 BR	0.50	99	91
ABG-6185	0.125	94	81
(1 mm)	0.25	96	94
lot 87/010 BR	0.50	100	96
ABG-6185	0.125	86	68
(1 mm)	0.25	100	96
lot 88/034 BR	0.50	100	100
ABG-6185	0.125	94	63
(1 mm)	0.25	98	84
lot 88/039 BR	0.50	100	98
Check	--	0	0

[a/] Mortality readings were taken after 48 h of exposure.

99% and complete control of *Cx. peus* prevailing in high numbers in the ponds at the Riverside facility at the rates of 0.25, 0.5 and 1.0 lb/acre respectively. Against the same species, the primary powder (ABG-6184) yielded more superior results than BSP-2, causing complete control of mosquito larvae at the rates of 0.05 and 0.1 lb/acre. At all rates applied, both formulations suppressed the larval population for more than 2 weeks (Table 6).

In the Coachella Valley facility, BSP-2 produced 83% reduction of *Cx. tarsalis* at the rate of 0.5 lb/acre, and poor results were obtained at the low rate of 0.25 lb/acre 2 days after treatment. These rates produced excellent control of *Cx. peus* larvae at the Riverside facility, and failure to produce similar results against *Cx. tarsalis* at the Coachella Valley could be attributed to several factors. The *Cx. peus* larvae could be more susceptible to *B. sphaericus* than *Cx. tarsalis*, or some of the spores of *B. sphaericus* were consumed by the nontarget organisms which were present in large numbers in the naturally vegetated ponds at the Coachella Valley facility. Ponds at Riverside were free of vegetation, and the number of nontarget organisms in the ponds was minimal. In addition, water temperature at the Coachella Valley during these studies was in the range of 17-24°C compared to 20-30°C at Riverside.

The high-spore and low-spore concentrates produced similar results at the rates of 0.25 and 0.5 lb/acre, producing excellent reduction of *Cx. tarsalis* for one week, but the population began to recover 14 days after treatment. The primary powder (ABG-6184) produced 85% and complete control of *Cx. tarsalis* at the rates of 0.05 and 0.1 lb/acre respectively. Both rates remained active for one week, but activity declined 14 days after treatment (Table 6).

From the data presented, it is evident that ABG-6184 primary powder appears to be equally effective against the larval populations in Coachella Valley and Riverside facilities. However, BSP-2 is somewhat less active against the Coachella Valley population. ABG-6184 primary powder could be used effectively against larvae of *Culex* species in clear water situations at the rates of 0.05-0.1 lb/acre, while BSP-2 could be used at 0.5-1.0 lb/acre. The latter preparation is a dilute formulation, and dosages indicated are in the range of practical rates required for satisfactory mosquito larval control.

In recent field evaluations against floodwater mosquitoes, BSP-2 and the primary powder ABG-6184 displayed excellent activity against *Psorophora columbiae, Aedes melanimon* and *Ae. nigromaculis* in irrigated fields in the Coachella Valley of southern California and in the central San Joaquin Valley in Tulare and Kings Counties, California (Mulla et al. 1987). *Psorophora columbiae* was reported to be as susceptible to the primary powder ABG-6184 as the *Culex* species, but *Ae. nigromaculis* and *Ae. melanimon* were 5-fold less susceptible. Complete control of these species, however, was obtained at the rate of 0.5 lb/acre. The liquid formulation BSP-2 was 2-fold less active against the floodwater mosquito spec-

Table 4.-Evaluation of granular formulations of *B. sphaericus* 2362 against 4th-instar larvae of *Cx. quinquefasciatus* in the laboratory.

Material and formulations	Theor. conc. granules mg/liter	(%) Mortality after addition (days)[a/] 4 hr A	1A	2A	6A	6B	12A	12B
ABG-6185	0.125	93	55	35	34	63	19	71
(1 mm)	0.25	98	73	59	43	80	25	85
lot 87/010 BR	0.50	100	94	79	66	95	35	93
ABG-6185	0.125	34	21	10	10	58	13	50
(3 mm)	0.25	60	34	23	14	79	13	85
lot 87/009 BR	0.50	83	65	35	30	98	23	93
Check	--	0	0	6	1	1	3	3

[a/] Mortality readings were taken after 48 h of exposure.

A: Water from treated jars and checks were obtained without stirring.

B: Water from treated jars and checks were obtained after stirring.

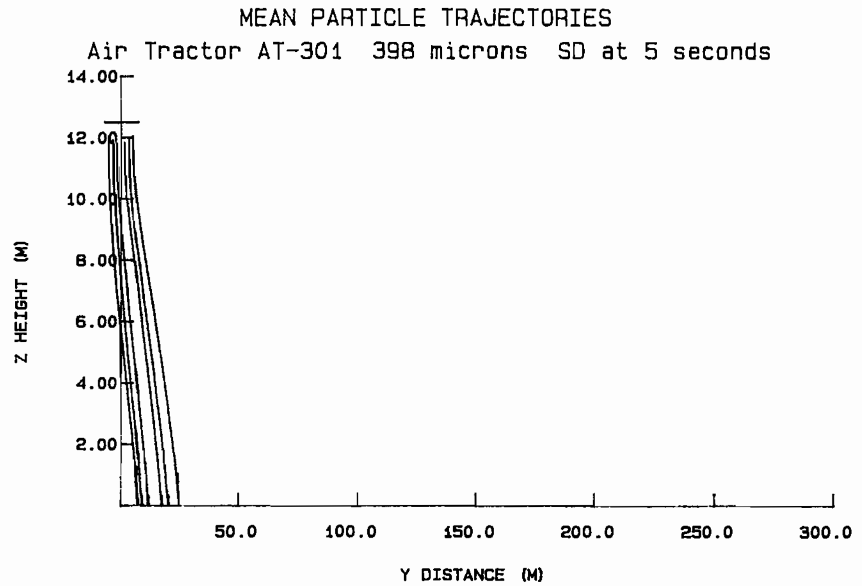

Figure 4.-Theoretical trajectories of 398 micrometer drops based on AGDISP simulation of test on 9/22/86.

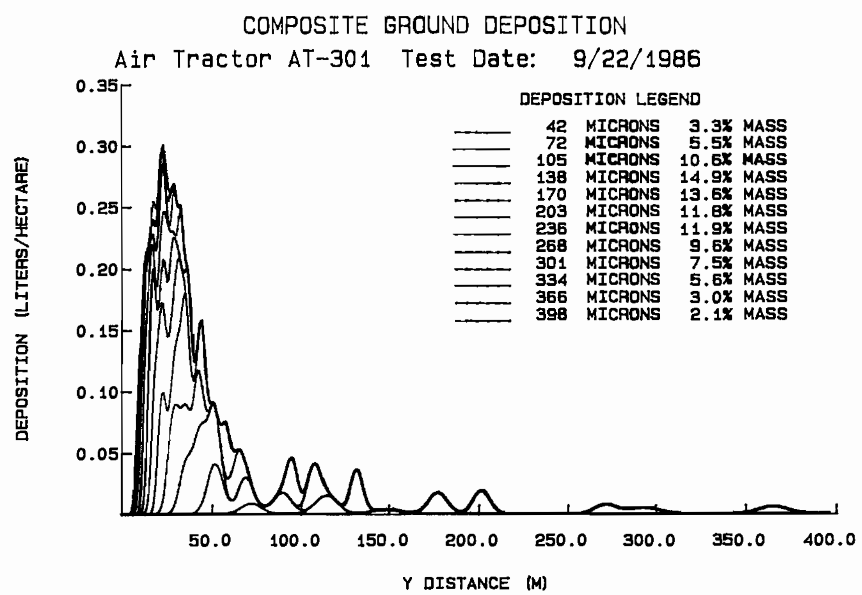

Figure 5.-Theoretical deposit pattern in liters/hectare based on AGDISP simulation of test on 9/22/86.

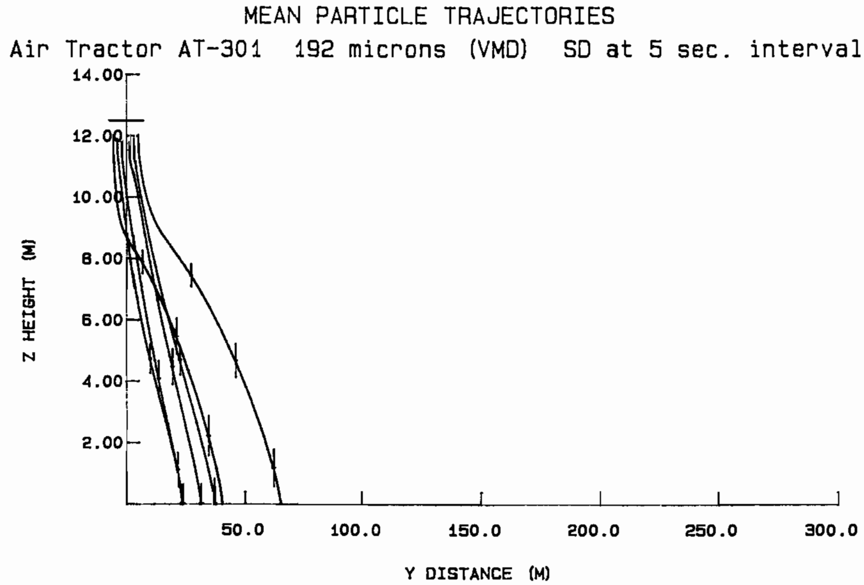

Figure 2.-Theoretical trajectories of 192 micrometer (volume median diameter) drops based on AGDISP simulation of test on 9/22/86.

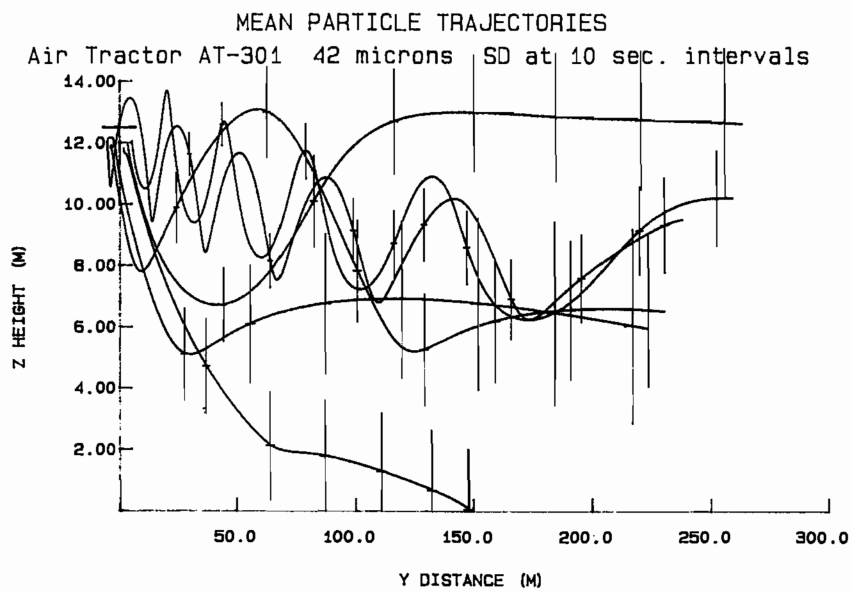

Figure 3.-Theoretical trajectories of 42 micrometer drops based on AGDISP simulation of test on 9/22/86.

Table 2.-Aircraft Characteristics.

Gross weight	2,270 kg (5000 lb)
Wing span	13.75 m (45.1 ft)
Air speed	49.2 m/s (110 mph)
Flight height	12.5 m (41 ft)
Circulation	26.8 m^2/sec (289 ft^2/sec)
Circulation decay factor	0.41
Drag coefficient	0.09
Wing area	25.1 m^2 (270 ft^2)
Propeller Eff	80%
Propeller RPM	2000
Propeller radius	1.33 m (4.38 ft)

Table 3.-Meteorological Conditions.

	Speed		Height	
	m/s	(mph)	m	(ft)
Wind velocity profile	2.4	5.4	1.2	4
	2.8	6.3	2.4	8
	3.1	6.9	4.9	16
	3.7	8.3	9.8	32

Additional background turbulence, q^2 : 0.059 m^2/sec^2

Max. turbulent macroscale, A : 30 m

Wind direction: 81° relative to flight direction

Stability Ratio: -0.29

Table 4.-Drop Characteristics.

Nozzle Position	Horiz. Dist. from Centerline		Vert. Dist. from Wingtip	
	m	ft.	m	ft.
3 & 4	1.63	5.3	-0.70	-2.3
2 & 5	3.45	11.3	-0.59	-1.9
1 & 6	5.28	17.3	-0.47	-1.5

Drop specific gravity 1.23
Drop downward velocity 13.5 m/s (30 mph)

diameter. The horizontal axis represents distance from the center of the aircraft as it passed the sample line, 0 meters being directly under the center of the aircraft. The vertical axis is height above the ground surface. The horizontal line at approximately 12.5 meters height represents the wing of the aircraft. There are six trajectories plotted on the figure, each representing the path predicted for a droplet released from one of the nozzles on the boom. The crosses appearing at intervals on the trajectories indicate the magnitude of the standard deviation of position about the predicted mean position. Five seconds are elapsed between each of the marks, and five seconds between release and the first mark.

Looking at the trajectories, the four inboard nozzles have their droplets all deposit within 50 meters of the flight line. The outboard nozzle on each side of the aircraft are held up by interaction with the wingtip vortices and have their descent slowed. As a result, there is more displacement of these droplets due to the crosswind, blowing from left to right. The outboard nozzles take approximately 17 seconds to reach the ground, while those closest to the fuselage deposit within 10 seconds of release.

Drops with a diameter of 42 microns are represented by Figure 3. The axes are the same as in Figure 2, with the aircraft and the release points in the same position. Each cross on the predicted trajectories now represents a 10 second interval with a total simulated time in excess of 70 seconds. These smaller drops clearly follow different paths than those representing the VMD. Only one of the six drops followed ever hits the ground. It is also the only mean trajectory to come within 5 meters of the ground. Drops released from the two outboard positions have clearly been entrained in the wing tip vortices and will be carried far away from the flight line. Similarly, the trajectories from the other three nozzle positions have all been strongly affected by the crosswind. The small settling velocity of 42 micron drops is not sufficient to overcome the wake turbulence immediately behind the aircraft. As a result, these drops will also be carried far downwind.

The largest drops simulated, a 398 micrometer diameter, are represented by the predicted mean trajectories plotted in Figure 4. They all fall almost directly under the aircraft, within the first 25 meters. The simulated time to impact is less than eight seconds for all positions.

Composite deposition for all 12 drop sizes is shown in Figure 5. The legend indicates the drop diameters used in the simulation and the percentage of the total mass represented by each. The drop diameters in the range of 105 through 268 microns make up a majority of the mass, and contribute nearly all of the mass in the predominant peak, depositing between 0 and 70 meters. A general description of the prediction would include a peak value of approximately 0.30 1/ha at 25 meters downwind, falling off to a "hole" at 80 meters, with secondary peaks between 90 and 140 meters and small, residual deposits continuing out to nearly 400 meters from the flight line.

Note should be made of the fact that only discrete drop sizes can be modeled, but the actual drop size spectrum emitted from a nozzle will be continuous. As a result, there will be some smoothing of the values where strong peaks are plotted. The peaks would not be as high, nor the low points as shallow, if a continuous spectrum was modeled. The general shape and trends would not change however, and in areas where there is not a major difference in the behavior of sequentially larger drop sizes, there would not be a substantial difference between discrete sizes and a continuous spectrum. One such area that would not change is the primary peak deposit occurring in the first 70 meters downwind.

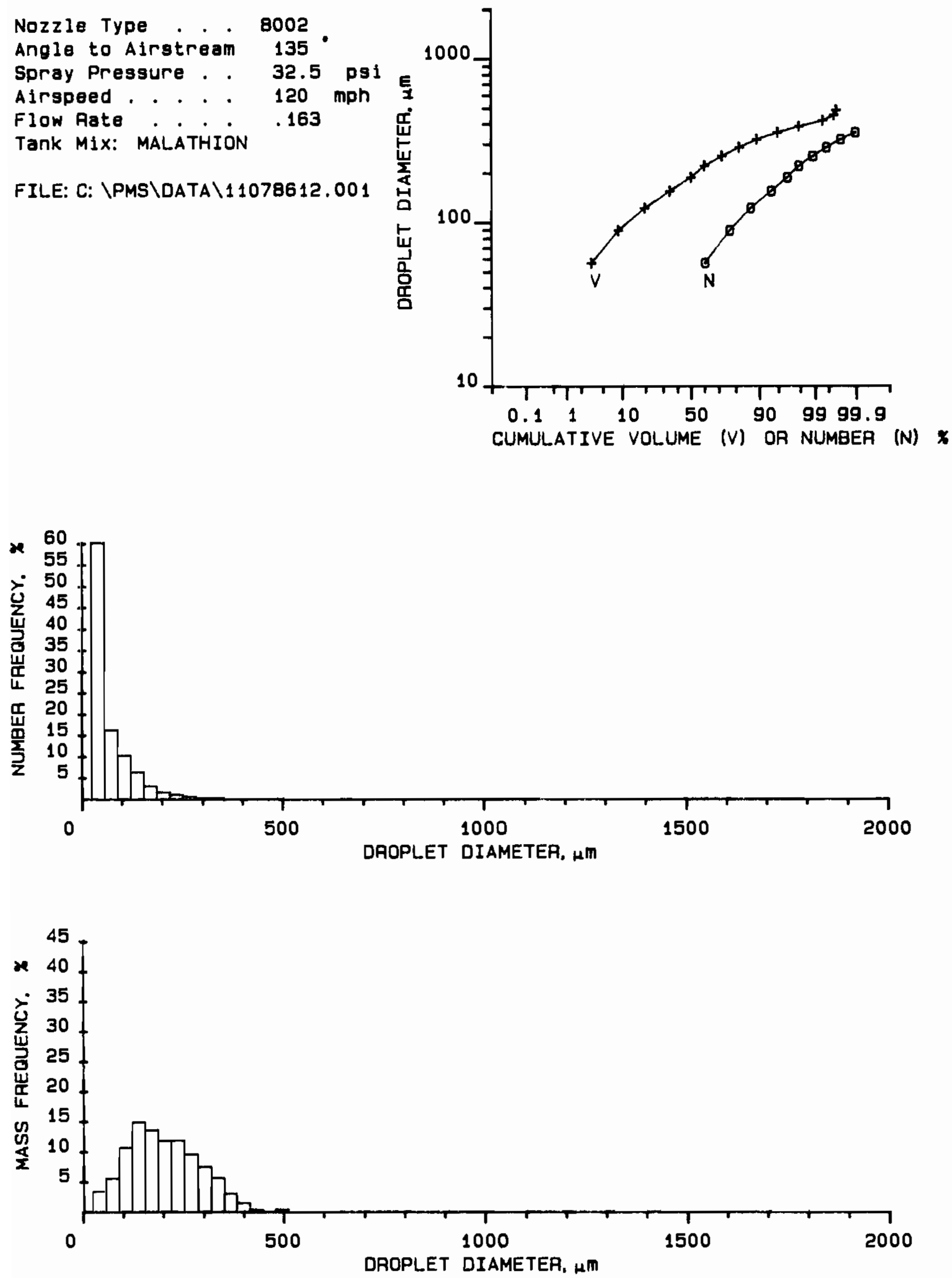

Figure 1.-Drop size spectrum from an 8002 nozzle directed 45 degrees down and forward into an airstream of 49.2 m/s with a neat malathion formulation at 224 kPa.

Table 1.-Drop Size Spectrum for 1986 Mosquito Trials with Aircraft.

Nozzle	8002	Slice Rate	4 MHz
Angle to Airstream	135 degrees	AVG	100
Spray Pressure	32.5 psi	DFM	1 cm
Airspeed	120 mph	BAR	1.5
Flow Rate	.163 gpm	Distance to Probe	46 cm
Tank Mix	MALATHION	Sample Interval	420 sec
		Number of Samples	1
		Number of Scans	12
FILE:	C:\PMS\DATA\11078612.001	Scan Spacing	2.5 cm
Number of Tests Combined:	1	Scan Length	40 cm
		Number of Sample Rings	0

UPPER LIMIT	N(RAW)	N/SEC	Gm/SEC	% N	% VOL	ACCUMULATED % N	ACCUMULATED %VOL
56	2712	7.21E+06	0.24	60.24	3.39	60.24	3.39
89	4724	1.95E+06	0.39	16.31	5.55	76.55	8.95
122	3287	1.23E+06	0.75	10.27	10.68	86.82	19.63
154	2577	761577	1.04	6.36	14.92	93.18	34.55
187	1901	367823	0.95	3.07	13.62	96.26	48.17
220	1367	189791	0.83	1.59	11.89	97.84	60.06
252	940	122094	0.83	1.02	11.91	98.86	71.96
284	538	66868	0.67	0.56	9.61	99.42	81.58
318	299	36497	0.52	0.31	7.51	99.73	89.09
351	181	20222	0.40	0.17	5.66	99.90	94.75
382	70	8388	0.21	0.07	3.07	99.97	97.82
414	29	3102	0.10	0.03	1.46	99.99	99.28
447	6	536	0.02	0.00	0.32	100.00	99.60
479	3	61	0.00	0.00	0.05	100.00	99.64
512	1	362	0.02	0.00	0.33	100.00	99.97
545	0	0	0.00	0.00	0.00	100.00	99.97
578	1	21	0.00	0.00	0.03	100.00	100.00
Total	1.86E+04	1.20E+07	6.99				

TOTAL ACCEPTED RAW PARTICLES / TOTAL IMAGES = 18636/ 22790 = 81.8%

NUMBER MEAN DIAM = D_{10} .....69.57 μm
VOLUME MEAN DIAM = D_{30} ....103.74 μm
SAUTER MEAN DIAM = D_{32} ....153.30 μm

NUMBER MEDIAN DIAM= $D_{N.1}$.......<56 μm
$D_{N.5}$.......<56 μm
$D_{N.9}$....138.10 μm

VOLUME MEDIAN DIAM= $D_{V.1}$.....92.19 μm
$D_{V.5}$....191.98 μm
$D_{V.9}$....323.61 μm

RELATIVE SPAN = 1.21

AGDISP

AGDISP (AGricultural DISPersal), is a trajectory model originally developed by NASA with recent modifications and enhancements undertaken by the U.S.D.A. and the U.S. Forest Service. AGDISP assumes that particles or droplets are influenced primarily by two forces; weight and aerodynamic drag. Aerodynamic drag is due to the difference in velocity between the droplet and the surrounding air. After an initial particle relaxation time, the particle, or droplet, is assumed to be travelling at the same speed and direction as the combination of ambient wind and the aircraft wake field. By following the motion of this field and time-stepping through the process from release to deposition, the mean path taken by the particle can be predicted. Turbulent fluctuations in the atmosphere can be approximated and used to calculate a Gaussian or normal distribution of deposition about that mean. By modeling the flight of all drop sizes from all nozzle positions, and summing the deposition from each, we can arrive at a simulated composite deposition. The results can be compared with field data.

AGDISP inputs include information concerning the characteristics of the aircraft, the atmosphere, the application under study, the droplets and the target. Aircraft inputs include the wingspan, the type of wing loading, the strength of the wing tip vortices, the drag coefficient of the aircraft, the propeller diameter, efficiency, rpm, and whether the aircraft is a monoplane or a biplane. Atmospheric parameters needed include the crosswind profile, the level of background turbulence present and the maximum length scale for that turbulence. The application characteristics used as input values are the height and speed of flight and the rate of decay of the aircraft-induced circulations.

Particle or droplet characteristics needed are initial droplet diameter, the specific gravity of the droplet, the initial position and velocity relative to the wing and whether or not evaporation is to be simulated. If evaporation of a water based spray is to be included in the model, the wet bulb depression must be included or the evaporation rate otherwise parameterized, as well as the minimum drop size, the diameter at which evaporation will cease. Other inputs required for AGDISP are the canopy height, the canopy density profile as a function of height and the height at which deposition is considered to have occurred.

The outputs from AGDISP can be used to plot the position of the droplets as a function of time. Deposition for each drop size can also be determined in units of either liters/hectare, or drops/square centimeter. Optionally, several drop sizes can also be combined to give a composite deposition representing the predicted deposit from a particular application.

VALIDATION STUDY METHODS & PROCEDURES

A field application was conducted on September 22, 1986 near Davis, CA. The field was flat, open ground with no vegetative cover. The roughness length of the field, z_0 was determined to be 0.004 meter. The flight path was 400 meters (0.5 mile) long and perpendicular to a sample line of collection devices. The sample line extended from the center of the flight line to 400 meters downwind.

Data collected from the field included deposition on 7.6 cm x 45.7 cm Mylar sample sheets, magnesium oxide coated microscope slides, Rotorods, Staplex high volume air samplers with glass fiber filters, and sentinel mosquitoes (*Culex pipiens* adults). The Mylar sheets and coated microscope slides were placed at ground level, and the Rotorods, Staplex air samplers, and the sentinel mosquitoes at a height of approximately 1.2 meters above the surface. Rotorods are a rotating "U"-shaped brass bar with height of 6 cm and face width of 0.159 cm. They are powered by a 6 volt dry cell battery. The Staplex air samplers are powered by 110 volts AC power and fitted with a 8.9 cm diameter glass fiber filter. In addition, at 50 meters downwind from the flight line, a tower 17 meters in height was fitted with eight samplers fitted with 5.9 cm diameter glass fiber filters. The tower provided a vertical profile of the spray cloud as it passed the 50 meter position.

Weather data was collected during the test from a 9.75 meter mast and a 4.8 meter mast. Data collected included a vertical profile of wind speed and temperature, wind direction, barometric pressure and wet bulb depression. The data was recorded for later analysis.

The aircraft used in this test was an Air Tractor AT-301 with a Pratt & Whitney R1340 engine. An independent spray system consisting of a CO_2 pressure tank, a 7.6 liter spray tank, a pressure regulator and a solenoid to control flow was fitted to the aircraft. A 1/2-inch I.D. hose supplied material to 6-8002 nozzles oriented 135 degrees into the airstream. Nozzle pressure was 224 kPa (32.5 psi). Material used was Cythion (Malathion, 91% a.i.). A flow rate of 617 ml/min/nozzle (0.163 gpm/nozzle) was obtained.

The drop size emitted from the nozzle was measured in our dropsize measurement facility using the Particle Measurement System OAP-2D-GA1, with a range of 28 to 2062 micrometers diameter. The drop size distribution is presented in Table 1 and Figure 1. The volume median diameter was 192 micrometers.

RESULTS AND DISCUSSION

The AGDISP program was used to simulate the field application. Additional specific inputs to the program are listed in Tables 2, 3, and 4. The simulation was run for the 12 drop sizes used to represent the emitted drop size spectrum, ranging from 42 micrometers to 398 micrometers. In addition, the VMD of 192 microns was also simulated. Trajectories were plotted for several drop sizes as well as the composite deposition in l/ha and drops/cm^2 for the entire drop size spectrum.

Figure 2 shows the predicted trajectories as computed by AGDISP for a drop of 192 micron

Ballistic or Trajectory Models.-Trajectory models simply sum the forces acting on a particle at any particular time to arrive at an equivalent acceleration vector acting on the particle. The acceleration vector has properties of magnitude and direction. By applying Newton's Second Law (F=ma) to the particle, and multiplying by the appropriate time interval, the position of the particle can be traced.

One version of this type of model is already available. NASA and the U.S. Forest Service have, over the past several years, funded the development of a computer program called AGDISP (AGricultural DISPersion), (Holmes 1978, Morris et al. 1982, Teske 1984, Bilanin and Teske 1984, and Teske 1986). The Agricultural Engineering Department of the University of California at Davis has obtained a copy of the most recent version of AGDISP, MOD 4.0, modified it to run on an IBM PC AT, and added the necessary screen and plotter graphics to interpret the output. Hardware requirements include an 80287 math co-processor, an Enhanced Graphics Adapter and Enhanced Color Display, and a Hewlett-Packard HP-7475A Plotter. A hard disk is also necessary in the AT for input and output data storage and manipulation.

Ballistic models require a large amount of detailed input and are highly dependent upon the accuracy of the input. They can give very detailed information as to the position of particles as a function of time, for the given set of input conditions, but do require large amounts of computer time and memory to operate. They are most appropriate for large particles, under the influence of highly complex wind fields, as would be found in an aircraft wake.

Similarity Theory.-Similarity theory, or dimensional analysis, has been successfully applied to several problems in the atmosphere, including dispersion from the point of release for a gas (Horst 1979). The principle of the model is that there are a limited number of variables that describe and control the motion of the atmosphere in the surface layer. By grouping these variables into dimensionless parameters, the theory says that we can describe other quantities, properly combined into non-dimensional parameters themselves, as functions of the dimensionless parameters. Similarity theory does not postulate as to the shape or form of those functions, only that they exist. The functions themselves must be empirically derived from experimental data.

This technique has been successfully applied to a non-reactive gas in the atmosphere. It may be applicable for the dispersion of a limited range of spray drop sizes. Different drop sizes may require different functions to describe their dispersion. This should most likely be a valid technique for the small drop sizes, of less than 20 micrometer diameter. At these sizes, settling velocity is very small compared to the atmospheric turbulence, and the theoretical restrictions on the model should be satisfied.

Particle-In-Cell Techniques.-This type of model sets up a grid of cells over the area of interest. The spray dispersion is then modeled by following a mass of droplets as they are advected and diffused through the grid. Concentration within any of the elements of the grid can be computed at any time. The technique has been applied successfully in the past to such diverse problems as a heavier-than-air gas release and also potential releases of nuclear waste and contamination.

Particle-in-cell models have the advantage of being able to model complex terrain within the grid and also to vary the wind field, handle area emissions, multiple sources, and changing atmospheric conditions. This technique does require a large amount of computer power, however, as the mass in each cell must be monitored for each time interval, the wind field must be updated, and the intermediate results must be checked and altered to account for such factors as deposition and adherence to the conservation of mass.

APPROPRIATENESS OF THE MODELS

Adulticiding for mosquito control can be divided into two distinct categories, those applications made from ground based equipment, and those made from aircraft. The two types of applications are vastly different and are controlled by different phenomena. Based upon the characteristics of each type of application, a selection as to the appropriate model can be made.

Ground Based Equipment.-Applications from ground equipment, such as cold foggers, are characterized by an initial velocity as the material leaves the atomizer, and the lack of any strong, imposed turbulent field from the application device itself. Such an application could most accurately be modeled by either similarity theory or a particle-in-cell model. Both of these models have been successfully used to model pollutants with characteristics similar to cold fogger adulticide application as it disperses from a ground source. These models are currently under further investigation and development at the Agricultural Engineering Department, University of California, Davis. The plan of work includes model development, coding and obtaining computer solutions, and validate the predictions by comparing them to experimental data collected from field studies.

Aerial Application.-During application by aircraft, the droplets emitted from the atomizers are subject to the complex wake field generated behind an aircraft. Commonly, these are larger drops and have a broader size range. For most of these drops, gravitational settling cannot be ignored and the aircraft wake will have a major effect on the drops. Clearly a more comprehensive and complex model must be employed. For these reasons, the trajectory or ballistic model is a good choice to adequately model the complex phenomena and force fields acting upon the droplets during the initial period, while other models may be useful after the aircraft wake has decayed.

MODELING THE BEHAVIOR OF A ULV ADULTICIDE APPLICATION

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ABSTRACT

The characteristics of an Ultra-Low Volume application for control of adult mosquitoes are discussed, along with the general concepts of modeling the dispersion of material in the atmosphere. Three types of models are described, and suggestions as to when each is an appropriate model to use are given.

One of the three types, AGDISP, a trajectory model, is described in more detail. Results of a field trial of an adulticide ULV application are given and compared to predictions about that application from AGDISP. Results are generally in good agreement and show promise of using AGDISP for computer analysis of applications.

INTRODUCTION

The atmospheric dispersion of a spray designed for control of adult mosquitoes is a complex phenomenon. The exact characteristics of the dispersion of each application depend upon many factors, some of which are under the control of the operator, and some of which are not. The combination of all forces acting upon the spray drops and the characteristics of the drop spectrum will determine their motion in the atmosphere.

Major variables under the control of the operator include: 1) the application rate and, 2) the drop size spectrum emitted (to the extent allowed by the given equipment). In addition, the applicator always has the option to not make the application, but once the application is underway, several factors are removed from the operator's control.

These factors outside of the control of the operator, include: 1) the atmospheric temperature profile, 2) the wind speed and direction, 3) the vertical wind profile, 4) the stability of the atmosphere, and 5) the operating and turbulence generating characteristics of the application equipment. The spray will also be affected by evaporation, gravity, chemical reactions, interaction with the plant canopy or an insect population, coagulation, Brownian diffusion, electrostatic attraction, interception, and impaction.

The resulting motion and behavior of the spray cloud is the result of all the forces acting upon the particles that make up the cloud. When adulticiding mosquitoes, several of the concerns mentioned above can be ignored. In order to model the motion of the cloud and the variations of concentration within the cloud, assumptions and simplifications must be made. Among the assumptions most often made are that the concentration of pesticide within the cloud is low enough to ignore impaction, interception, and coagulation of drops onto other drops. It is also commonly assumed that the pesticide does not change the ambient atmospheric conditions (temperature, relative humidity) inside the cloud. For an oil-based material, evaporation can be ignored as well. The resulting set of predominant factors influencing the droplets can be described as gravity, the induced force field due to an aircraft wake or initial velocity from a cold fogger and the atmospheric conditions.

MODELING TECHNIQUES AND APPROACHES

In order for a model to be useful in a management context, it must exhibit several characteristics. First, it must be realistic. This means that it must contain all pertinent factors in its formulation and must have the relationships between the variables correct. A useful model must also be simple to use. The intended users of the model must have access to the machines necessary to run the model. Similarly, the model must be designed to make sense to the intended user and to accept inputs from the users and provide output in a form easily interpreted.

Management models provide a forecast to the user. This forecast must be on the safe side, that is the model must be conservative in its predictions. Lastly, a good model should be general enough to be applicable over a wide range of possibilities and not be limited to use under a very narrow range of circumstances.

Several approaches to modeling the dispersion of material in the atmosphere can be taken. Most of the theoretical development and basic modeling techniques has been undertaken by persons interested in air pollution. Experimental work to validate models and test their applicability has been carried out for several different types of models. Other types of models have been explored by agricultural engineers and others interested in agricultural and forestry spray dispersion. Listed below are several of the general approaches that have been utilized or suggested and comments as to the advantages, disadvantages, applicability and limitations of each.

General reviews of modeling the dispersion of material in the atmosphere can be found in such references as Nieuwstadt and van Dop (1982), Pasquill and Smith (1983), Panofsky and Dutton (1984), and Seinfeld (1986).

Table 11.-Percent mortalities of caged *Cx. pipiens* (Lab) and *Cx. tarsalis* (Wild) following nonthermal aerosol applications of malathion, Colusa MAD, August 12 and 27, 1986.

	12 hour mortalities (%)					
	95% Malathion		95% Malathion		56% Malathion	
Distance (ft)	Lab	Wild	Lab	Wild	Lab	Wild
0	100	100	100	87	100	31
200	100	90	100	69	100	77
400	100	73	100	80	97	32
600	100	59	100	68	95	46
1320	100	57	100	55	96	16
2640					100	44
3960					70	27
5280					71	48
Control	3	25	3	25	10	3
lb AI/100 ft		0.124	0.087			0.033
Wind velocity (mph)		4	4			1
Inversion (°F)		0.2	0.3			3.0
Stability ratio		0.6	0.8			100

ACKNOWLEDGMENTS

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Table 9.-Mortality (%) of caged *Cx. pipiens* and *Cx. tarsalis* following nonthermal application of permethrin, Colusa MAD, July 8, 1986.

	12 hour mortality (%)	
Distance (ft)	*Cx. pipiens*	*Cx. tarsalis*
0	100	100
200	100	100
400	100	100
600	100	100
1320	100	100
2640	69	73
3960	27	50
5280	23	55

Wind velocity (mph)	1-2
Inversion (°F)	1
Stability ratio	13

Table 10.-Mortalities (%) of *Ae. nigromaculis* (Wild) and *Cx. pipiens* (Lab) following nonthermal aerosol application of chlorpyrifos, Zumwalt pasture, Colusa MAD, June 26, 1985.

	12 hour mortality (%)			
	Line A		Line B	
Distance	Lab	Wild	Lab	Wild
0	100	86	100	62
200	100	75	92	68
400	100	71	88	61
600	100	24	100	71
800	88	65	100	78
1000	94	61	100	94

Wind velocity (mph)	1
Inversion (°F)	0.5
Stability ratio	15

Table 7.-Mortality (%) of caged *Cx. pipiens* and *Ae. melanimon* at selected sites following nonthermal aerosol application of 2 fl oz/min undiluted Scourge[TM], Big Pine, September 24, 1985.

		12 hour mortality (%)			
		Line 1		Line 2	
Distance (ft)	Site	Lab	Wild	Site	Lab
0	Open	100	100	Open	100
150	Creek	60	100	-	-
200	Fence	100	100	Mid block	100
300	Backyard	60	73	-	-
350	Side yard	96	100	Open	100
350				Shrubbery	96
350				Shrubbery	43
375				Open	64
400	Open	100	100	Open	100
Wind velocity (mph)			1		
Inversion (°F)			1		
Stability ratio			25		

Table 8.-Percent mortality of caged mosquitoes following nonthermal aerosol application of 2 fl oz/min Scourge[TM], (1.51 lb resmethrin/gal), Owens River, Inyo County, September 25, 1985.

		12 hour mortality (%)	
Distance (ft)	Placement	*Cx. pipiens*	*Ae. melanimon*
0	Open, top of bank	100	
100	Open, over bank	100	100
150	Under trees	87	
170	Grass, water area	100	
200	Road, 4 ft vegetation	100	
300	Road, 4 ft vegetation	57	100
400	Road, 4 ft vegetation	46	75
400	Road, 4 ft vegetation	41	
450	Trees	10	
500	Trees, at river	20	
Control		0	0
Inversion (°F)	1		
Wind velocity (mph)	0.5 (Slight drift toward river)		
Stability ratio	100		

Table 6.-Percent mortalities of caged *Cx. pipiens* and *Ae. melanimon* following nonthermal aerosol application of resmethrin, Inyo County, September 23, 1985.

Distance (ft)	12 hour mortality (%) *Cx. pipiens*	*Ae. melanimon*
0	-	10
500	100	100
750	100	100
1000	95	100
1500	8	58
2000	17	-
Wind velocity (mph)	2 - 3	
Inversion (°F)	9	
Stability ratio	50	

Three applications of Scourge™ were made in three areas of Inyo County in September, 1985: a pasture; a small community; and a river bottom wooded area. Table 6 presents data on the pasture application against caged *Cx. pipiens* (VSCB lab) and wild *Ae. melanimon*. Scourge™ was diluted 1:6 with diesel oil and applied at 7 fl oz/min. The vehicle speed was 5 mph and the dosage rate was calculated at 0.00306 lb AI/100 ft of vehicle travel. Both mosquito species responded equally to the application. High mortalities were obtained to 1,000 feet. Unexpected cross-winds caused an insecticide "miss" on the lower end of the pasture and the caged mosquitoes.

Scourge™ was applied undiluted at 2 fl oz/min in the town of Big Pine on September 24, 1985. The vehicle speed was 5 mph and the resmethrin application was 0.0054 lb AI/100 ft. Two lines of both *Cx. pipiens* (VSCB) and wild *Ae. melanimon* were set through residential areas to a distance of 400 ft downwind from the line of application. Table 7 shows mortality of the mosquitoes at 12 hours. Both species responded equally to the applications. Average mortality for all cages (96% *Aedes* and 86% *Culex*) indicated good kills in the open at 400 ft and a fair amount of shrubbery penetration at 350-375 ft.

Scourge™ was applied to a wooded and weed-covered area along the Owens River bottom on September 25, 1985 to evaluate penetration of the vegetation. Two fluid ounces of undiluted Scourge™ were applied at a vehicle speed of 5 mph (0.0054 lb AI/100 ft). Both laboratory reared *Cx. pipiens* and wild *Ae. melanimon* were used during this application. However, only 4 cages of *Ae. melanimon* were filled that evening. Twelve hour mortalities and cage placements are shown in Table 8. Penetration to 300 feet under these conditions was encouraging.

Permethrin.-One experimental application of permethrin, (Pramex 57%; 5 lb AI/gal), was made on a test line north of Colusa. The material was diluted 1:1 with a light viscosity oil and applied at 4.25 fl oz/min. The vehicle speed was 7 mph and the dosage rate was 0.0135 lb AI/100 ft. Both *Cx. pipiens* (VSCB lab) and wild *Cx. tarsalis* were used in this run. Twelve hour mortalities of caged mosquitoes and weather data are shown in Table 9. Mortalities of 100% were observed on both species for a distance of 0.25 mile.

Chlorpyrifos.-Dursban® Mosquito Fogging Concentrate (6 lb chlorpyrifos/gal) was diluted with a light viscosity oil to a 1 lb AI/gal field formulation and applied to the Zumwalt pasture, Colusa MAD on June 26, 1985. The vehicle speed was 6 mph and output rate was 12 fl oz/min (0.0178 lb AI/100 ft). This formulation and the output rate have been operational at Colusa for over ten years. Both field collected *Ae. nigromaculis* and laboratory reared *Cx. pipiens* were used on this test. Cages were placed in two lines across the pasture at 200 ft intervals to a distance of 1,000 feet. Mortalities of the caged mosquitoes are shown in Table 10.

An overall mortality of 97% was observed in the *Cx. pipiens* with 100% mortality up to 1,000 feet. The average mortality for *Ae. nigromaculis* was 68% with no 100% mortality being observed. This indication of chlorpyrifos resistance in *Ae. nigromaculis* prompted further test work with malathion.

Malathion.-Nine applications of undiluted malathion at various flow rates and vehicle speeds were made on test lines against *Cx. pipiens* (VSCB lab) and wild *Cx. tarsalis*. Both 95% and 56% malathion were used in these test lines north of Colusa, Colusa MAD during the 1986 season. The two highest dosages and the lowest dosage applied will be reviewed. Mortalities of the caged mosquitoes are shown in Table 11. High control mortalities of 25% occurred on the 1/4 mile runs and may have been due to holding the wild mosquitoes for 3 days prior to the tests.

These tests produced high mortalities in the laboratory-reared, susceptible *Cx. pipiens* and unacceptably low mortalities in the wild *Cx. tarsalis*. The nine malathion runs at Colusa gave clear evidence of malathion resistance in adult *Cx. tarsalis*.

DISCUSSION AND CONCLUSIONS

Under the considerable threat of adult mosquito resistance, it is necessary to include wild caught target species in nonthermal aerosol application evaluations. New chemicals to replace those to which mosquitoes have developed resistance are slowly becoming available. Several materials were evaluated which produced excellent operational control and high mortalities in test lines. It is evident, however, that further evaluations are necessary to insure operational success.

Table 4.-Percent mortality of caged *Cx. pipiens* at 3 output rates of resmethrin (undiluted Scourge™) applied by cold fogger, Colusa MAD, June 4 and 5, 1985.

Distance (ft)	12 hour mortality (%) at output/min: 4.3 fl oz	3.8 fl oz	1.1 fl oz
0	100	100	100
100	100	100	100
200	100	100	100
300	100	100	100
400	100	100	100
600	100	100	100
1320	100	100	0
2640	71	97	0
3960	97	7	0
5280	82	23	0
Control	0	3	0
Inversion°F	0.5	0.5	0.5
Wind velocity (mph)	4	3	6
Stability ratio	0.8	3	0.5
lb AI/100 ft	0.0043	0.0041	0.0007

Table 5.-Mortalities of caged *Cx. pipiens* (Lab) and *Cx. tarsalis* (Wild) following nonthermal aerosol applications of Scourge™ (1.51 lb resmethrin/gal), Colusa MAD, July 7 and August 12, 13 and 27, 1986.

	12 hour mortality (%)							
	7-7-86		8-12-86		8-13-86		8-27-86	
Distance(ft)	Lab	Wild	Lab	Wild	Lab	Wild	Lab	Wild
0	100	100	100	100	-	100	100	100
200	100	100	92	54	-	100	100	79
400	100	100	89	63	-	100	94	71
600	47	29	91	33	-	64	100	100
1320	40	76	82	29	-	23	44	53
2640	50	41	-	-	-	-	-	-
2960	33	7	-	-	-	-	-	-
5280	17	37	-	-	-	-	-	-

	7-7-86	8-12-86	8-13-86	8-27-86
Dilution	1:9	1:9	1:7	1:7
lb AI/gal	0.15	0.15	0.19	0.19
Output (fl oz/min)	4.25	4.1	4	8
Vehicle speed (mph)	8	8	8	8
lb AI/100 ft	0.00071	0.00069	0.00084	0.00169
Inversion (°F)	1	2	0.8	0.4
Wind velocity (mph)	1	1	1	4
Stability ratio	29	70	25	1.0

Table 2.-Results of field applications of bendiocarb against *Cx. pipiens* (Lab) and *Ae. nigromaculis* (Wild) at Fitzpatrick pasture, Shasta MAD, July 10, 1985.

		Average Cage Mortality (%)		Leg Counts		
Line	Swath	Lab	Wild	Pre	Post	% Reduction
A	900 ft.	97	94	10	2	80
B	600 ft.	100	100	50	20	60
C	800 ft.	100	100	50	1	98

of 100 percent were obtained at distances up to 1,300 ft downwind. The application rate was calculated at 0.0056 lb AI/acre.

One operational application of bendiocarb was made on the Fitzpatrick pasture, Shasta MAD on the evening of July 10, 1985. The pasture was traversed at three intervals to make swath widths of 900 ft, 600 ft and 800 ft. Both wild *Ae. nigromaculis* and laboratory reared *Cx. pipiens* were caged and placed at 200 ft intervals parallel to the wind direction and perpendicular to the line of application. Undiluted Ficam® ULV was applied at 3 fl oz/min at a vehicle speed of 5 mph (0.0089 lb AI/100 ft). Wind speed was 1-2 mph with an inversion of 1.2°F. The average mosquito mortalities for lines A, B and C are shown in Table 2. The overall mortalities for the caged wild and laboratory reared mosquitoes were 98% and 99% respectively.

Pre- and post-application leg counts (Table 2) showed an overall reduction of 80% in the field population. The relatively low reduction of the wild mosquitoes indicated by the pant leg count as compared to high mosquito mortalities in the cages was at least partially due to a continuous hatch of mosquitoes in a nearby pasture.

Three test line applications of Ficam® ULV, were made in the rice country north of Colusa on June 4 and 5, 1985. Susceptible *Cx. pipiens* (VSCB lab) were used in all tests. Cages were set out for a distance of one mile downwind. Mortality of the caged mosquitoes, output rates and weather data are shown in Table 3. Atmospheric stability ratios varied widely on runs 1 and 2. A 27-fold increase in the stability ratio (S.R.) produced a 300% increase in swath width as measured at the 100% mortality point downwind. Line 3 with a doubled output rate of 8 fl oz/min effectively doubled the swath. The necessity of proper atmospheric conditions for aerosol application and the need for these measurements is indicated by these data.

Resmethrin.-Three test line applications of undiluted Scourge™, (1.51 lb resmethrin/ gal), were made northeast of Colusa on June 4 and 5, 1985. Susceptible *Cx. pipiens* (VSCB lab) were used in all lines. The resmethrin was applied at output rates of 4.3, 3.8 and 1.1 fl oz/minute. Mortalities of the caged mosquitoes are shown in Table 4. Applications at 4.3 and 3.8 fl oz/min were made in an effort to determine an effective dosage for wide swath field applications. In spite of relatively low stability ratios during these applications, the high level of control out to 1/2 and 3/4 mile was encouraging. Under more stable atmospheric conditions, these outputs would be operational. The 1.1 fl oz/min output was tried in an effort to determine a low dosage for in-town operations. One hundred percent control at 600 ft would be satisfactory, even at the 0.8 S.R. during the application. However, the small orifice of the in-line disk needed for the one ounce output rate caused problems by plugging.

Resmethrin was applied in test lines north of Colusa on the evenings of July 8, August 12, 13 and 27, 1986. Scourge™ was diluted 1:9 and 1:7 with a light viscosity oil. Laboratory reared susceptible *Cx. pipiens* and wild *Cx. tarsalis* were used in these tests. Mortalities of the caged mosquitoes, application data, and weather data are shown in Table 5.

In all Colusa tests, both diluted and undiluted resmethrin was equally effective against susceptible *Cx. pipiens* and malathion resistant *Cx. tarsalis*.

Undiluted Scourge™ was applied against laboratory reared *Cx. pipiens* and chlorpyrifos resistant *Ae. nigromaculis* on the Zumwalt pasture, Colusa MAD on June 26, 1986. Two lines of caged mosquitoes were placed across the pasture. Cages were placed at 200 ft intervals to a distance of 1,000 ft. The output rate was 4.25 fl oz/min and the vehicle speed was 6 mph, which corresponds to 0.0095 lb AI/100 ft of vehicle travel. A wind velocity of 2.5 mph and inversion of 4°F gave a stability ratio of 25. One hundred percent mortality was observed in all cages.

Table 3.-Application data and mortalities of caged *Cx. pipiens* following three applications of bendiocarb, (undiluted Ficam® ULV), Colusa MAD, June 4 and 5, 1985.

Distance (ft)	12 hour mortality (%)		
	Run 1	Run 2	Run 3
0	100	100	100
100	100	100	100
200	100	100	100
300	89	100	100
400	35	97	100
600	41	100	100
1320	0	20	92
2640	0	0	66
3960	0	0	8
5280	0	-	0
Control	0	1	0
Output rate (fl oz/min)	4.1	3.9	8.0
Wind velocity (mph)	5	2	2
Inversion (°F)	0.2	1.0	0.5
Stability ratio	0.3	8.0	4.0
lb AI/100 ft	0.0051	0.0053	0.0132

EFFECTS OF NONTHERMAL AEROSOL APPLICATIONS OF SELECTED CHEMICALS AGAINST CAGED MOSQUITOES

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INTRODUCTION

Over the years, the village of Colusa (population 4,210), has been periodically infested with (fogger) researchers: Akesson et al. (1971), Womeldorf et al. (1973), Townzen (1975), Lusk et al. (1976), and Atkins et al. (1981), who have been in search of a way, or a better way. Other contributors to the Colusa experience have included G. Mount, P. Gillies, E. Fussell, S. Husted, J. Mulrennan, R. Washino and E. Zboray and other District personnel. Whitesell (1973, 1984) described the Sacramento Valley area of Colusa, the equipment, operations and techniques of cold fogging. At times, Colusa MAD's personnel have cooperated in studies in other geographic areas.

Based on the continuing "need-to-know" criteria, efficacy data were gathered on several chemicals during the 1985 and 1986 mosquito seasons. Test line and operational field cold-fogger applications were made against field collected and laboratory reared mosquitoes in cooperation with the Colusa, Burney Basin, and Shasta MADs and the Inyo County Health Department. Test results on bendiocarb, resmethrin, permethrin, chlorpyrifos, and malathion are presented in this paper.

METHODS AND MATERIALS

Vehicle mounted cold foggers were used in all tests. Colusa and Shasta MADs' units were home built, utilizing Roots or Cummins blowers powered by Crosley engines and equipped with AFA venturi assemblies. Insecticides were supplied to the venturi assemblies by pressurizing the insecticide tank with air bled from the blower. Flow rates were maintained by Spraying Systems Co. TeeJet® interchangeable orifice plates inserted into the chemical line as flow restrictors. Various orifice sizes were utilized to maintain the desired flow rate. Applications at Burney Basin MAD and in Inyo County were made with commercial Microgen® foggers. Various formulations and chemical concentrations were used, therefore, each application will be described separately. Flow rates, vehicle speeds and weather conditions will be described. Also, a common figure of pounds of active ingredient delivered during 100 ft of spray vehicle travel (lb AI/100 ft) will be used.

Mortalities of caged mosquitoes and leg count reductions were used as evaluation tools. Disposable paper and nylon net cages as described by Townzen and Natvig (1973) were used. Susceptible *Culex pipiens* (VSCB Sacramento Laboratory Colony) were used as air samplers and indicated operational effectiveness of the application. Native *Aedes spp.* or *Culex tarsalis* were collected by aspiration or CDC trapping and used as indicators of the operational effectiveness of the chemical used. Approximately 20 mosquitoes were placed in each cage. Portable weather instrumentation was maintained at each site and runs were made only under acceptable conditions.

DESCRIPTION AND RESULTS

Bendiocarb.-Operational field runs with bendiocarb were made at Goose Valley (Burney Basin MAD) and Fitzpatrick Pasture (Shasta MAD). Test lines were run in the rice country 5 miles northeast of Colusa, Colusa MAD.

Two runs were made in the north portion of Goose Valley, a 5,000 acre mountain valley in northern California. Weather conditions were optimal on both evening runs, wind velocity was 1-5 mph with an inversion of 3-6°F. Ficam® ULV, (1.67 lb bendiocarb/gal), was applied undiluted at 5 fl oz/min and the vehicle speed was 5 mph (0.0148 lb AI/100 ft). On the first night, 43 cages were dispersed over 23 sites to cover the target area. Twenty-three cages at 12 sites were used the second night. The average mortality of the caged *Cx. pipiens* (VSCB lab), wild *Ae. nigromaculis* and pre- and post-leg counts of the wild mosquitoes are shown in Table 1. Mortalities

Table 1.-Results of field applications of bendiocarb against *Cx. pipiens* (Lab) and *Ae. nigromaculis* (Wild) at Goose Valley, Burney Basin MAD, June 10 and 11, 1985.

	Cage Mortality (%)		Leg Counts		
Date	Lab	Wild	Pre	Post	% Reduction
6-10-85	85	-	500	25	95
6-11-85	100	99.5	25	1	96

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dipping and mortalities of sentinel larvae placed in the fields. The mean reduction of sentinel larvae was 96% for the 1200 ITU material and 97% for the 600 ITU material.

REFUGE AND PASTURE STUDIES

Two other habitat types the District treated with B.t.i. this year were wildlife refuges and irrigated pastures. Formulations of B.t.i. included a commercially obtained aqueous suspension and a sand core granule produced by the District using purchased technical grade powder. A total of 117 wildlife refuge ponds were treated by both air (3) and ground (114) applications, which amounted to 905 acres. There were 6 pasture treatments by both air (2) and ground (4) applications, which amounted to 617 acres.

The pre-inspections, treatments, and post-inspections made by operational District personnel totaled 213 hours and cost $1,540 in wages. Air applications were performed by Micro Spray at a cost of $1.62 per acre, totaling $715. One granular application was performed without cost by Butte County Mosquito Abatement District. Ground applications were made by District personnel using two newly acquired pieces of equipment: a Birchmeier® backpack blower ($550) and Billy Goat® blower modified for granular applications ($541).

Operational bioassays using cups of lab-reared *Culex pipiens* larvae resulted in an adjustable swath width, from 5 to 100 feet from both Birchmeier® and Billy Goat® blowers.

Sampling-mandated treatments for each habitat were assessed individually and took into consideration: impact on populated areas; availability of aircraft, personnel, and material; density of larvae present; and the cost of larviciding versus adulticiding.

Aircraft applications of B.t.i. were performed with both aqueous suspensions of 600 ITU material, and 4% sand core granules formulated by the District from 5000 ITU technical powder. The B.t.i. in aqueous suspension was applied at 16 ounces per acre to both refuge and pasture habitats. A total of 55 gallons of material was used at a cost of $536 ($9.75/gal.). The granules were applied at 5 pounds per acre to one pasture. A total of 1000 pounds of granules were applied at a cost of $0.55 per pound, which included the cost for formulation labor and materials.

Ground applications of B.t.i. were performed using the District's 3 or 4% sand core granule formulated from 5000 ITU technical powder. The application rate of B.t.i. in refuge habitats ranged from 5 to 8 pounds per acre with 3% material, and 7 to 20 pounds per acre with 4% material. Most refuge applications were made using approximately 6 pounds per acre with 3% material. The application rate of B.t.i. granules in pastures was 6 pounds per acre of 4% material. A total of 7,494 pounds of material was used in ground applications to both pastures and refuges. The cost of 3% granules was $0.43 per pound, which included all formulation costs.

Overall, the B.t.i. in aqueous suspension applied by aircraft to both refuge and pasture habitats didn't perform adequately. The 16 ounce per acre application rate may not have been high enough given the high density of larvae present. The B.t.i. granules, however, performed well on both refuge and pasture habitats. The mean reduction in larvae for refuge habitat treated with B.t.i. granules was 86%. The mean reduction in larvae for pasture habitat treated with B.t.i. granules was 96% by ground application and 93% by aircraft application.

A study was conducted near the end of September to determine the longevity of B.t.i. granules applied to pre-flooded refuge ponds and pastures. The District's greenhouse was used to simulate the hot, humid conditions associated with these habitats. Our test showed that 3% B.t.i. sand core granules remained viable up to six days prior to irrigation. No granules were tested longer than this six-day period. More testing will be needed to determine if viability extended longer than six days.

In late September a side-by-side test was conducted to contrast granules formulated with Microbial Resources technical powder (Skeetal®) verus Abbott Laboratories technical powder (Vectobac AS®). The Skeetal® was formulated into a 5.3% sand core granule with 3750 ITU technical powder. The Vectobac AS® was formulated into a 3% sand core granule with 5000 ITU technical powder. Both materials were applied at 7.3 pounds per acre to a refuge pond at Sutter National Wildlife Refuge where *Aedes melanimon* larvae were present at a density of 30 per dip. The mean reduction in larvae for the Skeetal® granules was 66% and for the Vectobac® granules, 85%.

CONCLUSIONS AND RECOMMENDATIONS

B.t.i. has been demonstrated to be very effective against the major problem species of mosquitoes in the District.

In 1987, efforts will be made to improve the results of the research through: improvements in supervision; increased training of field personnel; re-evaluation of threshold limits; and further studies of the impact of treating all sources in a given area; employing light trap and biting count analyses.

With the planned changes in this program, modest increases in personnel, vehicles and larvicides are anticipated.

A PROGRESS REPORT ON THE USE OF B.T.I. IN VARIOUS HABITATS OF SUTTER-YUBA MOSQUITO ABATEMENT DISTRICT

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1986 B.T.I. PROGRAM

An operational program of mosquito control utilizing the bacterial agent *Bacillus thuringiensis israelensis* (B.t.i.) was initiated at the Sutter-Yuba Mosquito Abatement District for the 1986 season. Applications were made in both Sutter and Yuba Counties emphasizing the rice habitat. Approximately 52,000 acres of various source types were treated throughout the District. The text immediately following will address our rice field applications. Other habitat treatments will follow this discussion.

RICE STUDIES

A district-wide total of 596 rice fields (50,340 acres) was dip sampled for mosquito larvae every 7 to 10 days. These fields were edge dipped for ease of access, speed, and to minimize any conceivable damage to the rice stands. Dipping was always confined to four previously selected stations, with ten dips taken about each station. A limited study comprised of six fields showed no significant differences between edge dipping and dipping throughout the fields. Inspections were made at 24 and 48 hours prior to and after applications. Midway through the season, larval mortality increased between 24 and 48 hours post-application. Subsequent post-inspections were extended to 48 hours.

A sampling crew of 11 temporary employees hired in late May worked until early August for salaries totaling $24,798 (4,631 hours). Five vehicles were used, each with two assigned dipping personnel.

Maps identifying rice growers, fields, acreages, and locations were made using information obtained from county agricultural permits. Rice fields were plotted onto District zone maps for record keeping and use by dipping personnel. All rice fields were apportioned into six geographical subdivisions and mapped. These six maps were used by the pilot during applications.

MATERIALS AND METHODS

All rice fields in 1986 were aircraft-treated. The bidded contract was awarded to Micro Spray of Marysville, California, which employed a Piper Pawnee 250®. The aircraft had an average application speed of 105 mph and a payload capacity of 150 gallons. The cost for the applications, which included the adaptation of the District's Mini Micronair® spray system ($2,000-1985) and associated maintenance time, amounted to $1.02 per acre. The cost for all rice field applications totaled $49,402.

The initial sampling threshold mandating treatment was eight larvae per 100 dips. In mid-July the threshold was changed to two larvae per 100 dips due to the large numbers of rice field mosquitoes present in light traps and associated service requests.

Calibration of the aircraft was performed by Micro Spray and verified by the District several times during the season. The swath width of the aircraft was determined by bioassay using cups of lab-reared *Culex pipiens* larvae. The initial flight tests in June resulted in a 150 foot swath width and a decision was made to fly 120 foot swaths in the field to assure adequate coverage. Subsequent testing in mid-July found that wind currents as low as 1 mph caused downwind drift, as much as 50 feet with the aircraft flying at an altitude of 25 feet. This drift was compounded by the small size of the droplets produced by the Micronair® atomizers. A decision was made to increase the droplet size by slowing the speed of the rotary atomizers as instructed in the Micronair manual. Treatment efficacy data indicated that this adjustment decreased the drift problem. The pilot also made adjustments on his headlands pass at the upwind side of the fields.

B.t.i. in both an aqueous suspension and technical powder formulation was purchased from three different manufacturers during the 1986 season. These manufacturers were Abbott Laboratories (Vectobac-AS®, Vectobac technical powder®); Microbial Resources (Skeetal®); and Zoecon (Teknar®). The application rate was 4.5 ounces per acre of an aqueous suspension of 600 ITU (International Toxic Units) material, in all instances. A total of 48,547 acres of rice were treated with 1,707 gallons of B.t.i. at a cost of $16,641 ($9.75/gal.).

Analysis of dipping data showed a mean reduction in larvae of 69%. When the dipping station on the upwind side of the field was not included in the treatment efficacy calculation, an 87% mean reduction in larvae resulted.

With regard to treatment efficacy, New Jersey light trap data collected within the B.t.i. treatment area provided inconclusive results. The 1986 season showed higher numbers of rice field mosquitoes when compared to previous years.

The District performed a field trial between two B.t.i. formulations for Abbott Laboratories. In this test 1200 ITU material applied at 2.25 ounces per acre was compared side-by-side with a 600 ITU material applied at 4.50 ounces per acre. The treatment efficacy was the same for each material, as indicated by pre- and post-treatment ies, but excellent control of the three species studied was obtained at rates ranging from 1.0-1.5 lb/acre (Mulla et al. 1986 and unpublished data).

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Table 6.-Evaluation of *B. sphaericus* 2362 formulations against *Culex* mosquito larvae in experimental ponds.

Formulations	Rate lb/acre	Larvae/5 dips pretreatment	(%) Reduction after treatment (days) 2	7	14
		UC Riverside (Sept., 1986)[a/]			
BSP-2	0.25	126	95	86	87
liquid	0.50	148	99	92	91
	1.00	120	100	85	76
ABG-6184	0.05	83	100	77	82
primary powder	0.10	142	100	99	97
Check	--	148	0	0	0
		Coachella Valley (Oct., 1986)[b/]			
BSP-2	0.25	12	4	38	0
liquid	0.50	18	83	82	21
Low-spore liquid	0.25	27	91	97	47
conc.	0.50	19	100	96	60
High-spore liquid	0.25	23	57	93	76
conc.	0.50	34	98	95	71
ABG-6184	0.05	30	85	97	89
primary powder	0.10	30	100	97	74
Check	--	23	0	0	0

[a/] Population *Cx. peus* (95%), *Cx. tarsalis* (5%). Water temp. 20-30°C.

[b/] Population *Cx. tarsalis*. Water temp. 17-24°C.

Table 5.-Evaluation of granular formulations of *B. sphaericus* 2362 against *Culex* mosquito larvae in experimental ponds.

Formulations	Granules lb/acre	Larvae/5 dips pretreatment	(%) Reduction after treatment (days) 2	7	14
		Coachella Valley (May, 1986)[a]			
ABG-6185	1.0	20	96	56	0
(3 mm)	2.0	93	100	95	69
lot 87/009 BR	4.0	49	100	96	94
ABG-6185	1.0	26	100	81	23
(1 mm)	2.0	53	100	88	0
lot 87/010 BR					
ABG-6185	1.0	38	98	51	0
(1 mm)	2.0	59	100	89	90
lot 87/034 BR	4.0	84	100	84	73
ABG-6185	1.0	26	100	62	0
(1 mm)	2.0	74	100	94	98
lot 88/039 BR	4.0	90	100	98	96
Check	--	23	0	4	0
		UC Riverside (June, 1986)[b]			
ABG-6185 (3 mm)	0.5	21	88	0	--
lot 87/009 BR	1.0	92	99	61	--
ABG-6185 (1 mm)	0.5	33	100	27	--
lot 88/034 BR	1.0	14	100	0	--
ABG-6185 (1 mm)	0.5	21	98	0	--
lot 88/039 BR	1.0	17	100	0	--
Check	--	12	0	17	--

[a] Population *Cx. tarsalis*, water temp. 22-35°C.

[b] Population *Cx. peus* (53%), *Cx. tarsalis* (30%), *Cx. quinquefasciatus* (17%).

Water temp. 22-31°C.

Figure 6 is a plot, on the same scale, of actual field data for the trial conducted on September 22, 1986. Malathion deposited on the Mylar sheets has been converted to l/ha and plotted versus distance from the flight line. One value is off scale, and corresponds to the exact downwind location (24 m) predicted for the peak deposit.

Of particular note is the excellent agreement in the general shape of the predicted and actual deposits. The field data shows a strong peak at 24 meters, a "hole" at 80 meters, secondary peaks at 90 to 140 meters, and much lower deposits on out to 400 meters. The vertical scale shows good agreement on the magnitude of the secondary peaks, and, with the exception of the magnitude of the off-scale value, a good representation of the primary deposit also.

Figure 7 again shows the deposits predicted by AGDISP, but in units of drops/cm^2. If this is the method by which the pest is controlled, this prediction would be much more important. In this case, the pattern maintains a higher value farther away from the flight line. The smaller drop sizes, while representing a small fraction of the total mass, can still contribute many drops, as the mass per drop increases as a function of diameter cubed.

This scale emphasizes the problem with discrete drop sizes as opposed to a continuous spectrum. The peak centered at 150 meters is from a 42 micron drop, while the peak centered at 120 meters is due to drops of 72.5 micron diameter. Obviously, a smooth, continuous spectrum between these two sizes would result in a smooth deposition over that interval, instead of the two peaks with the "hole" between them.

This trial resulted in 100% mortality of the sentinel mosquitoes at 18 meters and beyond, with 0% mortality from the centerline to 18 meters.

SUMMARY

An overall view of atmospheric dispersion of sprays has been presented, along with a general description of some commonly used approaches to modeling the process. Similarity theory models and particle-in-cell models show promise from modeling the release from ground based equipment, such as cold foggers, for adulticiding with a ULV application. Trajectory, or ballistic models have been shown to model the important processes in flight of droplets released from aircraft.

The AGDISP model has been described as one choice for a trajectory model. Its inputs and

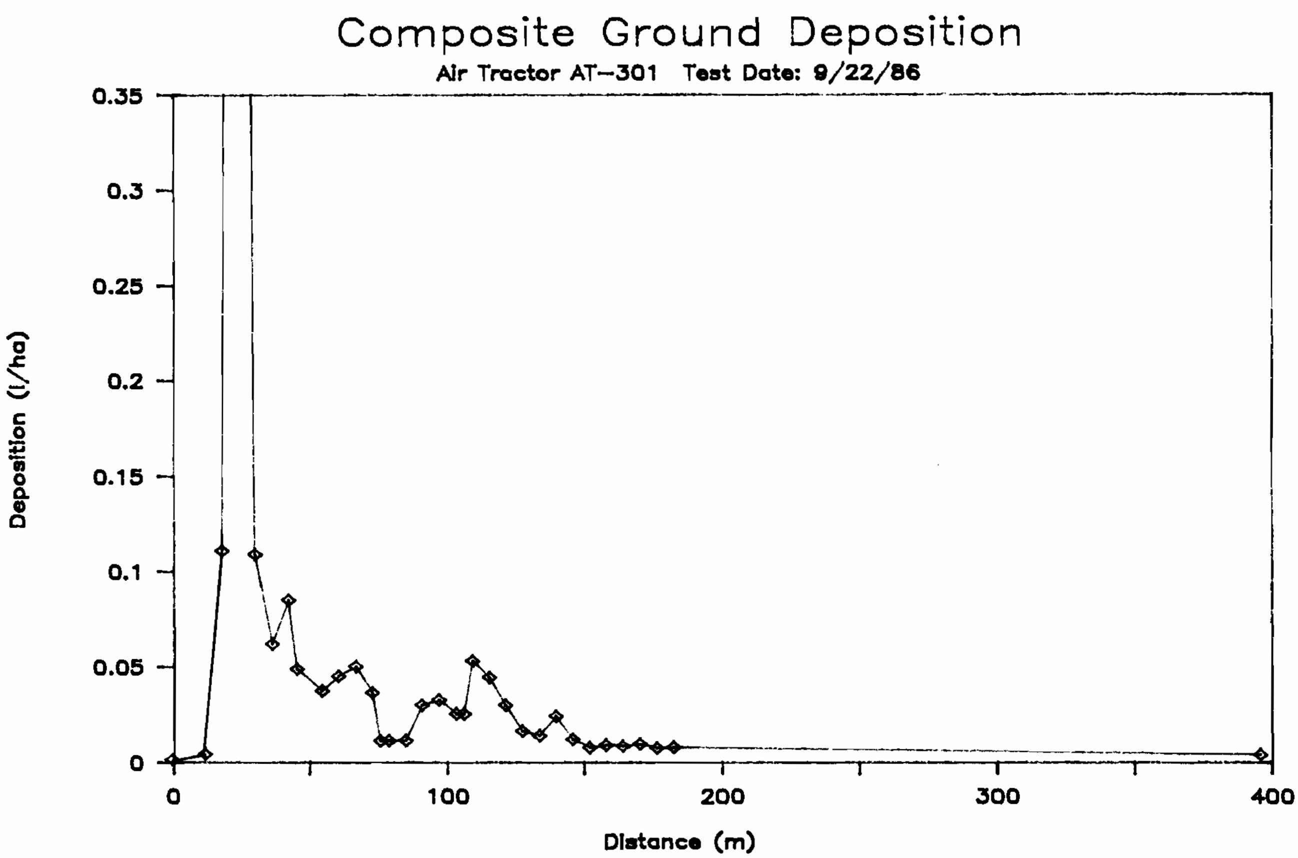

Figure 6.-Experimental data on downwind deposits on Mylar sheets for test on 9/22/86.

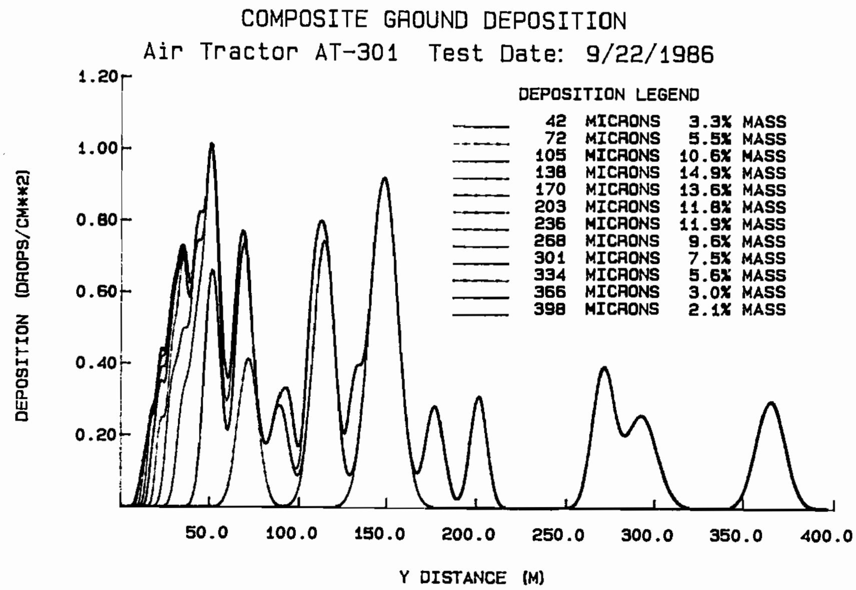

Figure 7.-Theoretical deposit pattern in drops/cm^2 based on AGDISP simulation of test on 9/22/86.

outputs have been described. One field trial has been simulated using AGDISP and the model results compared to the actual field data obtained. There is good overall agreement in the general shape of the deposition curve as a function of downwind distance, and AGDISP also predicts the correct order of magnitude for deposits. This type of model deserves further study, and may hold great promise as an analytical tool to help tailor aircraft set up and nozzle placement to achieve the desired spray patterns for successful control of adult mosquitoes with an ULV application.

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INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN CALIFORNIA:

STATUS OF ORGANOPHOSPHORUS RESISTANCE IN

LARVAL *CULEX TARSALIS* THROUGH 1986

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INTRODUCTION

The Vector Surveillance and Control Branch of the California Department of Health Services initiated an insecticide resistance surveillance program in 1963 (Brown et al. 1963; Gillies 1964). This program was a cooperative effort with local mosquito control agencies in response to their need for early detection of resistance (Womeldorf et al. 1966; Gillies et al. 1968a). However, the entire program was abruptly halted in 1979 due to severe budgetary cuts. Very limited testing was done after the program lost all three staff positions. Through reprioritization and in response to the demands of local mosquito control agencies, the program was restored in 1984. One staff position was assigned to the task of providing services to these agencies statewide. This level of effort is only a third of pre-1979 staffing, therefore, the restored program initially focused upon *Culex tarsalis* as the major species of public health importance. Testing of organophosphorus (OP) larvicides against larval *Cx. tarsalis* began in 1984 and continued through 1986, however, a few tests were performed in 1983. In this report, current findings are compared to those reported in the past and comparisons are made between geographic regions of the State.

METHODS AND MATERIALS

Larval collections of untreated and OP pressured populations were routinely tested in the laboratory. Bioassays were performed as described in detail by Gillies and Womeldorf (1968). Late third to early fourth instar larvae were placed in wax-coated 3-ounce cups (twenty per cup) containing 100 ml of water. Distilled (preferably) or good quality aged tap water was used. Cups were treated with a series of differentially increasing doses of weight/volume acetone-insecticide solutions to yield desired final concentrations expressed in parts per million (ppm). A standard bioassay included replicates of each dose unless precluded by a scarcity of larvae. A wide range of concentrations was used to achieve a range of mortality from zero to 100%. Larval mortality was recorded 24 hours after exposure. Data were evaluated by graphic analysis or probit analysis to obtain estimates of LC_{50}s and LC_{90}s. Tests were considered invalid if there were fewer than three data points, mortality above or below 50% was lacking, greater than 10% mixed species or pupation occurred or if statistically significant heterogeneity of the data points was present.

RESULTS AND DISCUSSION

Table 1 lists the highest level of OP resistance reported (for each of the four specified larvicides) at each of the 22 agencies of the current survey. For comparison to resistance levels reported in the past, these findings are listed together with the highest LC_{50} recorded at each of these agencies through 1978 (Zboray and Gutierrez 1979). However, the table published by Zboray and Gutierrez contains LC_{50}s for only 19 of the 22 agencies. Earlier unpublished data (prior to 1978) for Riverside and San Bernardino Counties (from desert areas along the Colorado River) therefore appear in Table 1 and not in the Zboray-Gutierrez table. No previous data were available for Marin-Sonoma MAD.

The year the highest LC_{50} was obtained and resistance status of that LC_{50} are also included in Table 1. Resistance status was determined by criteria previously set forth (Thompson 1985). Briefly, LC_{50}s are classified either susceptible, incipient or resistant depending upon thresholds and the LC_{90}/LC_{50} ratio (Brown et al. 1963, Womeldorf et al. 1966, Gillies et al. 1968b). These criteria are used to interpret laboratory data to predict the success or failure of an application of insecticide in the field. However, for the purpose of discussion in this report, LC_{50}s equal to or greater than thresholds of 0.1 ppm for malathion, 0.005 ppm for parathion and fenthion and 0.0025 ppm for chlorpyrifos are resistant.

Although the LC_{50}s in Table 1 are the highest recorded per agency, per larvicide, they do not necessarily identify resistant populations. Many are susceptible. The percentage of the LC_{50}s in Table 1 which are resistant (per larvicide) is shown in Table 2. Resistance to malathion and parathion has remained essentially unchanged since 1978, while fenthion resistance decreased, and chlorpyrifos resistance increased substantially. Most populations encountered in the current survey were susceptible to malathion, and about as many populations were susceptible to parathion and fenthion as were resistant. However, three of every four tested were resistant to chlorpyrifos.

The findings of the 1983-86 survey were

Table 1.-Highest LC_{50} (ppm) recorded at each of the indicated agencies for each of the specified organophosphorous larvicides tested against larval *Culex tarsalis* during 1983-86 compared to those recorded through 1978[1]. Resistance status: S = Susceptible; R = Resistant.

AGENCY	MALATHION			PARATHION			FENTHION			CHLORPYRIFOS		
	Year	LC_{50}	Status	Year	LC_{50}	Status	Year	LC_{50}	Status	Year	LC_{50}	Status
Marin-Sonoma	1985	0.092	S	1986	0.0031	S	1985	0.0042	S	1986	0.002	S
	—	—	-	—	—	-	—	—	-	—	—	-
Solano Co.	—	—	-	—	—	-	1986	0.0031	S	1986	0.0024	S
	1965	0.11	R	1965	0.0023	S	1969	0.0027	S	—	—	-
N. Salinas Valley	1986	0.26	R	1986	0.012	R	1986	0.014	R	1986	0.011	R
	—	—	-	1976	0.0033	S	—	—	-	1978	0.0037	R
Burney Basin	1984	0.025	S	1984	0.0025	S	1984	0.0038	S	1984	0.0014	S
	1974	0.015	S	—	—	-	1974	0.0026	S	—	—	-
Shasta[2]	1985	0.069	S	1984	0.0021	S	1984	0.0096	R	1986	0.0041	R
	1967	0.087	S	1977	0.0024	S	1977	0.013	R	1977	0.0028	R
Tehama Co.	1984	0.058	S	1984	0.0042	S	1984	0.0036	S	—	—	-
	1968	0.053	S	1964	0.0031	S	1971	0.0056	R	1972	0.00053	S
Butte Co.[2]	1986	0.042	S	1986	0.0022	S	1986	0.0037	S	1986	0.0015	S
	1966	0.1	R	1972	0.0045	S	1971	0.0079	R	1968	0.00028	S
Sacramento Co./Yolo Co.	1986	0.22	R	1986	0.0056	R	1986	0.007	R	1986	0.0029	R
	1966	0.11	R	1966	0.0032	S	1968	0.0031	S	—	—	-
San Joaquin Co.	1986	0.083	S	1986	0.0041	S	1986	0.0034	S	1986	0.0028	R
	1967	0.071	S	1977	0.0082	R	1972	0.0058	R	1975	0.0012	S
Fresno Westside	1986	0.15	R	1986	0.0033	S	1986	0.0076	R	1986	0.0038	R
	1972	0.23	R	1969	0.022	R	1969	0.023	R	1969	0.0089	R
Consolidated	1985	0.01	S	1986	0.0014	S	1986	0.0032	S	1985	0.0008	S
	1969	0.24	R	1970	0.023	R	1970	0.053	R	—	—	-
Kings	—	—	-	1984	0.012	R	1984	0.014	R	—	—	-
	1972	0.75	R	1972	0.054	R	1972	0.04	R	1972	0.027	R
Tulare	—	—	-	—	—	-	—	—	-	1986	0.0053	R
	1975	0.52	R	1971	0.0099	R	1969	0.017	R	1971	0.011	R
Kern	—	—	-	1986	0.008	R	—	—	-	1986	0.0044	R
	1964	0.065	S	1978	0.026	R	1976	0.013	R	1978	0.0061	R
Goleta Valley	1986	0.037	S	1986	0.0023	S	1986	0.0019	S	1986	0.0012	S
	1966	0.054	S	1966	0.0024	S	1969	0.0044	S	1966	0.00063	S
Ventura Co.	1986	0.07	S	1986	0.0042	S	1986	0.0054	R	1986	0.0034	R
	—	—	-	—	—	-	1976	0.0035	S	—	—	-
Northwest	1984	0.27	R	1984	0.044	R	1984	0.084	R	1984	0.11	R
	1971	0.016	S	1971	0.0016	S	1971	0.0026	S	1971	0.00024	S
Owens Valley	1985	0.017	S	—	—	-	1985	0.0033	S	1985	0.0013	S
	1969	0.026	S	—	—	-	1972	0.0059	R	1969	0.00045	S
San Bernardino Co.[3]	1984	0.44	R	1984	0.056	R	—	—	-	1983	0.0093	R
	1972	0.057	S	1972	0.0018	S	1972	0.0043	S	1972	0.0004	S
Riverside Co.[3]	—	—	-	1984	0.018	R	—	—	-	—	—	-
	1972	0.097	S	1972	0.0054	R	1972	0.0058	R	—	—	-
Coachella Valley	1986	0.061	S	1984	0.011	R	1986	0.0063	R	1984	0.0052	R
	1972	0.073	S	1970	0.016	R	1972	0.0086	R	1971	0.026	R
Imperial Co.[3]	1985	0.14	R	1983	0.009	R	—	—	-	—	—	-
	1972	0.21	R	1972	0.018	R	1972	0.017	R	1972	0.0082	R

1. Zboray and Gutierrez (1979).
2. Local mosquito control agency data; 1984-86.
3. Unpublished data; 1972.

Table 2.-Percentage of the larval *Culex tarsalis* LC_{50}s listed in Table 1 which are resistant[1] to the specified larvicides.

Larvicide	1963-78 Survey	1983-86 Survey
Malathion	36%	36%
Parathion	50%	56%
Fenthion	60%	47%
Chlorpyrifos	50%	75%

[1]Only those agencies reporting an LC_{50} for both survey periods (1963-78 and 1983-86) are included in this comparison.

Table 3.-Percentage of larval *Culex tarsalis* LC_{50}s in Table 1 which are resistant to the specified larvicides, 1983-86 survey.

Larvicide	Sacramento Valley (Northern CA)	San Joaquin Valley (Central CA)	Desert Areas (Southern CA)
Malathion	20%	33%	60%
Parathion	25%	40%	100%
Fenthion	40%	50%	67%
Chlorpyrifos	50%	100%	75%

segregated into regional areas of the State as shown in Table 3. Overall, resistance appears to be least in the north and greatest in the south. Most populations of the Sacramento Valley are susceptible to malathion, parathion and fenthion, but half are resistant to chlorpyrifos. The San Joaquin Valley has a substantial number of chlorpyrifos and fenthion resistant populations. Use of OP larvicides in the desert areas of Southern California is contraindicated. Table 3 suggests that none of these larvicides could be selected universally to control *Cx. tarsalis* in all of California. The decision to use OP larvicides should be made by individual agencies, and among other considerations should include the results of resistance surveillance.

ACKNOWLEDGMENT

I thank the managers of participating mosquito control agencies for providing the assistance and cooperation needed to conduct this survey. I also thank the technical staff of those agencies who helped perform the tests and in some cases supplied data for the survey.

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EVALUATION OF NEW EXPERIMENTAL INSECT GROWTH REGULATORS AGAINST MOSQUITOES (DIPTERA: CULICIDAE) AND MIDGES (DIPTERA: CHIRONOMIDAE) IN THE LABORATORY

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ABSTRACT

Five experimental benzoylphenylurea insect growth regulators (IGRs), UC-75118, UC-75150, UC-76721, UC-76724 and UC-86874, provided by Union Carbide were tested against larvae of eight species of laboratory-reared mosquitoes and two species of field-collected chironomid midges. Diflubenzuron was simultaneously tested (as a standard) against each mosquito and midge species. UC-76724 was the most toxic (including diflubenzuron) to *Aedes aegypti* (L.), *Anopheles albimanus* Wiedemann, *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, *Cx. salinarius* Coquillett, and *Wyeomyia mitchellii* (Theobald) mosquitoes and *Chironomus crassicaudatus* Malloch, and *Glyptotendipes paripes* Edwards midges; the LC_{90} values ranged from 1.1 to 15.4 ppb of UC-76724 for these species. The IGR, UC-75150, was also highly active against *Ae. aegypti, Cx. nigripalpus, Cx. salinarius, Wy. mitchellii,* and *C. crassicaudatus*, with LC_{90} values ranging from 2.7 to 9.8 ppb. The superior laboratory activity of UC-76724 and UC-75150 warrants field evaluation of these new IGRs against mosquitoes, midges, and other pest and vector dipterous insects.

In the past 15 years several insect growth regulators (IGRs) including chitin synthesis inhibitors and juvenile hormone analogs have been tested in the laboratory and under field conditions against dipterous insects of medical and economic importance (Ali and Mulla 1977, Ali and Lord 1980, Dame et al. 1976, Lacey and Mulla 1978, Mulla et al. 1974). Some of these IGRs, such as methoprene, diflubenzuron, Bay Sir 8514, and UC-62644 (2,6-difluoro-N-[[[4-[3-dichloro-5-trifluoromethyl-2-pyridinyloxy]-3,5-dichlorophenyl]amino]carbonyl]benzamide) exhibited excellent activity against mosquitoes and chironomid midges in a variety of habitats (Ali and Stanley 1981, Axtell et al. 1980, Mulla and Darwazeh 1975, 1979). These compounds are especially useful against organophosphorus-resistant strains of mosquitoes (Dame et al. 1976) and midges (Pelsue et al. 1974).

Recently, some new benzoylphenylurea IGRs became available for evaluation. The following laboratory study was conducted on the effectiveness of five new IGRs against eight species of mosquitoes and two species of midges. The activity of these IGRs against each mosquito or midge species was compared to that of diflubenzuron, also a benzoylphenylurea tested simultaneously as a standard.

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MATERIALS AND METHODS

The IGRs evaluated were Union Carbide materials, UC-75118, UC-75150, UC-76721, UC-76724, and UC-86874. The exact chemical structure of each of these compounds is presently not disclosed by Union Carbide. Technical grade material of each IGR (91-96% purity) was provided by Union Carbide while technical grade diflubenzuron (90% purity) was obtained from Uniroyal Chemical Company.

One percent stock solution (w/v) and 6-7 serial dilutions of each technical grade material were made in acetone. For mosquito bioassays, 4th instars of *Aedes aegypti* (L.), *Ae. taeniorhynchus* (Wiedemann), *Anopheles albimanus* Wiedemann, *An. quadrimaculatus* Say, *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, *Cx. salinarius* Coquillett, and *Wyeomyia mitchellii* (Theobald) were utilized. These species were maintained at the Florida Medical Entomology Laboratory at Vero Beach, Florida. For midge bioassays, field-collected 4th instars of *Chironomus crassicaudatus* Malloch and *Glyptotendipes paripes* Edwards were used. Larvae of the former species were collected from Lake Monroe, as described in Ali and Baggs (1982), while *G. paripes* was obtained from Lake Jessup located 5-6 km distance from Lake Monroe, Seminole County, Florida.

Mosquito bioassay methods were generally the same as described by Mulla et al. (1974). Twenty mosquito larvae were placed in a 120-ml disposable cup containing 100 ml of tap water. Distilled water (pH 6.9) was used in tests concerning *Wy. mitchellii* because of the possibility of its larval mortality in tap water (Nayar 1982). Five or six different concentrations of each experimental IGR and diflubenzuron were tested against each mosquito species each time. Each concentration was replicated three times and three untreated checks were maintained in each test which lasted for 3-7 days. Larvae in the cups were examined daily and the final larval or pupal mortality or adult emergence in each treated cup was recorded at the time of complete adult emergence in the checks. One ml of 1% hog liver + yeast (3:2) was added to each cup at 2-day intervals. The midge bioassays were conducted in 1200-ml clear plastic rearing units previously described by Ali and Lord (1980). Each unit received twenty 4th instar larvae, 150 g of sterilized fine sand and 500 ml tap water, and was continuously aerated to maintain an air flow rate of 40 ± 10 ml/min. The method of IGR treatments of midge larvae was the same as used for mosquito bioassays. For midge food, 0.1 g of ground dog food (Dog Kisses®, Hartz Mountain Products Corp.) was added to each unit at 2-day intervals. Dead larvae, pupae and living or dead adults in each unit were counted and removed daily. The experiment was maintained for 5-7 days until no living larvae or pupae remained in the checks. Each mosquito or midge bioassay was repeated at least three times; evaluation against chironomid species had to be repeated 5-6 times because of high mortality encountered in checks on some occasions. A 14-h photoperiod and 27 ± 2°C were maintained in the evaluation room during the experiments. The corrected mortality (against checks) of each mosquito and midge species at different concentrations of an experimental IGR or diflubenzuron was subjected to log probit regression analysis to determine the LC_{50} and LC_{90} values.

RESULTS AND DISCUSSION

Table 1 presents activity of the experimental IGRs and diflubenzuron against 4th instars of *Ae. aegypti* and *Ae. taeniorhynchus*. UC-76724 was the most active against *Ae. aegypti*, while UC-75150, UC-76721, UC-86874, and UC-75118, in that order, were effective against *Ae. aegypti* with LC_{90} values ranging from 1.1 ppb (UC-76724) to 21.0 ppb (UC-75118). Against *Ae. taeniorhynchus*, UC-86874 (LC_{90} = 4.2 ppb) and UC-76724 (LC_{90} = 4.9 ppb) were the most active, while UC-75118 (LC_{90} = 23.6 ppb) was the least effective. Generally, *Ae. aegypti* was more susceptible to the new IGRs as well as to diflubenzuron than was *Ae. taeniorhynchus*, and at least three of the new IGRs showed superior activity than diflubenzuron against both *Aedes* species (Table 1).

Anopheles albimanus and *An. quadrimaculatus* larvae were less susceptible to all the experimental IGRs as compared to diflubenzuron (Table 2). Among the different test compounds, UC-76724 was the most effective against *An. albimanus* (LC_{90} = 15.4 ppb) as well as against *An. quadrimaculatus* (LC_{90} = 5.8 ppb). The levels of activity (LC_{90}) of other IGRs were 3-8 and 4-12 times lower than UC-76724 when tested against *An. albimanus* and *An. quadrimaculatus*, respectively. Diflubenzuron was twice more active against *An. albimanus* than was UC-76724; the latter IGR showed similar activity as diflubenzuron against *An. quadrimaculatus*.

Data in Table 3 indicate that larvae of all three species of *Culex* were susceptible to the experimental IGRs. UC-76724 was the most effective of the experimental materials against *Cx.*

Table 1.-Biological activity of five new experimental insect growth regulators (IGRs) and diflubenzuron against 4th instars (laboratory reared) of *Aedes* mosquitoes[a] exposed continously to the IGRs in the laboratory.

IGRs	Lethal concentration in ppb			
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
	Ae. aegypti		*Ae. taeniorhynchus*	
UC-75118	9.2	21.0	8.0	23.6
UC-75150	1.8	2.7	4.0	10.5
UC-76721	1.8	3.9	4.3	8.4
UC-76724	0.6	1.1	1.2	4.9
UC-86874	1.8	4.8	0.9	4.2
Diflubenzuron	2.0	4.7	1.8	9.9

[a]Maintained at the Florida Medical Entomology Laboratory at Vero Beach, Florida.

Table 2.-Biological activity of five new experimental insect growth regulators (IGRs) and diflubenzuron against 4th instars (laboratory reared) of *Anopheles* mosquitoes[a] exposed continuously to the IGRs in the laboratory.

IGRs	Lethal concentration in ppb			
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
	An. albimanus		*An. quadrimaculatus*	
UC-75118	19.8	124.6	--	--
UC-75150	7.3	42.3	6.3	22.2
UC-76721	14.7	69.5	--	--
UC-76724	3.7	15.4	2.0	5.8
UC-86874	14.4	54.3	11.3	69.1
Diflubenzuron	1.4	7.2	1.2	5.0

[a]Maintained at the Florida Medical Entomology Laboratory at Vero Beach, Florida.

Table 3.-Biological activity of five new experimental insect growth regulators (IGRs) and diflubenzuron against 4th instars (laboratory reared) of *Culex* mosquitoes[a] exposed continuously to the IGRs in the laboratory.

IGRs	Lethal concentration in ppb					
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
	Cx. nigripalpus		*Cx. quinquefasciatus*		*Cx. salinarius*	
UC-75118	5.3	32.6	14.4	30.1	4.5	14.5
UC-75150	2.0	7.9	4.4	13.9	1.9	9.8
UC-76721	8.3	27.4	15.2	30.8	5.2	23.4
UC-76724	1.3	4.3	1.9	4.7	1.5	5.5
UC-86874	1.3	4.6	3.6	12.9	3.8	11.5
Diflubenzuron	1.1	5.9	1.4	4.8	2.9	9.6

[a]Maintained at the Florida Entomology Laboratory at Vero Beach, Florida.

Table 4.-Biological activity of five new experimental insect growth regulators (IGRs) and diflubenzuron against 4th instars (laboratory reared) of *Wyeomyia mitchellii*[a] exposed continuously to the IGRs in the laboratory.

IGRs	Lethal concentration in ppb	
	LC_{50}	LC_{90}
UC-75118	20.8	42.2
UC-75150	2.8	6.8
UC-76721	21.0	54.8
UC-76724	1.4	3.3
UC-86874	14.8	67.2
Diflubenzuron	15.3	39.5

[a]Maintained at the Florida Medical Entomology Laboratory at Vero Beach, Florida.

nigripalpus (LC_{90} = 4.3 ppb), *Cx. quinquefasciatus* (LC_{90} = 4.7 ppb), and *Cx. salinarius* (LC_{90} = 5.5 ppb). UC-75150 and UC-86874 also showed superior activity against the three species, while UC-76721 and UC-75118 were the least active of the experimental IGRs (Table 3). The level of activity of UC-76724 was similar to that of diflubenzuron against *Cx. nigripalpus* and *Cx. quinquefasciatus*. The former IGR was twice more active than diflubenzuron against *Cx. salinarius*.

UC-76724 and UC-75150 were highly active against larvae of *Wy. mitchellii* as indicated by the LC_{90} values of 3.3 ppb (UC-76724) and 6.8 ppb (UC-75150) (Table 4). The other three experimental IGRs were 6-10 and 13-20 times less active than were UC-75150 and UC-76724, respectively. The latter two IGRs proved to be 6-12 times more toxic to *Wy. mitchellii* than was diflubenzuron.

The levels of biological activity of the experimental IGRs against midge species, *C. crassicaudatus* and *G. paripes* (Table 5) were generally similar to those achieved for the mosquito species. Against the midge larvae, UC-76724 was the most active, followed by UC-75150, UC-75118, and UC-76721, with LC_{90} values ranging from 4.5 - 25.3 ppb (*C. crassicaudatus*) and 3.1 - 34.2 ppb (*G. paripes*). Of all the experimental growth regulators tested, only UC-76724 was slightly more lethal to the midges than was diflubenzuron.

In a previous laboratory study, some benzoylphenylurea IGRs (diflubenzuron, Bay Sir 6874, and Bay Sir 8514) had proven to be highly effective against mosquitoes (Mulla and Darwazeh 1979). Diflubenzuron caused complete inhibition of adult emergence of *Cx. p. pallens* and *Cx. tritaeniorhynchus* at 0.2 and 0.1 ppb, respectively (Takahashi and Ohtaki 1976). The LC_{50} values of the same IGR against *Ae. albopictus*, *Ae. subalbatus*, *Cx. p. molestus*, and *Cx. p. pallens* were 0.47, 0.3, 0.72, and 0.18 ppb, respectively (Ishita and Kurihara 1977). In laboratory evaluations conducted by Mulla and Darwazeh (1979), the LC_{90} values of diflubenzuron, Bay Sir 6874, and Bay Sir 8514 against *Cx. quinquefasciatus* were 1.5, 1.8, and 6.8 ppb, respectively, while *Culiseta incidens* was 2-5 times more susceptible to the IGRs than was *Cx. quinquefasciatus*. In another study, the LC_{50} value of Bay Sir 8514 against the larvae of *Ae. aegypti* was found to be 51.4 ppb (Herald et al. 1980).

Against chironomid larvae of the species, *G. paripes* and *C. decorus*, the LC_{90} values of UC-62644, diflubenzuron, and Bay Sir 8514, respectively, were 3.1, 4.1, and 7.6 ppb (*G. paripes*) and 5.7, 6.0, and 22.0 ppb (*C. decorus*) (Ali and Stanley 1981).

The present laboratory study has demonstrated that among the new benzoylphenylurea IGRs, UC-76724 was the most toxic to a wide variety of mosquito and midge species. These species included *Ae. aegypti, An. albimanus, Cx. nigripalpus, Cx. quinquefasicatus, Cx. salinarius,* and *Wy. mitchellii* mosquitoes, and *C. crassicaudatus* and *G. paripes* midges. The LC_{90} values of these species ranged from 1.1 to 15.4 ppb of UC-76724. UC-75150 also proved superior in toxicity as indicated by the relatively low LC_{90} values of this IGR against *Ae. aegypti, Cx. nigripalpus, Cx. salinarius, Wy. mitchellii,* and *C. crassicaudatus*. The standard, diflubenzuron, was highly effective against all mosquito and midge species except for *Wy. mitchellii* which was relatively tolerant to diflubenzuron with an LC_{90} value of 39.5 ppb. A general comparison of activity of UC-76724 and diflubenzuron indicated that UC-76724 was more toxic than diflubenzuron against six (out of a total of eight) species of

Table 5.-Biological activity of four new experimental insect growth regulators (IGRs) and diflubenzuron against field-collected 4th instars of chironomid midges exposed continuously to the IGRs in the laboratory.

IGRs	Lethal concentration in ppb			
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
	Chironomus crassicaudatus[a]		*Glyptotendipes paripes*[b]	
UC-75118	4.4	12.6	10.6	22.4
UC-75150	3.6	9.4	2.9	10.6
UC-76721	11.6	25.3	10.3	34.2
UC-76724	1.2	4.5	1.6	3.1
Diflubenzuron	2.6	7.4	2.0	5.4

[a]Field populations drawn from Lake Monroe, Sanford, Seminole-Volusia Counties, Florida.

[b]Field populations drawn from Lake Jessup, Sanford, Seminole County, Florida.

mosquitoes and both species of midges. Thus, the experimental IGR, UC-76724, and perhaps, UC-75150, having demonstrated good activity against mosquitoes and midges in the laboratory, warrant field evaluation against these and other dipterous pest and vector insects.

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MOSQUITO PROBLEMS IN SEWAGE TREATMENT PLANTS USING AQUATIC MACROPHYTES IN CALIFORNIA

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ABSTRACT

Problems associated with mosquitoes breeding in wastewater treatment facilities using macrophytes jeopardize the development of an otherwise efficient technology. Sound strategies are lacking at present for both prevention of mosquito breeding in such facilities, as well as for efficient control of existing mosquito problems. Long-term ecological studies are needed to develop strategies, but improved methods of sampling and assessment of predator-prey relationships are needed, as well as studies of the relationship of physical factors in water to all biological components. Finally a systems approach to management to these facilities should be developed.

INTRODUCTION

Wastewater treatment in ponds containing various species of aquatic plants (macrophytes) has been promoted as a feasible and economic alternative to conventional treatment using chemical and physical technology (Dinges 1978, Gupta 1980). The use of macrophytes for wastewater treatment grew out of the successful use of stabilization (oxidation) ponds using algae dating back to the 1920's. Numerous species of macrophytes in a number of configurations have been proposed for use, but water hyacinth (*Eichornia crassipes*) has been the macrophyte most commonly used. Economic savings are considered to result from lower capital investment, lower operating manpower requirements and lower energy inputs. Initial assessments of feasibility, however, did not consider adequately the public health problems which might arise from production of mosquitoes in such ponds. In California and also in other states, mosquito problems have occurred in pilot plants using macrophytes, and satisfactory long-term mosquito control has yet to be achieved (Townzen and Wilson, 1983). In California, pilot plants in Contra Costa, Napa, Placer, and Sonoma Counties were placed into operation in the late 1970's and early 1980's, only to be closed a few years later because of unsolved problems with mosquitoes (Table 1). It is only in those plants where influent to the macrophyte ponds has already received advanced treatment that mosquito breeding has not been a problem (Los Banos and Mountain View).

Presently in California, wastewater treatment plants using macrophytes are operating only in San Diego, using water hyacinth, and in Gustine and Mountain View, using cattail and bulrush. In these cases, however, the continued operation of the plants is contingent upon successful mosquito control.

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The objectives of this paper are to review the present status of mosquito control options for macrophyte-based wastewater treatment plants, and to discuss future studies needed to design practical and long-lasting control of mosquitoes without the need for repeated treatments with conventional chemical pesticides. Fundamentally, there are two approaches which can be taken to avoid mosquito problems in wastewater treatment plants. One is to design the plants in a way that is not conducive to mosquito breeding. The second is to intervene in some way after mosquito breeding has reached a level which will subsequently result in adult mosquito populations of pest or disease transmission significance. Unfortunately, we lack sufficient information presently to employ either approach satisfactorily.

There is a strong need for the development of alternatives to the present expensive and energy-intensive methods of wastewater treatment. Although federal and state grant programs for plant development are being phased out, low-interest loan programs for communities will probably take their places. Given the competition for tax dollars for public services such as schools, streets, solid waste disposal, police protection, water supply, and storm drainage, small communities simply can not afford the large outlays of capital needed for conventional treatment facilities. Mosquito control in ponds using macrophytes, therefore, represents a challenge to engineers and biologists with a high potential payoff.

BIOLOGICAL ASSOCIATIONS OF MOSQUITOES, PLANTS AND POLLUTED WATER

Effects of vegetation.-Vegetation present in aquatic habitats can influence mosquito populations in a number of ways. Plants can directly affect water temperature, evaporation, surface characteristics, and chemical composition (WHO 1967). Presence or absence of plants may affect predation rates of mosquito larvae. Plant growth can also influence oviposition by mosquitoes. This can be because of shade produced by plants, or by presenting a physical barrier to oviposition (Russell and Rao, 1942). There can

Table 1.-Pilot plants for wastewater treatment using macrophytes in California.

City	County	Begun	Ended	System
Mountain View	Contra Costa	1974	Operating	Cattail/bulrush
Forestville	Sonoma	1979	1982	Water hyacinth
Occidental	Sonoma	1980	1982	Water hyacinth
St. Helena	Napa	1980	1980	Water hyacinth
Hercules	Contra Costa	1980	1980	Water hyacinth
Roseville	Placer	1981	1983	Water hyacinth
San Diego	San Diego	1981	Operating	Water hyacinth
Los Banos	Merced	1982	1986	Bulrush
Gustine	Merced	1985	Operating	Cattail/bulrush

be an actual toxic or inhibitory factor produced by plants. Bates (1949) discusses aquatic plants which are inimical to mosquito breeding, such as *Utricularia* (bladderwort). Finally, plants present in aquatic environments may complicate control of mosquito larvae by chemical means because of reduced penetration of toxicants.

From the standpoint of wastewater treatment plant design, it would be very useful to be able to correlate the occurrence of different plants with the occurrence of various species of mosquitoes in aquatic environments. This has been a goal of mosquito researchers for a long time. Bates (1949) summarized available studies as follows: "Studies with this object in view have, however, generally given rather unsatisfactory results, in that there seems rarely, if ever, to be a working correlation between the incidence of a particular species or complex of plants and particular mosquito species". Nevertheless, general associations of plants and mosquito breeding are known to exist. Barnes and Gibula (1979) discussed the subjects of classification of vegetation and the relationship of vegetation and mosquito breeding as related to remote sensing. They cite examples of types of vegetative patterns associated with mosquito breeding, and types where mosquito breeding is absent. These and other authors point out that vegetation may be an indicator of total ecological conditions, and not always a direct cause of mosquito breeding (WHO, 1967). There have, moreover, been successful attempts to correlate the occurrence of certain life forms of plants with density of mosquito larvae. Hess and Hall (1945) discussed the relationship of plant forms with occurrence of anopheline larvae and offered a classification of aquatic plants from this standpoint. Hess and Hall (1943) and Rozeboom and Hess (1944) proposed that the linear amount of air-water-plant interface, which they called the intersection line, for a given plant life form in aquatic environments was positively correlated with the density of anopheline larvae present. Dr. Vincent Resh, of the University of California at Berkeley, has extended these studies to show that the amount of intersection line present is related to mosquito predation rates by mosquitofish.

It is interesting that water hyacinth has a life form (floating mat) which Hess and Hall (1945) would have classified as high production potential for anopheline mosquitoes and that this species is associated also with high production of culicine mosquitoes. Plant forms which Hess and Hall (1945) considered to have low anopheline mosquito production potential include floating leaf plants such as *Potamogeton diversifolius* (pondweed) and erect naked plants such as *Scirpus americanus* (three-square bulrush) and *Typha latifolia* (common cattail). Studies by Dr. Charles Schaefer at Gustine have shown, however, that treatment ponds containing bulrush and cattail produce mosquito problems when growth of plants become extremely dense or become lodged so that fish and other mosquito predators cannot gain access to mosquito larvae.

The other side of the coin in terms of wastewater treatment is the effectiveness of various types of vegetation in treating wastewater. Unfortunately, there have not been many studies which have correlated vegetative life form with wastewater treatment efficiency. Stowell et al. (1981) comment on wastewater treatment potential of some macrophytes, including water hyacinth, water primrose, cattails, and bulrush.

Effects of water quality parameters.-The literature on the relationship of various water quality parameters and occurrence of species of mosquito larvae is voluminous. Nevertheless, the current status of our knowledge in this area is not satisfactory. Most studies represent correlations between various water quality parameters and density of various mosquito species (e.g. Hagstrum and Gunstream, 1971), and there have been few studies which have offered physiological or ecological explanations for the associations. An exception to this is in the case of studies of salinity tolerance in mosquito larvae. Studies such as Garrett and Bradley (1984) have shown that mosquito species occupying habitats of differing salinities differ in their osmoregulatory mechanisms. A detailed study on the relationship of the occurrence of mosquito species to levels of water pollution existing in Oregon log ponds was made by McHugh et al., (1964). These authors classified degrees of pollution in terms of specific water quality parameters and showed that some factors, such as chemical oxygen demand, were highly correlated with presence or absence of mosquito species, while many others, including biochemical oxygen demand, total solids, color, turbidity, conductivity, pH, and a number of salts (including chlorides) did not.

In spite of the lack of detailed studies in this area, the association of various mosquito species with polluted water habitats is fairly well known in general terms, even to the extent of knowing which species will occur on the basis of the degree of pollution. What is not known, however, is which specific factors associated with pollution influence mosquito breeding to the extent that one might predict the effect of alteration of specific factors on mosquito breeding.

CONTROL OF MOSQUITOES IN POLLUTED HABITATS

Use of chemical pesticides.-Generally, chemical pesticides are not considered a satisfactory solution for long-term mosquito control in wastewater ponds or other polluted mosquito breeding sources. Oils have been used in the past, but are expensive because they have little residual activity. Organophosphates, and other conventional chemicals, are avoided because of the danger of inducing high levels of physiological resistance in mosquito populations. Furthermore, some chemicals do not provide satisfactory control in highly polluted water, or do so for only limited lengths of time. Axtell et al. (1980) found that malathion and temephos provided unsatisfactory control of *Culex quinquefasciatus* in anaerobic animal waste lagoons, and chlorpyrifos and Flit MLO gave good control only at relatively high doses. Certain "biorational" compounds such as insect growth regulators (IGR's) have shown promise, but also only at relatively high doses (Axtell et al. 1980, Williams and Palmisano, 1981).

Unfortunately, little specific information is available on the efficacy of various chemical pesticides in wastewater. Some investigators have reported differences between laboratory results and results from tests in wastewater environments, but have only been able to speculate on the causes (i.e. pH differences, photodegradation, etc.). Little concrete information is available on the effect of physical and chemical factors on efficacy of pesticides in aquatic environments generally. Muirhead-Thompson (1971) reviewed this complex subject, and discussed the impact of factors such as temperature, pH, water hardness, silt, and type of insecticide formulation.

Use of microbial pesticides.-The microbial insecticides *Bacillus thuringiensis* serotype H-14 (B.t.i.) and *Bacillus sphaericus* (Bs) may be effective in wastewater ponds, especially if used in conjunction with other methods such as mosquitofish. B.t.i. has been shown to be effective even in highly polluted water sources such as log ponds (Eldridge and Callicrate, 1982) and dairy waste lagoons (Mulla et al. 1982), but at dosages higher than would be needed for freshwater sources. Recommendations for use of B.t.i. in polluted environments commonly call for dosages of up to four times that recommended for unpolluted ones.

Use of biological control.-Few biological control agents have been tested in polluted environments, and some that have are not effective. Jaronski and Axtell (1982) found that *Lagenidium giganteum* did not infect mosquitoes even at relatively low levels of pollution. Interestingly, they found that nitrogen (as ammonia) and phosphorus, but not chemical oxygen demand, correlation with infection levels. *Gambusia affinis* is the most commonly used fish in biological control programs for mosquitoes, but this species is relatively intolerant of polluted water, and especially to low levels of dissolved oxygen (Sjogren, 1972). Mian et al. (1986) have experimented with two other species of fish, *Cyprinodon macularius* (desert pupfish) and *Poecilia reticulata* (guppy) in a sewage treatment pond, and showed that both these species can survive over a mosquito breeding season. The guppy has long been touted as a pollution-tolerant fish suitable for mosquito control in aquatic environments containing various kinds of pollutants, including insecticides (Bay and Self, 1972). Large numbers survive and reproduce in the secondary clarifiers of the U.C. Davis and Chico State University wastewater treatment plants.

FUTURE DIRECTIONS IN RESEARCH

There are two basic approaches which can be taken in research on methods of mosquito control. One is the correlative approach in which a number of variables are measured and recorded at a number of different sites, and then certain of the variables are regressed against a variable which represents an estimate of mosquito control (larval density, light trap catches of adult mosquitoes, etc.). Another approach is the manipulative, or experimental approach, in which certain parameters are isolated and varied, and then estimates of control success are recorded. This approach requires the use of experimental controls. Presently, there are several constraints to the use of either method. One is the present lack of reliable sampling methods for mosquito larvae as well as other biological components of aquatic environments. Service (1976) has presented an excellent summary of the problems with sampling of mosquito larvae, and expands on the subject in a later paper (Service, 1985). He points out that most of the problems stem from the clumped (contagious) distribution of larvae, and the lack of satisfactory methods to treat such data. Service (1985) has also shown experimentally that the degree of contagion by mosquito larvae varies with stage of development, with earlier stage larvae showing more clumping than later stage larvae, and with pupae showing the least amount of clumping of all immature stages.

Further problems result from sampling methods used for predators as well as methods used to estimate actual predation rates. Some studies of predators have used standard aquatic biological methods (e.g. dip net samples) to estimate density and survival of predators, and have equated these estimates to predation rates. Presence, and thus survival, can not be used as indicators of predation of course, because there are many factors which may affect predation rates, such as metabolic rate and physiological status of predators, which would not be detectable by sampling *per se*.

On the assumption that there is a high correlation between larval density and adult abundance in situations where mosquito populations are fairly well isolated, estimates of larval mortality have commonly been used to assess control success where mosquito problems have developed. But Agudelo-Silva and Spielman (1984) showed that in laboratory experiments which simulated larval control, removal of larvae actually resulted in an increase in the number of adults which emerged. The explanation for this is that mosquitoes, being "r" strategists, can compensate for regulation by density-dependent mortality factors while maintaining stable population sizes. Among others, Service (1985) suggests that control strategies which target younger instars may produce this effect, and that IGR's, filarial parasites, and fish, all of which cause mortality in older instars, should therefore be used in preference to microbial insecticides and other methods which are more toxic to younger instars.

Other factors may cause poor correlations between estimates of larval mortality following control and adult abundance. Dow et al. (1965) showed that intense larviciding of *Culex tarsalis* larvae did not result in a satisfactory reduction in the adult population because of migration of adults into the treated area. Eldridge et al. (1985) showed little reduction of estimates of adult abundance by either light trapping or human bait trapping after intensive larval control measures in snow pools, and also attributed the disparity in part to movement of adult mosquitoes into the treated area.

Studies are badly needed which account for all trophic levels represented in wastewater ponds. In most cases, all components of the food chain are not known, and this further complicates interpretation of sampling results. Studies which use mechanical exclosures of various sizes would be very useful in assessing the impact of various predators on mosquitoes and other organisms. Also, the availability of experimental ponds which can be under the complete control of the investigator is essential to valid research using manipulative techniques. This must include availability of experimental control ponds in which mosquitoes can be permitted to breed in the absence of the experimental intervention being evaluated. Unfortunately, many of the studies which have been conducted in wastewater treatment ponds have not produced useful results because public health considerations have required insecticidal treatment of all ponds.

Finally, studies of mosquito control in wastewater ponds are needed which take a systems approach, especially from the standpoint of economics. Certainly, the engineers which have designed these ponds must have done analyses of capital construction costs and maintenance and operational costs. Likewise the total cost of mosquito control using various options should be determinable. Obviously, if all costs associated with wastewater treatment using macrophytes plus all costs associated with mosquito control exceeds the costs associated with conventional wastewater treatment using mechanical and chemical means, then the former method is not feasible. Estimates of the U.S. Environmental Protection Agency suggest that the cost gap between macrophyte systems and conventional is wide, however. They suggest that construction costs for a conventional activated sludge plant are 20 times greater than for a water hyacinth pond system, with operating and maintenance costs about four times as great for the conventional plant (EPA, 1980).

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ECOLOGY OF IMMATURE *CULEX TARSALIS* AT BREEDING SITES IN KERN COUNTY, CALIFORNIA, 1986[1,2]

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ABSTRACT

The population ecology of the immature stages of *Culex tarsalis* Coquillett was investigated at 6 representive breeding sites on the floor of the San Joaquin Valley: 1) overflow pools along the Kern River, 2) subterranean seepage into the dry Goose Lake bed, 3) a thermally polluted sump at Poso oil field, 4) canal spillage into desert at the Kern National Wildlife Refuge, 5) percolation ponds near Lake Buena Vista, and 6) ponds at the Eureka Duck Club. Study emphasis was placed on 1) comparing adult (females per CO_2 trap night) and immature (nos. of larvae and pupae per dip) abundance, 2) estimating immature life stage duration in predator exclusion cages and the age structure of the natural population, 3) constructing stage-specific life tables from dip counts using the method of Lakhani and Service (1978), 4) identifying the possible causes for immature mortality, and 5) monitoring adult quality at emergence (wing length and autogeny status).

During 1986, 68,159 larvae were collected in 3,150 dips taken at the 6 study areas, of which 97% were identified as *Cx. tarsalis* (abundance = 21 larvae + pupae/dip). Immature and adult abundance were not correlated among study sites ($r = 0.45$, $P>0.05$) due to immature sampling difficulties and seasonal changes in the composition of the adult population. Adult wing length was longest at sites having the coolest water temperature. Autogeny rates were highest at sites having the highest estimates of phytoplankton and periphyton standing crops.

Immature developmental rates were slowest during early spring and fall when water temperatures were coolest. The relative lack of food markedly delayed 4th instar development at Buena Vista, but not at the remaining study areas where the addition of supplemental food to cohorts in predator exclusion cages did not markedly alter immature development. Similarly, the addition of supplemental food significantly increased the male and female wing length and the autogeny rate of females emerging from pupae reared at Buena Vista.

Vertical life tables were calculated at each site from stage-specific dip counts which were corrected by stage-specific developmental rates, an index of sampling probability. Survivorship from eclosion to emergence ranged from 0.3% at Goose Lake to 17.4% at Kern River (Table 1). By comparing vertical life table survivorship for uncaged cohorts with horizontal survivorship for fed and unfed cohorts in predator exclusion cages, it was possible to grossly indicate the impact of selected mortality factors. Mortality related to poor water quality ranged from 31% at the Kern Refuge to 90% at Goose Lake (Table 1). Poor water quality at Goose Lake was related to elevated concentrations of bivalent salts (conductivity = 16,167 µmhos/cm, alkalinity = 2,314 ppm of $CaCO_3$) and ammonia (NH_4 = 3.93 ppm). Mortality related to the relative lack of food was highest at Buena Vista (15.3%) where supplemental food also markedly increased the immature development rate and adult quality at emergence. Mortality related to predation was highest at the Kern Refuge (66.3%) and lowest at Goose Lake (3.7%) where the diversity and abundance of the predator community was reduced markedly. At the remaining study areas, the coleopteran larvae *Laccophilus* (abundance = 1.0 - 2.5/dip) and *Tropisternus* (abundance = 0.2 - 0.4/dip) were the most abundant predator taxa. Unlike our observations at a permanent foothill source during 1985, odonate naiads were never abundant at ephemeral breeding sites on the floor of the San Joaquin Valley.

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[2]Research during 1985 and 1986 is being prepared for publication in the Journal of Medical Entomology.

Table 1.-Relative impact of mortality factors on immature *Culex tarsalis* populations estimated by comparing survivorship in fed and unfed predator exclusion cages with life table survivorship at Kern County study sites, 1986.

	1986 Study Sites					1985[a]
	Kern River	Goose Lake	Poso Sump	Kern NWR	Buena Vista	Poso West
L1 - A survivorship (%)						
1. Fed cages	nd[b]	10.0	57.6	69.0	66.0	89.7
2. Unfed cages	58.0[b]	4.0	58.0	68.6	50.7	77.0
3. Life tables	17.4	0.3	6.5	2.3	10.2	7.3
Relative impact of mortality factors (%)						
Water (100 - 1)	nd	90.0	42.4	31.0	34.0	10.3
Lack of food (1 - 2)	42.0[c]	6.0	0.0	0.4	15.3	12.7
Predation (2 - 3)	40.6	3.7	51.5	66.3	40.5	69.6

[a] Data averaged from May to October.

[b] Cage data from Poso Creek. nd = not done.

[c] Pooled effects of water quality and lack of food.

INTERACTIONS AMONG MOSQUITOFISH (*GAMBUSIA AFFINIS*), SAGO PONDWEED (*POTAMOGETON PECTINATUS*), AND THE SURVIVORSHIP OF *ANOPHELES* MOSQUITO LARVAE

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INTRODUCTION

The mosquitofish, *Gambusia affinis*, has long been used for the biological control of mosquito larvae (e.g. Krumholz 1948, Gall et al. 1980, Gerberich and Laird 1985). However, the level of control provided by *Gambusia* is not always satisfactory. Abundant aquatic vegetation is one factor that may reduce the effectiveness of *Gambusia* (e.g. Bay 1967, Gerberich and Laird 1985), especially in controlling anopheline mosquito larvae (Tennessee Valley Authority 1947).

Previous research has indicated that aquatic plant cover may provide anopheline larvae with a refuge from fish predation (Collins et al. 1983, Collins and Resh 1984, Curtin et al. 1984). Sago pondweed (*Potamogeton pectinatus*), with its many narrow floating leaves, provides a favorable habitat for *Anopheles* larvae (Collins et al. 1983, Balling and Resh 1984), probably because of the large amount of intersection line (i.e. air-water-plant interface) created by the architecture of this plant (Rozeboom and Hall 1944, Balling and Resh 1984, Collins and Resh 1985).

In this study, we experimentally examined the effects of *Gambusia* density and amount of aquatic plant cover on the survivorship of *Anopheles freeborni* larvae. In particular, we hypothesized that the floating canopy of *Potamogeton pectinatus* (hereafter referred to as pondweed) provides *Anopheles* larvae with a physical refuge from mosquitofish predation. This hypothesis predicts that *Anopheles* survivorship will increase as amount of pondweed cover (i.e. mosquito refuge) increases.

STUDY SITE

These experiments were conducted at Coyote Hills Marsh, a man-made freshwater marsh located in Coyote Hills Regional Park (Fremont, Alameda County, California). Extensive open water regions in the marsh interior are bordered by stands of cattails (*Typha angustifolia* and *T. latifolia*). From late spring to early autumn these interior regions are usually dominated by a dense floating canopy of pondweed (Balling and Resh 1984), although the extent of pondweed canopy has been greatly reduced in recent years because of grazing by crayfish (Feminella and Resh 1986). The pondweed canopy provides habitat for a variety of invertebrates (Lamberti and Resh 1984), including two species of *Anopheles* mosquitoes, *Anopheles freeborni* and *An. occidentalis* (Collins et al. 1983, Balling and Resh 1984). A variety of fish species, including *Gambusia affinis*, occur in the pondweed community (Schooley 1983, Page and Schooley 1984, Orr and Resh 1986).

METHODS

The effects of *Gambusia* density and pondweed cover on *Anopheles* larval survivorship were examined in a series of enclosure experiments. Each experiment used a factorial design combining three levels of *Gambusia* density (0, 2, and 8 fish per enclosure) with three levels of pondweed cover (0 or 10%, 50%, and 100% of natural cover). Enclosures were cylindrical cages (60 cm diameter x 91 cm high; surface area 0.25 m^2) that enclosed a complete column of water, extending from bottom sediments to the water surface. The walls of the enclosures were made of an upper band of 6 mil polyethylene sheeting and a lower band of fiberglass window screening (1 mm mesh) sewn together to form an open-ended sleeve that fit over a cylindrical welded-wire frame. Enclosures were installed in a fully developed pondweed bed located in 35-40 cm deep water at Coyote Hills Marsh. When in position, the enclosures were embedded approximately 10 cm into the sediment, with the band of fiberglass screen extending about 25 cm above the sediment. The upper polyethylene band was adjusted so that it extended only 5-10 cm above the water surface; this upper band of polyethylene prevented emigration of surface-dwelling *Anopheles* larvae. The lower band of fiberglass screening allowed exchange of water and small invertebrates between enclosures and the surrounding pondweed bed. Preliminary studies using this type of enclosure showed no significant alteration of temperature or dissolved oxygen vertical profiles in 80 cm deep water (Collins et al., unpublished data).

Each experiment followed the same procedure: (1) two weeks prior to experimentation, enclosures were placed into the pondweed bed. Any fish present in the enclosures were removed by repeated dip-netting. All damaged pondweed stems were also removed. (2) Enclosures were then left undisturbed for 11-12 days to allow pondweed regrowth and formation of a full surface canopy (=100% natural cover). (3) Treatments were assigned randomly to enclosures, and pondweed plants were removed as necessary to achieve the desired levels of floating canopy that approximated 0, 10, 50, and 100% natural cover; all pondweed plants were shaken vigorously before removal from the enclosure to minimize the effects of plant manipulation on epiphytic invertebrate densities. (4) Medium-sized female *Gambusia* (35-40 mm standard length) were then added at the desired densities (0, 2, or 8 fish per enclosure), and allowed to adjust to the microcosms for 2-3 days. (5) Each experiment began with the addition of 100 first instar *Anopheles freeborni* larvae that had hatched in the laboratory within the previous 24-36 hours; each experiment ended when the first pupae were observed. Surviving larvae and pupae were isolated by gently depressing the pondweed canopy; surfacing larvae and pupae were then collected with a fine mesh net. This procedure was repeated until 5 consecutive sweeps yielded no additional *Anopheles* larvae or pupae.

Experiments 1-3 were run sequentially during late summer and early autumn 1986 (see Table 1). The duration of each experiment varied (Table 1); experiments 2 and 3 were longer than experiment 1 because the normal seasonal decrease in water temperature caused reduced developmental rates of *Anopheles* larvae.

RESULTS AND DISCUSSION

All three experiments showed the same basic trends in *Anopheles* larval survivorship (Figure 1): both *Gambusia* density and amount of pondweed cover significantly influenced *Anopheles* survival (Table 1).

Influence of Fish Density.-In general, as fish density increased, mosquito survivorship decreased (Figure 1). The differences in survi-

Table 1.-Summary statistics from two-way analysis of variance on the proportion of *Anopheles freeborni* larvae surviving to the end of each experiment. Angular transformation of the proportion of larvae surviving was used in the analysis.

EXPERIMENT 1: 10%, 50%, 100% natural cover; 4 replicates for each of nine treatments (3 levels of cover x 3 fish densities); began 23 August 1986; 10 days duration.

Gambusia density	$F_{2,27} = 102.31$	p=0.0001
Pondweed cover	$F_{2,27} = 11.55$	p=0.0002
Interaction	$F_{4,27} = 0.41$	p=0.7972

EXPERIMENT 2: 0%, 50%, 100% natural cover; 2 replicates per treatment; began 17 September 1986; 15 days duration.

Gambusia density	$F_{2,9} = 51.72$	p=0.0001
Pondweed cover	$F_{2,9} = 64.88$	p=0.0001
Interaction	$F_{4,9} = 4.56$	p=0.0275

EXPERIMENT 3: 0%, 50%, 100% natural cover; 2 replicates per treatment; began 23 September 1986; 18 days duration.

Gambusia density	$F_{2,9} = 48.38$	p=0.0001
Pondweed cover	$F_{2,9} = 25.24$	p=0.0002
Interaction	$F_{4,9} = 0.49$	p=0.7422

vorship between controls (0 fish) and fish treatments (2 or 8 fish/enclosure) for each level of pondweed cover were significant in all cases except one: larval survivorship was not significantly different between the 0 and 2 fish treatments with 100% pondweed cover in experiment 2 (Duncan multiple range test for each experiment, $\alpha=0.05$).

Surprisingly, an increase from 2 to 8 *Gambusia* per enclosure resulted in only small decreases in larval survivorship. None of the paired comparisons between the 2 and 8 fish treatments within a given level of pondweed showed a statistically significant difference (Duncan multiple range test for each experiment, $\alpha=0.05$). Thus, the mean number of larvae killed per fish was much lower in the high-density *Gambusia* treatments. This type of response at high predator densities may be due to behavioral interference among individual fish or a shift in feeding preference for alternative prey. Although these results suggest that the benefits of enhancing *Gambusia* density above a minimum threshold may be marginal, further research is required to test whether this relationship is generally true for natural, uncaged populations or is merely an artifact of the scale of enclosure used in these experiments.

Influence of Pondweed Cover.-As hypothesized, larval survivorship increased with increasing pondweed cover (Figure 1). The differences in mean larval survivorship between low (0 or 10%) and high (100%) cover treatments within a given level of fish density were significant in all cases except for controls (0 fish) in experiment 1 (Duncan multiple range test for each experiment, $\alpha=0.05$). The positive relationship between *Anopheles* survival and amount of cover in the two fish treatments (2 and 8 *Gambusia*/enclosure) reflects the effectiveness of pondweed cover as a refuge from predation.

The increase in *Anopheles* survivorship observed with increasing plant cover in the control (0 fish) treatments (Figure 1) indicates a beneficial aspect of pondweed cover to *Anopheles* larvae even in the absence of *Gambusia*. Pondweed cover may provide *Anopheles* larvae with a refuge from adverse effects of physical disturbance, such as water turbulence caused by wind and rain (Collins et al. 1985). Pondweed may also serve as a rich source of food for mosquito larvae; Hess and Hall (1943) suggested that microbial food may be especially abundant within the intersection line produced by floating vegetation.

Survivorship of *Anopheles* larvae was generally highest in experiment 1 and lowest in experiment 3. This was probably the result of two related factors: First, the duration of the experiments increased as the season progressed due to decreased larval development rates; thus, larvae in the later experiments were exposed to potential sources of mortality, including predators, for a longer period of time. Second, physical disturbance caused by two late September storms probably increased larval mortality.

Our findings with *An. freeborni* are similar to those obtained in laboratory experiments using *An. occidentalis* (Curtin et al. 1984). The results of these two studies indicate that interactions between mosquitofish and pondweed can have a major influence on the survival of *Anopheles* larvae. Future research is planned to test the general validity of these basic relationships using other species of aquatic macrophytes.

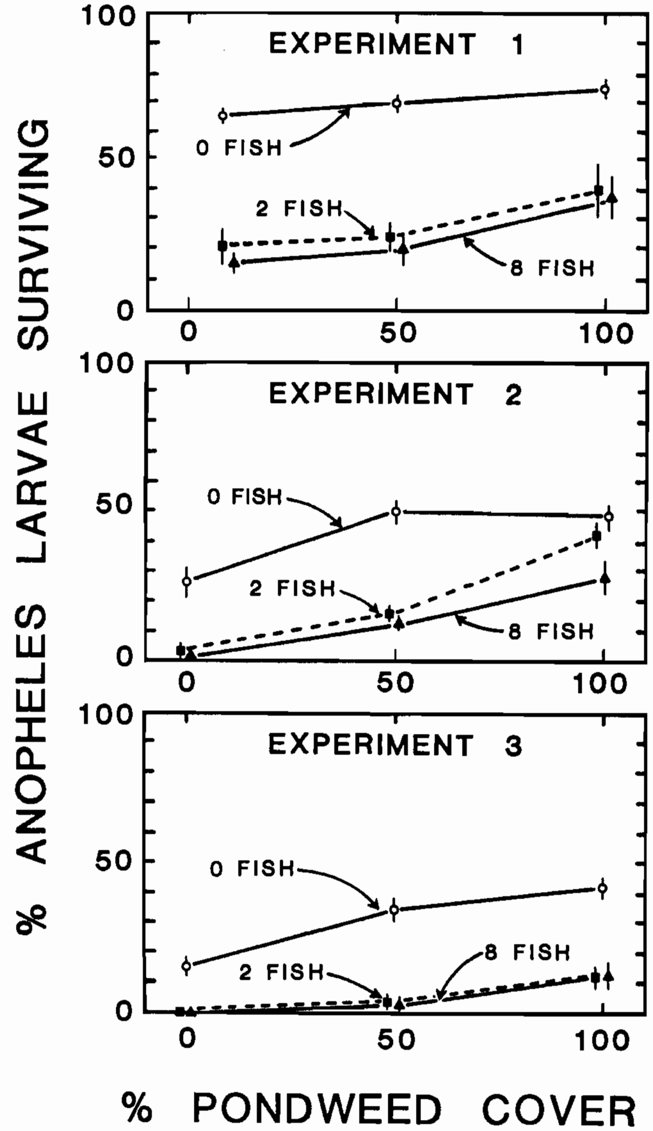

Figure 1.-The influence of *Gambusia* density and amount of pondweed cover on mean percent survivorship of *Anopheles freeborni* larvae in experiments 1, 2, and 3. Vertical lines indicate ± 1 standard error.

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A COMPARISON OF IMMATURE MOSQUITO POPULATIONS, AQUATIC PREDATORS, AND THE DEVELOPMENTAL RATE OF *CX. TARSALIS* IN WILD VERSUS WHITE RICE FIELDS IN THE CENTRAL VALLEY

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ABSTRACT

There were significantly greater (χ^2; $p<.001$) *Culex tarsalis* and *Anopheles freeborni* populations in wild than white rice fields during the last three weeks of the wild rice growing season. Aquatic predator population trends were, in general, inconsistent. *Culex tarsalis* larvae developed faster in wild than white rice fields (10 versus 17 days from first instar to pupation) despite similar water temperatures in both rice systems.

INTRODUCTION

Commercial varieties of white rice *Oryza sativa* have been cultivated in California for many years whereas wild rice *Zizania palustris* has been introduced relatively recently (Oelke, 1982, Winchell and Dahl, 1984). Wild rice is grown primarily in the Central Valley, Shasta and Lake Counties and the acreage under cultivation is expanding rapidly, thus creating new breeding sites for mosquito larvae in regions of California where rice has not previously been grown. In addition to the introduction of wild rice into new areas, acreage is being converted from white to wild rice in the Central Valley.

Cultivation practices of wild and white rice plants are similar although wild rice has a shorter growing season (approximately 100 versus 150 days). The most striking difference between the two plants is their appearance. Wild rice can grow to a height of three meters and appears much fuller than the shorter white rice, which grows to approximately one meter. We hypothesized that the differences between the two plants could correspondingly affect their aquatic insect communities. This study therefore compared population patterns and species composition of mosquito larvae and some aquatic predators in wild and white rice fields. Also evaluated were the comparative developmental rate of *Culex tarsalis* larvae and the ovipositional preference of adults.

METHODS

Adjacent wild and white rice fields were selected for study in the Central Valley near Nicolaus, Sutter County. Seeding dates were similar; the white rice was planted on May 6, one week before the wild rice. Four contiguous fields of each rice type were monitored throughout the growing season. The wild rice fields were approximately 5.2 acres and the white, 6.3 acres. Water was supplied from the Sacramento River. The wild rice fields were harvested on August 12 and the white rice on October 3.

Mosquito populations were assessed weekly by taking standard dips around three sides of each field (600 dips per rice type). Dip samples were concentrated and the contents identified and counted in the laboratory. Minnow traps (1/8" mesh) were set overnight (three/field) on a biweekly basis to evaluate the aquatic predator population. Water temperature, water depth and plant height were also monitored.

The developmental time of *Cx. tarsalis* larvae was determined by observing larvae placed in fine mesh floating cages, which were placed in three fields of each rice type (3-4 cages/field). Newly hatched first instar larvae were obtained from a two-week old *Cx. tarsalis* colony at the Sutter/Yuba MAD and released directly into the cages, 5 per cage, 50 per rice type. The larvae were observed and the high/low temperature monitored every 2-3 days. This study was carried out during the latter part of the wild rice growing season.

Water was collected from both rice field types on August 5 and brought immediately to the Sutter/Yuba MAD to evaluate the ovipositional preference of *Cx. tarsalis*. Water of each type was placed in three separate cages containing male and female *Cx. tarsalis* and the number of subsequent egg rafts counted.

RESULTS AND DISCUSSION

During the first seven weeks of sampling, from mid-May to the end of June, no mosquito larvae were found in either wild or white rice fields (Fig. 1). Thereafter, larvae were found on every sampling date. *Anopheles freeborni* appeared prior to *Cx. tarsalis* in both rice types. As the season progressed the anopheline population slowly increased to a peak during the first week of August with 0.18 and 0.04 immatures/dip in the wild and white rice respectively. The *Cx. tarsalis* population built up rapidly in the wild rice fields to a peak of 0.65 immatures/dip by the end of July, greatly outnumbering the white rice

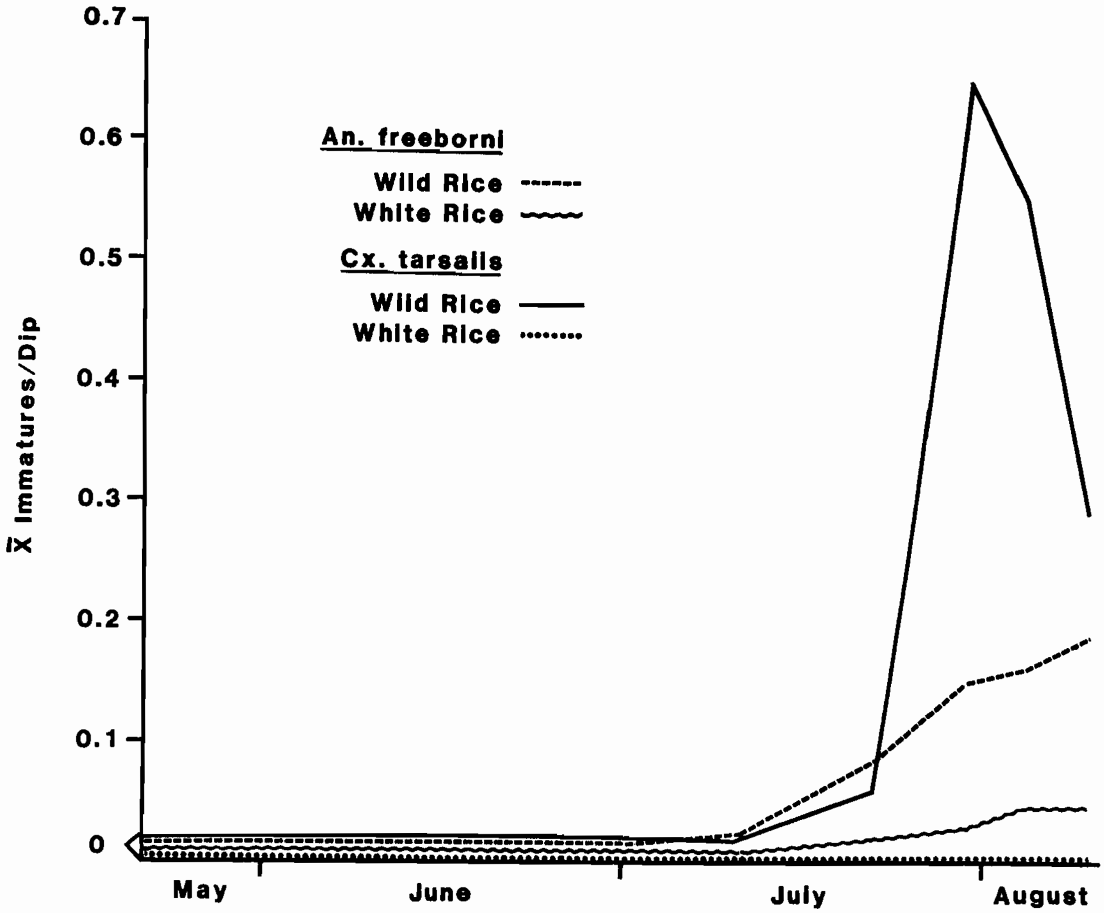

Figure 1.-Population densities of mosquito larvae in wild and white rice fields, Sutter County, 1986.

population, which never exceeded 0.005. Both the *An. freeborni* and *Cx. tarsalis* populations were significantly higher (χ^2; p<.001) in the wild rice fields than in the white rice fields during the last three weeks of the study. These dates correspond to maximum plant heights of 2.5 meters or more in the wild rice and 0.8 meters in the white rice. The total peak larval counts were 0.81 immatures/dip in the wild rice at the end of July and 0.04 immatures/dip during the first week of August in the white rice fields. The wild rice mosquito population was thus well above the 0.08 larvae/dip treatment level used by the Sutter/Yuba MAD in white rice fields.

Pesticides (Bolero, Parathion, Basagran) were applied to the white rice fields on three occasions during May and early June. We do not believe these pesticides were a factor in this study since they were applied 30 or more days before the mosquito population appeared in either rice type. No pesticides were used in the wild rice fields. The average water depth was greater in the wild rice (18 cm) than the white rice (12 cm). High/low water temperatures taken throughout the season were similar in both rice systems.

Aquatic predators were assessed using minnow traps and by dipping. Only general conclusions can be drawn from the minnow trap data because trap collections were highly variable. On certain dates, adult beetle and backswimmer populations were significantly different in the wild versus white rice fields but clear trends were not apparent. The hydrophilid beetle *Tropisternus lateralis* was the most abundant insect. Five species of fish were recovered, but always in low numbers.

Predators collected from dip sampling showed more consistent trends. Populations of dragonflies, damselflies, backswimmers and belostomatids increased sharply during the last three weeks of sampling, paralleling the mosquito population trend. Greatest variability among sampling dates and between rice types was again within the beetle population. Dragonflies and damselflies were significantly more numerous (χ^2; p<.01) in wild than white rice fields whereas the opposite trend was apparent for the belostomatids. Mayflies were more abundant in the wild rice system.

Cx. tarsalis placed in floating cages in the wild rice fields pupated five to seven days earlier

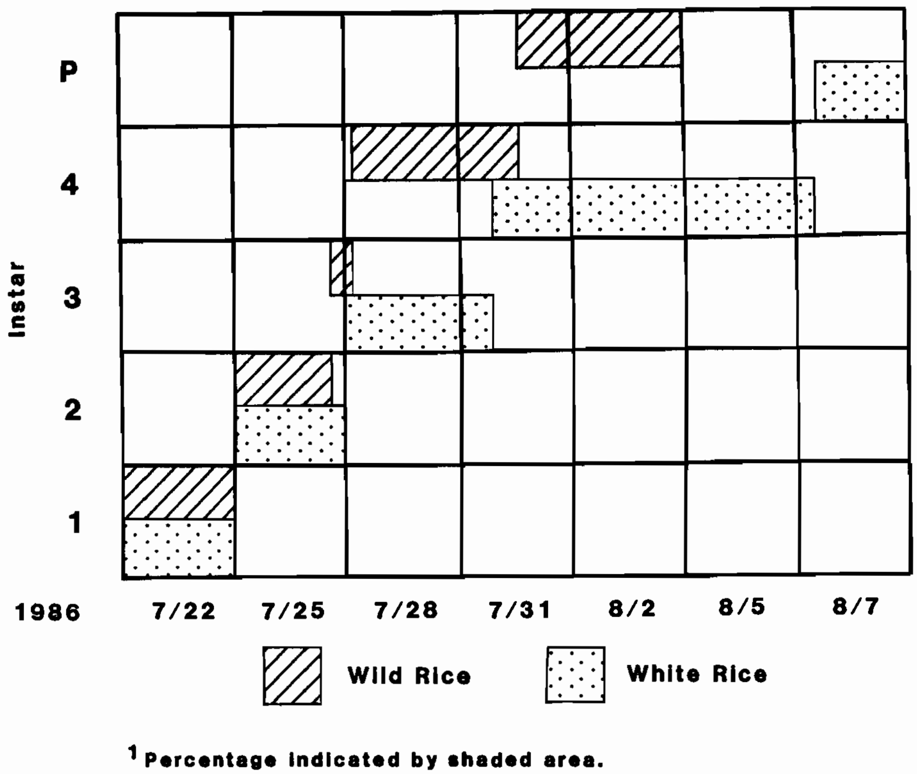

Figure 2.-Percentage[1] of surviving *Cx. tarsalis* larvae at different stages of development in wild vs. white rice fields, Sutter County, 1986.

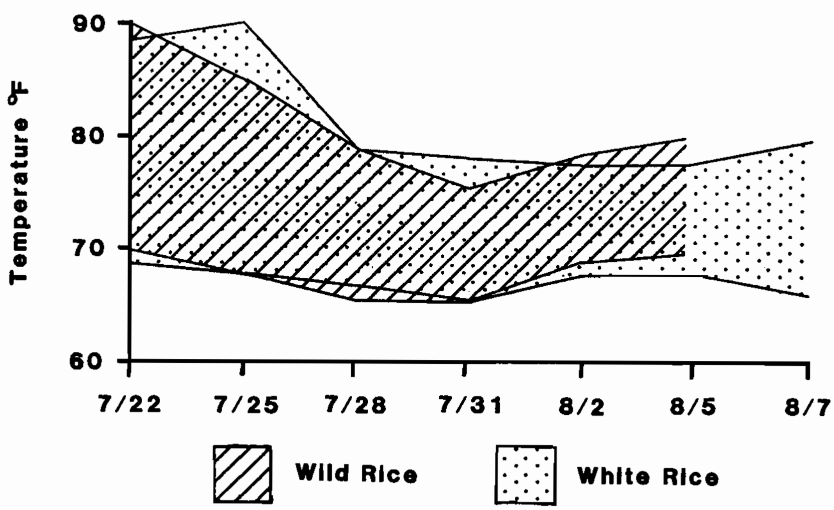

Figure 3.-High/low water temperatures in wild and white rice fields, Sutter County, 1986.

than larvae developing in the white rice fields (Fig. 2). Minimum developmental time from first instar to pupation was 10 days in the wild rice versus 17 days in the white rice. The water temperatures were very similar in both systems (Fig. 3). The most dramatic difference observed was in the time spent in the fourth instar (Fig. 2). Mortality was highest during the early instars, with an overall rate of 48% in the wild rice and 60% in the white rice. This mortality was due in part to predation as cages were left uncovered to minimize the effect of the cage on the immediate habitat of the larvae. The disparity in developmental time may have been due to differences in the nutritive content of the water.

Only a very preliminary assessment can be made on the ovipositional preference of *Cx. tarsalis* since only one of three laboratory colonies under test survived the course of the study, which was carried out near the end of the wild rice growing season. Females in the remaining colony showed a strong preference for the wild rice water by laying 71 rafts in the wild rice field water versus 8 in the pan with white rice water. Further comparative studies on the ovipositional preference and developmental rate of *Cx. tarsalis* are planned for 1987.

In conclusion, there was a significantly higher mosquito population and shorter larval development time in wild versus white rice fields. The quicker turnover time of larvae combined with the greater numbers in wild rice implies a much larger emerging adult population. The shorter growing season and the generally earlier planting dates for wild versus white rice in the Central Valley may partially ameliorate this effect.

ACKNOWLEDGMENT

We thank the personnel at the Sutter/Yuba Mosquito Abatement District for their assistance.

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FACTORS AFFECTING LARVAL MOSQUITO ABUNDANCE IN NORTHERN CALIFORNIA RICE FIELDS

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ABSTRACT

The relationship of water quality and aquatic plants with the abundance of *Culex tarsalis* and *Anopheles freeborni* larvae was investigated during two separate studies. The results from single and multiple linear regression methods suggested that the concentration of calcium, magnesium, and phosphate and the occurrence of the macrophytic alga, *Chara*, may be associated with the larval abundance of these two mosquito species in northern California rice fields. No correlation was observed between *Culex tarsalis* and *Anopheles freeborni* larval abundance during both studies.

INTRODUCTION

The number of mosquito larvae inhabiting rice fields may be quite variable from field to field and from year to year. Numerous field studies have attempted to determine the reasons for this variability. For many mosquito species water quality has been shown to influence oviposition and larval development (Bates 1949; Clements 1963) and several investigations have suggested that the distribution of mosquito larvae in naturally occurring habitats may be associated with various water chemistry parameters (Udevitz et al. 1987; Vrtiska and Pappas 1984; Hagstrum and Gunstream 1971). In addition, water chemistry may also influence the effectiveness of pathogenic fungi (Jaronski and Axtell 1982; Merriam and Axtell 1982) and nematodes (Petersen 1982), which are potential biological control agents of mosquitoes.

The type and density of aquatic vegetation in larval habitats may also affect the abundance of mosquito larvae through their effect on water temperature, surface characteristics, water chemistry, and predation rates (Eldridge and Martin 1988). Associations between plant species and mosquito breeding are known to exist. Rioux et al. (1968) produced a phytoecological map based on the association of 30 plant communities with mosquito breeding habitats in southern France. Following this initial study phytoecological maps have been developed in the Rhone-Alps and Atlantic coastal regions of France, in Korea, the eastern United States and Canada (Hayes et al. 1985). Along similar lines, Barnes and Cibula (1979) discussed the relationship of salt marsh vegetation and the breeding habitat of *Aedes sollicitans* in Louisiana and related these results to geographic features defined using remote sensing technology.

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This paper summarizes two separate studies designed to identify the relationship of water quality and weed diversity with mosquito abundance northern California rice fields and attempts to quantify these relationships using multivariate methods.

MATERIALS AND METHODS

Water Quality.-Twenty fields in south Sutter County were monitored for mosquito larvae weekly, from June 20 through September 19, 1983. Each field was dipped by three people using a standard mosquito dipper (0.437 liter), each taking 60 dips. Mosquito larvae were identified to species and recorded as total number larvae per 180 dips. Water chemistry was monitored every two weeks by removing from each field three 550-ml water samples for chemical analysis (pH, calcium, magnesium, sodium, chloride, sulfate (SO_4), phosphate (PO_4), boron, carbonate (CO_3+HCO_3), and conductivity). All water chemistry analyses were performed by Land, Air, and Water Resources Soil Extension Laboratory, University of California, Davis.

Vegetation.-In 1985, 46 fields located throughout Sutter County were monitored for larval mosquito abundance and the occurrence of weed species. Each field was sampled for mosquito larvae by three people each taking 10 dips each using a standard mosquito dipper. The weed canopy was identified on site and the contribution of each weed species to the total canopy was estimated by assigning a number between 0 and 7, with 0 being absent and 7 being very abundant. Each field was monitored every two weeks, from July 12 through August 30, 1985.

In order to determine the relationship of water quality and larval abundance, a linear regression model was constructed in a stepwise fashion for each mosquito species. Each model was created from the previous model by adding the variable with largest significant ($P<0.15$) partial correlation. Variables already in each equation were removed if their adjusted partial correlation coefficient was no longer significant ($p<0.15$).

RESULTS

Water Quality.-In general the rice field water sampled had a fairly low concentration of inorganic ions; none of the variables measured exceeded state guidelines concerning irrigation water. The concentration of dissolved ions was dominated by carbonate which is typical for hard water found in the Sacramento Valley. Correlation analysis indicated that many of the water chemistry variables were intercorrelated and it appeared that two groups may exist. Variables within group 1 (the upper group in Table 1) were highly intercorrelated ($r=0.55-0.93$) and tended to positively vary together, that is, when one was high, the others were also found to be high. Variables within group 2 (the lower group in Table 1) were not strongly correlated among themselves nor among the variables in group 1 and thus appeared to be independent.

The seasonal abundance of both *Culex tarsalis* and *Anopheles freeborni* larvae was variable with time, and each species exhibited its characteristic seasonal dynamics. The data was compared on a field by field basis so that the overall abundance of larvae for each species was summarized as the total number of larvae per 180 dips, averaged over all sampling dates. Correlation analysis (Table 1) indicated that *Cx. tarsalis* larval abundance was positively correlated with calcium ($r=.464$) and chloride ($r=.427$) concentrations and that *An. freeborni* was positively correlated with pH ($r=.557$), carbonate ($r=.443$), calcium ($r=.400$), magnesium ($r=.597$) and phosphate ($r=.409$). No other statistically significant correlations ($p<.05$) were observed. There was no significant correlation ($r=0.02$; $p<.93$) between *Cx. tarsalis* and *An. freeborni* larval abundance in these fields.

The multiple linear regression models select-

Table 1.-Water chemistry parameters monitored during July through August, 1983 in Sutter County, California. The correlation (r) and level of significance (α) for these parameters are listed for each mosquito species.

Parameters	Mean	Range	Culex	Anopheles
Calcium (mg/1)	15.87	7.816 - 41.884	0.464**	0.399*
Magnesium (mg/1)	10.17	3.405 - 23.347		0.597**
pH	6.2	5.3 - 7.2		0.557**
Conductivity (millimhos/cm)	0.23	0.07 - 0.58		0.396*
Carbonate (CO_3+HCO_3) (mg/1)	121.25	36.002 - 257.504		0.443**
Sodium (mg/1)	24.81	5.748 - 77.476		
Chloride (mg/1)	13.15	0.00 - 73.027	0.427*	
Boron (mg/1)	0.61	0.252 - 1.514		
Sulfate (SO_4) (mg/1)	7.59	0.00 - 18.251		
Phosphate (PO_4) (mg/1)	1.65	0.317 - 3.166		0.409*

*α =.10 **α =.05

Table 2.-Multiple linear regression models relating water chemistry to larval mosquito abundance (n=20).

Species	Intercept	Ca	Coefficients[a] (SE) Mg	P-PO_4	R^2	Prob.>F
Cx. tarsalis	15.40 (85.98)	28.33 (12.12)	-25.58 (16.17)	0	0.32	0.0396
An. freeborni	-406.62 (380.30)	-115.78 (38.90)	223.40 (51.88)	14724.00 (165.39)	0.68	0.0003

[a]Ion concentrations in mg/1

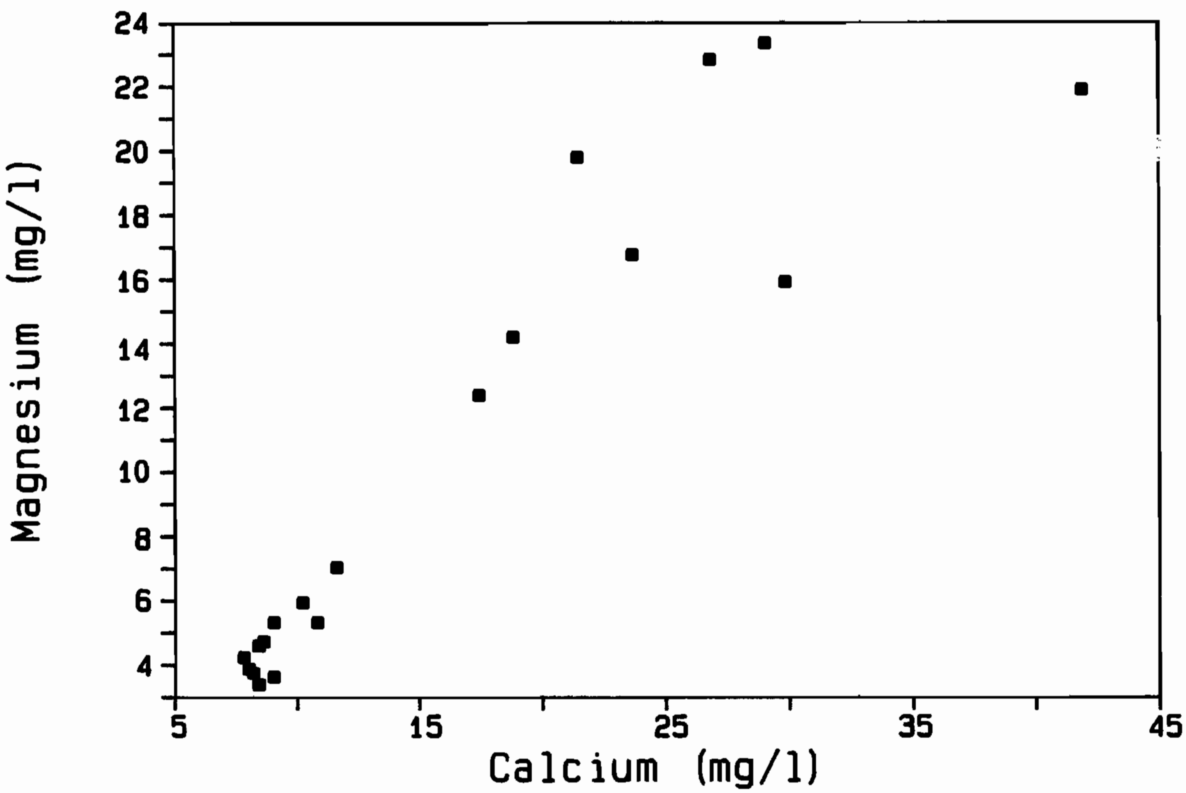

Figure 1.-Plot of magnesium concentration vs. calcium concentration for 20 fields in Sutter County, California.

ed through the stepwise procedure are presented in Table 2. For *Cx. tarsalis*, the model contained two variables: calcium and magnesium concentrations; for *An. freeborni*, the model contained three variables: calcium, magnesium, and phosphate concentrations. Both models were statistically significant ($p<0.05$; $n=20$). Even though chlorine had a significant zero-order correlation with *Cx. tarsalis* larval abundance, it did not enter the analysis. Once calcium entered the equation, the addition of chlorine contributed no new information. The same phenomenon occurred in the second analysis with many variables that, when alone, were significantly associated with *An. freeborni* abundance.

Interestingly, the coefficients for calcium and magnesium in the regression model for both mosquito species were opposite in sign even though their zero-order correlations were both positive. Calcium and magnesium were highly intercorrelated ($r=0.93$) and thus some information was shared by both variables. The switch in signs will often occur when two independent variables are more highly intercorrelated with each other than either with the dependent variable alone. However, since the unique relationship of both variables with the dependent variable was statistically significant, this suggests that the relationship between calcium and magnesium was not consistent among fields. The plot of magnesium vs. calcium shown in Figure 1 suggests that there may have been two groups of fields: those having low calcium and low magnesium located in the lower left hand corner of the graph, and those having high magnesium but variable concentrations of calcium. These differences may be due to fields being irrigated from different water sources (e.g. wells or surface ditches) or from a geographic variation in the mineral content of the soil bed. Thus, the information provided by the addition of the second variable in the linear regression model was probably not chemical but spatial. In general, the relationship between water chemistry and larval abundance was stronger for *An. freeborni* than for *Cx. tarsalis* (Table 1).

Vegetation.-During 1985, the abundance of both *Cx. tarsalis* and *An. freeborni* larvae was variable over time, similar to that observed in the 1983 study discussed above. As in the previous study, the total seasonal production of mosquitoes was estimated by averaging the total number of larvae collected over all sample dates. A total of 28 macrophytes and algae were identified in the 46 fields; the number of vascular plant species other than rice in a single field ranged from 2 to 15. The plant species most commonly encountered, as indicated by incidence (the number of fields where present out of 46 sampled) in Table 3, were *Echinochloa crusgalli* (Barnyardgrass), *Sorghum* (Johnsongrass), *Polygonum* (Smartweed), *Sagittaria* (Arrowhead), and *Spirogyra*. The most abundant aquatic plants as indicated by the mean index of cover were *Echinochloa crusgalli* (Barn-

Table 3.-The occurrence of aquatic vegetation in rice fields in Sutter County, California, during July and August, 1985. Incidence is reported as the number of fields out of 46 sampled in which each plant species was present. The units of abundance (0-7), with 0 being absent and 7 being very abundant, are qualitative. The correlation (r) and levels of significance (α) for each plant species are listed for both mosquito species.

		Incidence	Abundance		Culex	Anopheles
			Mean	Range		
GRASSES					-0.248*	
Echinochloa crusgalli	(Barnyardgrass)	32	1.05	0 - 6.00		
Leptochloa fascicularis	(Bearded Spangletop)	3	0.04	0 - 0.70		
Leptochloa imbricata	(Mexican Spangletop)	18	0.22	0 - 1.40		
Paspalum distichum	(Knotgrass)	5	0.04	0 - 0.70		
Sorghum spp.	(Johnsongrass)	32	0.79	0 - 4.30		
SEDGES						
Cyperus difformis	(Nutgrass)	19	0.99	0 - 5.70		
Eleocharis spp.	(Spikerush)	2	0.02	0 - 0.50		
Scirpus spp.	(Bulrush)	22	0.75	0 - 4.30		
Typha spp.	(Cattail)	8	0.10	0 - 1.30		
OTHER EMERGED						
Alisma triviale	(Water plantain)	19	0.45	0 - 5.30		
Ammannia coccinea	(Redstem)	15	0.37	0 - 2.70	0.307**	
Echninodorus cordifolius	(Burhead)	4	0.05	0 - 1.00		
Polygonum spp.	(Smartweed)	31	0.45	0 - 2.70		
Sagittaria spp.	(Arrowhead)	31	1.01	0 - 5.00		-0.277*
TOTAL EMERGED (GRASS+SEDGE+OTHER EMERGED)						-0.254*
FLOATING						
Azolla spp.		3	0.03	0 - 0.70		
Bacopa rotundifolia	(Waterhyssop)	7	0.13	0 - 2.00		
Heteranthera limosa	(Ducksalad)	12	0.21	0 - 2.20		
Lemna spp.	(Duckweed)	12	0.51	0 - 4.70		
Potamogeton spp.	(Pondweed)	1	0.01	0 - 0.70		
SUBMERGED						
Callitriche spp.	(Water-Starworts)	21	0.52	0 - 4.30		
Heteranthera limosa	(Ducksalad)	12	0.21	0 - 2.20		
Najas spp.	(Naiads)	20	0.94	0 - 5.30	0.273*	
Potamogeton spp.	(Pondweed)	1	0.01	0 - 0.70		
BLUE GREEN ALGAE						-0.243*
Anabaena spp.		20	0.25	0 - 1.50		
Gloeotrichia spp.		19	0.52	0 - 5.00		
Oscillatoria spp.		8	0.09	0 - 1.50		
GREEN ALGAE						
Chara spp.		13	0.37	0 - 3.30	0.249*	0.345**
Hydrodictyon spp.		3	0.03	0 - 0.70		
Nitella spp.		6	0.09	0 - 1.70		
Spirogyra spp.		27	0.62	0 - 4.00		

*α =.10 **α =.05

yardgrass), *Cyperus difformis* (Nutgrass), *Scirpus* (Bulrush), *Sagittaria* (Arrowhead) and *Najas*.

The results of the correlation analysis indicated that the abundance of *Cx. tarsalis* larvae was positively correlated with *Ammannia coccinea* (Redstem) (r=.307), *Najas* (r=.273), and *Chara* (r=.249) and was negatively correlated with the Total Grasses (r=-.248), but not any individual grass species. *An. freeborni* larval abundance was positively correlated with *Chara* (r=.345) and negatively correlated with *Sagittaria* (Arrowhead) (r=-.277), total blue-green algae (r=-.243), and total emerged weeds (r=.254) (but not any individual species). No other statistically significant zero-order correlations were observed. As observed in 1983, *Cx. tarsalis* and *An. freeborni* larval densities in rice fields were not correlated (r=.13; p>.10).

A model to predict larval abundance by the species mix of the plant canopy was created for each mosquito species using stepwise linear regression methods. The resulting models are shown in Table 4. Only the green alga, *Chara* was selected for both models. The utility of these models appears much better for *An. freeborni* (R^2=.46; p<.05) than for *Cx. tarsalis* (R^2=.30; p<.05).

DISCUSSION

In general, the correlation coefficients of *Cx. tarsalis* or *An. freeborni* larval abundance and aquatic plant species were low, and there doesn't appear to be a strong association of mosquito abundance with any particular plant species. However, both *Cx. tarsalis* and *An.*

Table 4.-List of the coefficients for the multiple linear regression models predicting mosquito abundance from the density of weed species. The variables are listed according to order of entry.

Culex tarsalis (R^2 = 0.30; p<05)

Species	Coefficients	SE
Intercept	6.629	9.1124
Ammannia coccinea	33.802	10.0311
Chara	28.393	9.5321
Cyperus difformis	-9.872	4.7712
Nittella	-49.913	24.9472

Anopheles freeborni (R^2 = 0.46; p<.05)

Species	Coefficients	SE
Intercept	23.685	7.0613
Chara	22.099	4.8557
Total Bluegreen Algae	-11.859	3.6270
Total Floating Macrophytes	-9.937	2.7692
Najas	8.524	2.6221
Leptochloa fascicularis	64.766	25.0911
Leptochloa imbricata	21.609	11.8978
Scirpus	-5.025	2.9843

freeborni were positively correlated with *Chara*. The alga prefers hard water that is high in calcium (Prescott 1970). In the 1983 water quality study, both *Cx. tarsalis* and *An. freeborni* larval densities were found to be positively correlated with calcium. This suggests that *Chara* and *Cx. tarsalis* and *An. freeborni* larval abundance may be indirectly associated through their mutual association with calcium-rich water. No correlation was found between the larval abundance of *Cx. tarsalis* and *An. freeborni* in either study.

The objective of both studies was to survey and discover any relationships that may exist between water quality, vegetation, and larval abundance and it was for this reason that the error level in the stepwise regression analysis ($p<.15$) was set so high. Studies of this kind are preliminary and any associations found do not imply cause and effect.

In general, the predictive power of the linear regression models were low, especially for *Cx. tarsalis*. Undoubtedly larval abundance for both species is also related to environmental factors not considered in these studies. *Cx. tarsalis* is a widespread species and is generally more tolerant of variation in water chemistry than is *An. freeborni*. Results presented here provide a basis for further studies of biotic and abiotic factors influencing larval mosquito abundance in northern California rice fields.

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USE OF A SURFACE SAMPLER TO ASSESS POPULATION DENSITIES OF AQUATIC ORGANISMS IN RICE FIELDS

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ABSTRACT

The surface sampler demonstrated the ability to collect a wide variety of aquatic organisms from the rice field habitat, but was somewhat selective for organisms of the nekton component. All immature stages of *Culex spp.* and *Anopheles spp.* were collected by the device during the survey. Data suggests the seasonal prevalence of *Culex* immatures followed a bimodal curve while *Anopheles* immatures steadily increased during the sample period. Surface samplers were more efficient in collecting *Culex* than *Anopheles* immatures. Seven times as many *Culex* immatures were collected than *Anopheles* immatures.

INTRODUCTION

Rice fields are the primary source of the encephalitis mosquito, *Culex tarsalis* Coquillett and the western malaria mosquito, *Anopheles freeborni* Aitken in the Sacramento County/Yolo County Mosquito Abatement District. During 1986, 36,189 acres of white and wild rice were grown in 459 fields, ranging in size from 1 to 470 acres. The average field size in the District was 80 acres, with typical field layout either of contour or laser level design. Cultural practices, such as herbicide and fertilizer applications, vary between individual growers from "organic" to heavy reliance on insecticides and herbicides to control pest species.

The District plants mosquitofish at 0.1 pound an acre in the spring and from July until September routinely adulticides the fields with malathion or pyrethrins to manage mosquitoes emanating from this source. To augment these control strategies, the District hires 8 to 10 seasonal rice checkers from May until September to monitor the larval population in the rice fields. They are assigned 25 fields to sample on a weekly basis. Depending on field size, they take from 25 to 100 dips with a standard pint dipper, recording the number of mosquito immatures and beneficial organisms collected. When larval populations exceed a predetermined tolerance level (0.1 larva/dip), the fields are treated with Vectobac®-AS.

Numerous devices and techniques have been proposed for larval sampling (Service 1976), but other than the standard pint dipper, none are applicable to a large scale operational program. Dipping as a larval surveillance method has inherent limitations due to the human element (Horsfall 1946) and dipping efficiency (Ree 1982), so there is a need to develop an objective sampler that can be employed in a large scale rice field surveillance program.

During 1985, the District laboratory staff devised a inexpensive sampler that could be mass produced from readily available materials. The design is similar to a floating quadrat developed by Goodwin and Eyles (1942), but has been modified to float below the water surface and retrieve larvae by simply lifting the device instead of laboriously pumping or dipping out the sample, which is a major disadvantage of most quadrat or area sampling devices (Knight 1964).

A study was undertaken to determine if the surface sampler design would collect aquatic invertebrates and could be employed in the District's rice field surveillance program.

MATERIALS AND METHODS

Surface samplers were constructed using 10-inch plastic embroidery hoops with interlocking sections, 200-mesh polyester monofilament fabric (Tetko, Inc., product number HC7-475), 1-inch red and white plastic fishing floats (Plastilite Corp., product number P3-BK) and 12-pound test nylon fishing line.

To assemble the sampler, a 12.75-inch (32.38 cm) diameter piece of polyester fabric was cut out, then locked in place between the embroidery hoop sections. Next, four 1/16-inch (0.16 cm) diameter holes were drilled equidistant apart through the face of the hoop. A 4-inch (10.16 cm) length of fishing line was then tied around the top of each hole so that fishing floats could be anchored to the hoop (Figure 1).

When placed in the water, the floats suspend the sampler 0.5 inch (1.27 cm) below the water surface. This allows organisms to be collected from above or within the device. The surface area of the sampler is approximately 0.5 square foot (0.1575 m^2).

The study site was the Yolo By-pass portion of Heidrick Farm's Conway Ranch which is located in eastern Yolo County between Interstate 5 and U.S. 80 Freeways and the Conway and Tule canals. Eight crops (tomatoes, corn safflower, beets, barley, wheat, white and wild rice) are farmed on the ranch with the majority of the acreage devoted to rice cultivation. Rice fields are first flooded in May, drained for herbicide applications of Bolero or Ordram, reflooded, and continously irrigated until September.

Fourteen separate rice fields were chosen at random, ranging in size from 17 to 470 acres and totaling approximately 2,000 acres. All fields in the study area were laser planed and planted with

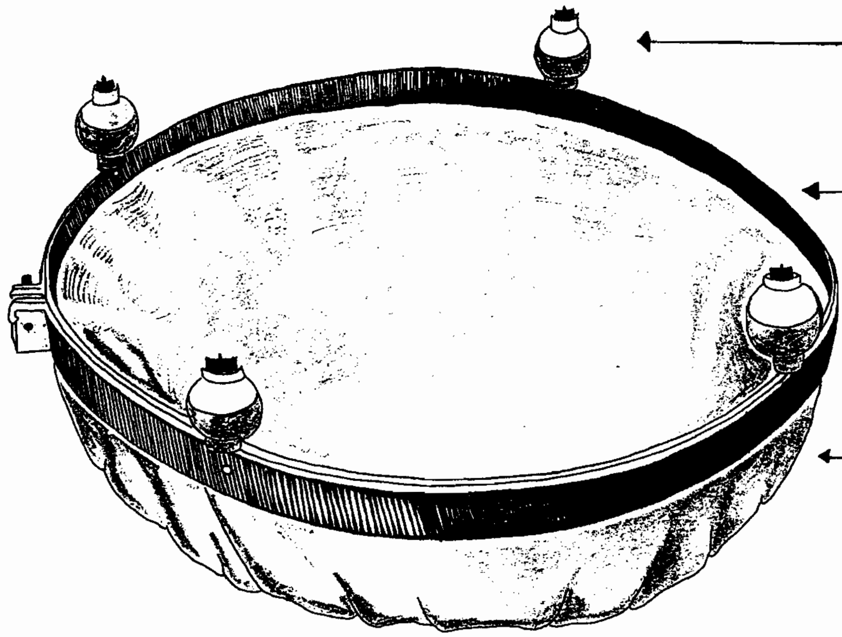

Figure 1.-Surface Sampler

either a M9 or 302 white rice variety.

Depending on acreage, each rice field was separated into either 1, 2 or 4 sampling areas. Fields less than 40 acres were considered as one area, while 40 to 80 acre fields were divided into two equal areas. Fields greater than 80 acres were separated into four equal areas.

Three surface samplers were randomly positioned within each area so that a 40 acre field contained three samplers; fields between 40 to 80 acres, six samplers; and fields greater then 80 acres, twelve samplers. In the study, a total of 123 surface sampler stations were established for an average of one sampler per 16 acres of surface area.

Surface samplers were placed in the fields during the week of July 16. The samplers were tethered to a wooded survey stake with a nylon fishing line to prevent drift, and were collected weekly from each field from June 23 through August 25.

To retrieve the contents of the sampler the collector lifted the device from the water, allowing excess fluid to drain through the fabric. Samplers were then taken from the field and inverted over a white enamel pan. Organisms and detritus adhering to the sampler were flushed with a pint wash bottle. To facilitate identification, organisms were then separated with a 3-ml plastic transfer pipet into plastic 8-ounce liver cups. The number of aquatic organisms collected were counted and recorded on a survey form.

RESULTS AND DISCUSSION

During the 10 week survey period, aquatic organisms from 12 different classes were collected by the surface sampler (Table 1). The list contains representatives from all five aquatic life habitats: benthos, periphyton, plankton, nekton and neuston. Members of the nekton component dominated the collections. The greatest diversity, 5 Orders containing 13 families were sampled from the class Insecta.

In a recent dipping survey of Fresno County rice fields, a more extensive list of aquatic organisms were collected (Miura et al. 1981). However, the diversity and community composition was similar to the results obtained by the surface sampler survey of the Sacramento County rice fields.

A total of 8,784 organisms was collected in the survey with an average of 10.2 per sampler (Table 2). Dytiscid larvae were the most prevalent organism collected by the sampler, followed by *Mesostoma spp.*, hydrophilid larvae, zygopterans, culicidae immatures, notonectids, dytiscid adults, anisopterans, hydrophilid adults, gerrids, *Hydra spp.*, belostomatids, *Dugesia spp.* and veliids. Two organisms, dytiscid larvae and *Mesostoma spp.* represented 50% of the total collection, while the remaining 50% was divided among 12 groups. Belostomatids, *Dugesia spp.* and veliids were rarely found in the survey. The mean number of organisms per sampler ranged from a high of 2.67 for dytiscid larvae to a low of 0.002 for veliids. Mosquito immatures (*Culex* and *Anopheles*) were frequently collected by the surface sampler and ranked fifth in abundance with an average of 0.62 per sampler.

The seasonal distribution of aquatic organisms (excluding mosquito immatures) collected by

Table 1.-List of aquatic organisms collected by surface sampler.

Rotifera		Hemiptera	
Hydroza			Veliidae
	Hydra spp.		Notonectidae
Turbellaria			Gerridae
	Mesostoma spp.		Belostomatidae
	Dugesia spp.		Corixidae
Oligochaeta		Coleoptera	
Hirudinea			Dytiscidae
Crustacea			Hydrophilidae
	Anostraca	Diptera	
	Notostraca		Culicidae
	Conchostraca		Syrphidae
	Cladocera		Stratiomyiidae
	Eucopepoda		Tendipedidae
	Podocopa	Gastropoda	
	Decapoda	Osteichthyes	
Insecta			Poeciliidae
	Ephemeroptera		Ictaluridae
	Odonata		
	Anisoptera		
	Zygoptera		

Table 2.-Population density of aquatic organisms collected by surface sampler (7 weeks).

		Number Collected	Mean Number/Sampler
1.	Dytiscid larvae	2301	2.67
2.	*Mesostoma spp.*	2108	2.44
3.	Hydrophilid larvae	1428	1.65
4.	Zygopteran	576	.66
5.	Culicidae immatures	535	.62
6.	Notonectid	497	.57
7.	Dytiscid adult	429	.49
8.	Anisoptera	409	.47
9.	Hydrophilid adult	301	.34
10.	Gerrid	129	.14
11.	*Hydra spp.*	65	.07
12.	Belostomatid	2	.002
13.	*Dugesia spp.*	2	.002
14.	Veliid	2	.002
	Total	8784	10.2

Table 3.-Seasonal distribution of aquatic organisms collected by surface sampler.

Week	6/23	6/30	7/7	7/14	7/21	7/28	8/4
Belostomatid						1	1
Gerrid	1	10	10	13	30	32	33[a]
Notonectid	17	78	106	122[a]	71	61	42
Veliid		1			1		
Dytiscid larvae	129	331	451[a]	443	375	351	221
Dytiscid adult	133	159[a]	36	35	26	23	17
Hydrophilid larvae	400[a]	261	241	210	148	92	75
Hydrophilid adult	44	10	82	85[a]	32	24	24
Anisoptera	58	62	51	48	55	75[a]	60
Zygopteran	32	56	32	68	84	148	156[a]
Dugesia spp.						1	1
Hydra spp.		40	2	1	22		65[a]
Mesostoma spp.	43	219	397	400	528[a]	365	155
Total	857	1227	1408	1425[a]	1372	1173	850

[a]Highest number collected during sampling period.

the surface sampler is shown in Table 3. Results from the sampler suggests that the aquatic communities population followed a bell-shaped growth curve with the highest population levels reached during the week of July 14.

Population peaks for each group varied throughout the sample period, most occuring in the last week of the survey. The hydrophilid larvae population peaked during the first week (June 23) of the survey; the dytiscid adults in the second week (June 30); the dytiscid larvae in the third week (July 7); the notonectids and hydrophilid adults in the fourth week (July 14); the *Mesostoma spp.* in the fifth week (July 2); the Anisoptera in the sixth week (July 28); and gerrid, zygopteran and *Hydra spp.* during the seventh week (August 4).

A total of 704 *Culex spp.* immatures were collected by the surface samplers (Table 4). Second instar larvae were the most frequently collected (183), followed by firsts (174), fourths (167), thirds (155) and pupae (25). An equal number of early instars (1st and 2nd) and late instars (3rd and 4th) were collected. The pupae represented a small percentage of the population.

The *Culex* first instar population was highest during the 7th week (August 4) of the survey, seconds in the 2nd week (June 30), thirds in the 8th week (August 11), fourths and pupae in the 3rd week (July 7).

During the survey, 113 *Anopheles* immatures were collected by the surface samplers; the population breakdown was comprised of 15 firsts, 26 seconds, 39 thirds, 29 fourths and 4 pupae (Table 5). The third instar larvae were the most frequently collected, followed by the fourths, seconds, firsts and pupae. The *Anopheles* first instar population was highest during the 7th week (August 4) of the survey, seconds and thirds in the 10th week (August 25), fourths and pupae in the 9th week (August 18).

Data from the surface sampler indicated that the *Culex* immature population frequency distribution followed a bimodal curve (Figure 2). There were two population peaks,; the first in the week of July 7, the second during the week of August 4. In contrast, the *Anopheles* population steadily increased from the sixth week (July 28) until the final week (August 25) of the survey. The seasonal population patterns for both *Culex* and *Anopheles* immatures are similar to trends observed in other rice field dipping surveys (Collins and Washino 1980 and Markos and Sherman 1957).

Of the 14 fields surveyed, 13 were positive for mosquito immatures. The mean number of mosquito immatures collected ranged from 0.0 to 1.60 per sampler (Table 6). Three fields had an immature population greater then 1.0 and only one field was negative for mosquito larvae. The majority of the fields ranged between 0.30 and 0.82 immatures per sampler, with four fields accounting of 50% of the larval production.

The mean number of *Culex* immatures collected by the surface samplers ranged from 0.0 to 1.41. Three fields had a population greater than 1.0 per sampler, and three fields less than 0.1 per sampler. The remainder of the fields were between 0.29 and 0.81 immatures per sampler.

The mean number of *Anopheles* collected by the surface sampler ranged from 0.0 to 0.4. The population in 5 fields was greater than 0.1 per sampler, and in 5 fields less than .01. Four fields were between .02 and .08 per sampler.

CONCLUSION

The determination of abundance and distribution of organisms during the rice field growing season present the basic problems facing

Table 4.-Seasonal distribution of *Culex spp.* collected by surface sampler.

	Week										
	6/23	6/30	7/7	7/14	7/21	7/28	8/4	8/11	8/18	8/25	
Stage											Total
1st		12	32	20	6	14	60[a]	17	13		174
2nd	2	53[a]	30	9	8	8	25	32	12	4	183
3rd	2	12	22	11	4	7	28	43[a]	19	7	155
4th	2	30	39[a]	25	15	5	14	18	17	2	167
pupae	2	2	11[a]	4	1	2	2	1			25

[a]Highest number collected during sampling period.

Table 5.-Seasonal distribution of *Anopheles spp.* immatures collected by surface sampler.

	Week										
	6/23	6/30	7/7	7/14	7/21	7/28	8/4	8/11	8/18	8/25	
Stage											Total
1st	0	0	0	0	1	1	6[a]	3	4	0	15
2nd	0	0	0	0	0	0	3	5	8	10[a]	26
3rd	0	0	0	0	0	1	3	12	9	14[a]	39
4th	1	0	0	0	0	0	1	7	10[a]	10[a]	29
pupae	1	0	0	0	0	1	0	0	2[a]	0	4

[a]Highest number collected during sampling period.

Table 6.-Field ranking, mean number of mosquito immatures per sampler.

Rank	Size(Acres)	*Culex spp.*	*Anopheles spp.*	Total
1	70	1.20	.40	1.60
2	440	1.41	.02	1.43
3	25	1.10	.10	1.20
4	255	.81	.01	.82
5	45	.73	.05	.78
6	162	.45	.20	.65
7	167	.47	.14	.61
8	152	.50	.08	.58
9	240	.29	.14	.43
10	185	.40	.01	.41
11	46	.30	.00	.30
12	139	.09	.07	.16
13	30	.06	.00	.06
14	17	.00	.00	.00

mosquito control efforts. Predictions can be made from reliable samples, however, sampling of organisms must be made in a consistant, unbiased manner to realistically estimate these population parameters.

Data collected from this first trial demonstrated the ability of the surface sampler to function objectively. Some selectivity for organisms present in the nectonic zone was noted, however, such selectivity was thought acceptable as most mosquitoes and their active predators inhabit this zone.

The samplers simplicity of design, low cost, easy maintenance and overall collection reliability indicates that it could be readily employed by mosquito abatement districts. Field trial results were encouraging and the surface sampler will continue to be evaluated.

ACKNOWLEDGMENTS

The authors thank G. Yoshimura and Susan Critchfield for their assistance in designing the

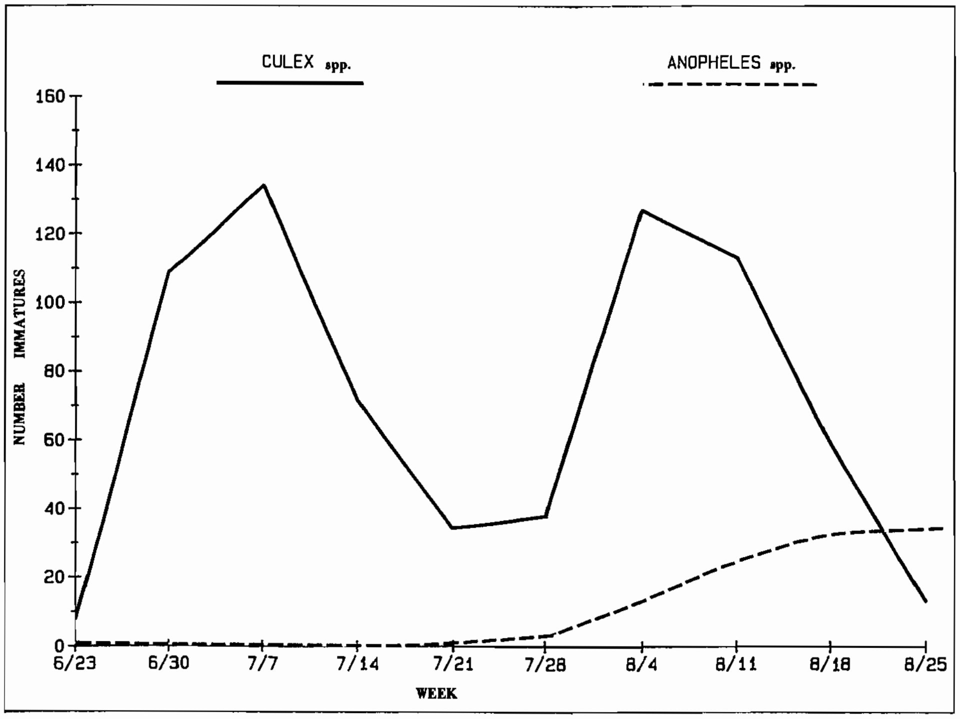

Figure 2.-Population curve of mosquito immatures collected by surface sampler.

sampler, Annett Eiffert for her help in constructing the traps and M. Tucker for his field assistance.

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THE MOSQUITOFISH (*GAMBUSIA AFFINIS*): POPULATION DYNAMICS STUDY IN SMALL EXPERIMENTAL RICE PLOTS

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ABSTRACT

In early May, three quantities (91,408 and 2722 g) of well-fed, predominantly gravid female mosquitofish were introduced into 6.1 m^2 rice plots, where plankton and immature insects were relatively abundant. The mosquitofish increased at an average rate of 1.4X per week for the next several weeks, reaching a peak of ca. 42,000 at the end of September. The population growth curves followed logistic curves fairly closely. Increased stocking rates generally produced an earlier population peak. Population peaks of stocked fish may never reach an equilibrium level (carrying capacity) because fields need to be drained yearly in September before harvest.

The mosquitofish, *Gambusia affinis* (Baird and Girard) have been widely used for controlling mosquitoes (Bay 1967, Fowler 1964, Hoy and Reed 1971). The merit of mosquitofish as mosquito-control agents can be attributed to many factors, the most important being a high reproductive capacity that produces a rapid population buildup from initial stockings of fish in mosquito breeding habitats. For example, ten weeks after Hoy and O'Grady (1971) planted 100 fish per acre, the population grew to an estimated 5900 ± 391 per acre. Twelve weeks after stocking at 1/8 lb/acre and ten weeks after an initial average count of 3 fish per trap, Reed and Bryant (1974) recorded increases averaging 183 per trap. Miura et al. (1982), using a capture/recapture method in late July, estimated absolute population increases from 4722 to 21,920 per acre in a period of only a month.

After an initial planting in rice fields, population increases of this species plotted over time normally follow a logistic growth curve with a degree of leveling off during the later half of the season. It seems that this leveling off is attributed to the maximum carrying capacity of rice fields, but whether it is or not, the fact remains that it has occurred on a number of occasions for this species (Miura et al. 1982, Norland and Bowman 1976, Reed and Bryant 1974, Stewart and Miura 1985). This leveling off represents the maximum potential for mosquitofish to prey on larvae due to density of the species and would be the level that would be desirable to coincide with peak populations of mosquito larvae.

During 1986, we conducted an experiment to determine the effects of the initial fish stocking rate on the final population density attained by the end of the rice season.

MATERIALS AND METHODS

Studies were conducted at the experimental rice plots at the University of California Kearney Agricultural Center near Parlier. The facility consists of 12 plots in 4 rows; each plot measures 6.1 m^2 x 0.5 m deep. Water to each plot was supplied from a reservoir through an underground pipeline and water depth was maintained by float valves (Fig. 1). Rice (Cal Pearl) was seeded in early May. Water temperature at 5 cm below the surface and air temperatures were taken with recording thermographs.

Six plots adjacent to each other were used to study the population growth of 3 stocking rates. Mosquitofish were introduced into the plots in early May. The source of fish was a drainage sump on Seventh Standard Road, northwest of Bakersfield, California. The fish were 88.3% gravid females and weighed an average of 1.48 g per fish (Table 1, Fig. 2). The rates stocked were 91,408 and 2722 g of fish per plot. The low rate was about 0.2 lb of fish per acre, which is the normal stocking rate used by the Fresno Westside MAD.

The fish population in each plot was monitored by trapping each week with 4 modified minnow traps (Miura and Takahashi 1975). From early June to late September, traps were set at 10 a.m. and retrieved at 1 p.m. Gravid females, fry and others (male, juvenile and female) were separately tabulated. After counting, all fish were returned to their respective plots. The traps were placed in the same arrangement in each plot and the same position each week.

Total population variances between the 3 stocking rates were analyzed by SAS GLM procedures on a 2550 Prime computer using analysis of variance, Duncan's multiple range, and equality of slopes tests. The data were also analyzed by linear regression of log transformations and also by comparing logistic growth curve models based on the data from the 3 different stocking rates.

RESULTS AND DISCUSSION

When the mosquitofish were initially introduced into the plots where plankton and immature insects were relatively abundant, the fish population increased at an average rate of 1.4X per week (Fig. 3) for the next several weeks, reaching a peak of ca. 42,000 in the end of August and then slowed down thereafter. This

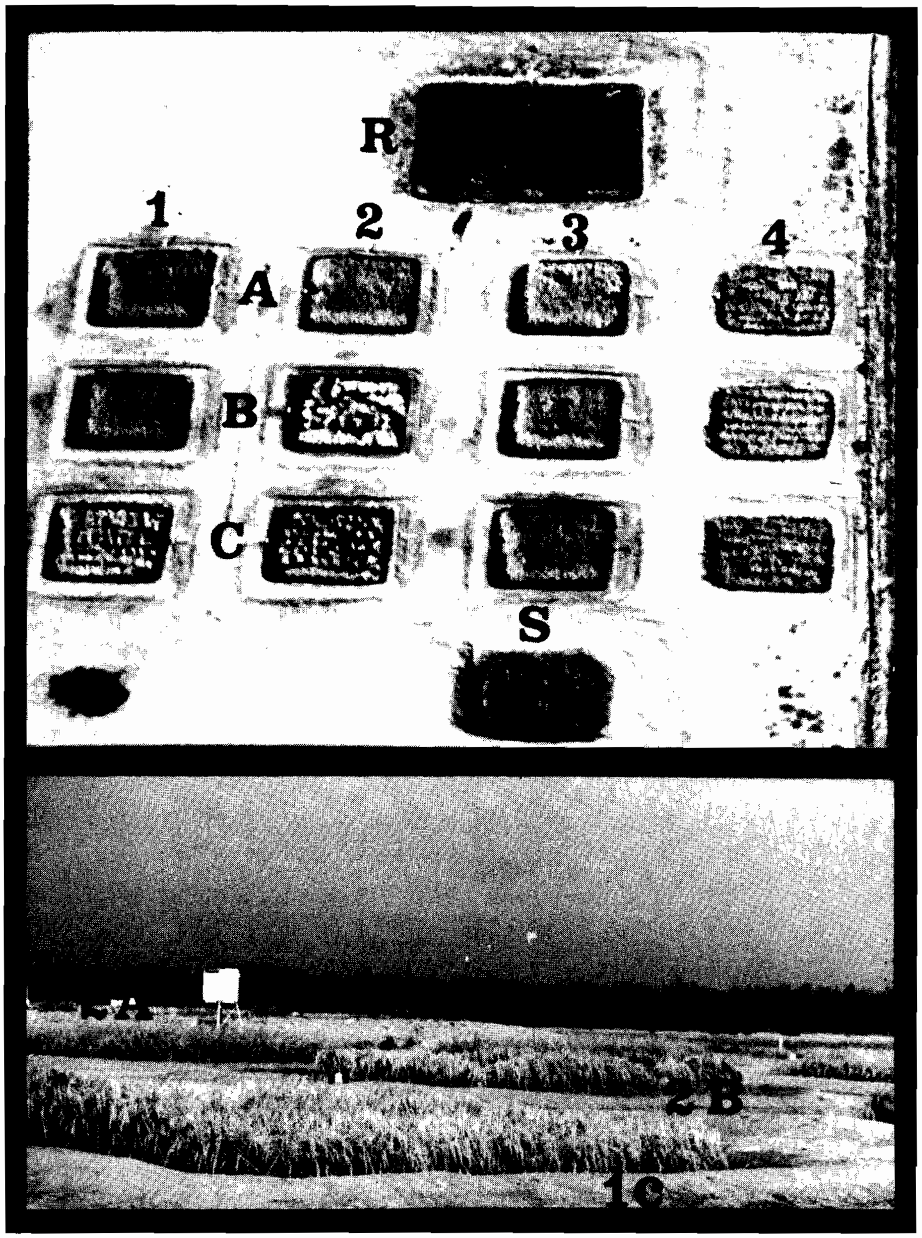

Figure 1.-Rice plots at the Kearney Agricultural Center: A, an aerial photograph showing layout of plots (1, 2, 3, 4, A.B.C.), reservoir (R) and sump (S). B, ground view of plots.

Table 1.-Statistics of mosquitofish.

		Standard length (cm)			Sex ratio
	N	Female	Male	Weight (g)	(F:M)
Stocked fish	74	4.3	2.7	1.48	79.1:20.9
Fish collected after tests	150	3.8	2.5	.46	53.4:46.6

Table 2.-Population growth of mosquitofish: number of fish captured by traps.

Date	Stocking Rate Low	Middle	High
June 5	8	26	24
12	13	16	19
20	77	65	82
26	113	81	107
July 3	67	176	122
9	392	582	476
16	372	780	1250
23	937	1229	1355
30	1096	1108	1561
August 6	1854	3179	2428
13	2177	3364	2535
20	1534	2516	2165
27	1851	2803	1785
September 3	1760	2640	1996
17	2722	2973	2701
25	2940	3021	2536

peak probably represented one of the upper fluctuation peaks, i.e., this peak is a departure from the equilibrium (the carrying capacity). The carrying capacity of an environment is the maximum number of animals that can be supported for a long time without damage to the environment. Since rice fields are artificially created, temporary bodies of water that are allowed to retain water for ca. 4 months during a year, population peaks of stocked fish never reach the equilibrium (Fig. 4). The fish collected at the end of the study were extremely light and slimmish (Table 1, Fig. 2), indicating deficiency of food resources brought about by overgrazing.

The results obtained from the stocking rate study showed generally good correlation between the number of fish caught and the stocking rate (Table 2); the middle rate had the largest mean followed by the high and then the low. Analysis of variance showed there was a significant difference between stocking rates ($F=50.51; P<0.0001$). Duncan's multiple range test ($\alpha=0.05$) also indicates all means were significantly different from each other. Linear regression of log transformed data and predicted logistic growth curves show that the slopes of all populations are approximate and parallel except for the high rate (Fig. 3).

We hypothesized that an increase in fish stocking rate would cause the subsequent population to reach the leveling off (upper fluctuating) peak at an earlier date. Our data generally support the hypothesis except for the high rate (Fig. 1); the peak of the high rate stocking was obtained between the peaks of the middle and low rate stockings. We reasoned the cause of this discrepancy as follows:

1. A sudden overcrowding by a high rate of stocking created a shortage of food and other requirements. This resulted in high mortality and reduced fecundity.

2. Trap avoidance by gravid females. Almost all females initially stocked were gravid (88.3%); they may have avoided the traps.

In summary, mosquitofish have a very high reproductive rate that produced rapid population build-ups from initial stocking rates. The population growth curves followed logistic curves fairly closely. Early in the season the growth rate was about 1.4X per week and leveled off in the late season. The growth curves indicate that an increased stocking rate generally produced an earlier population peak. The peaks approached at the end of the rice growing season were probably peaks of fluctuating level, they are not the equilibrium (the carrying capacity of rice fields). Mosquitofish populations in rice fields probably do not reach the equilibrium level because the rice season is of limited duration.

ACKNOWLEDGMENTS

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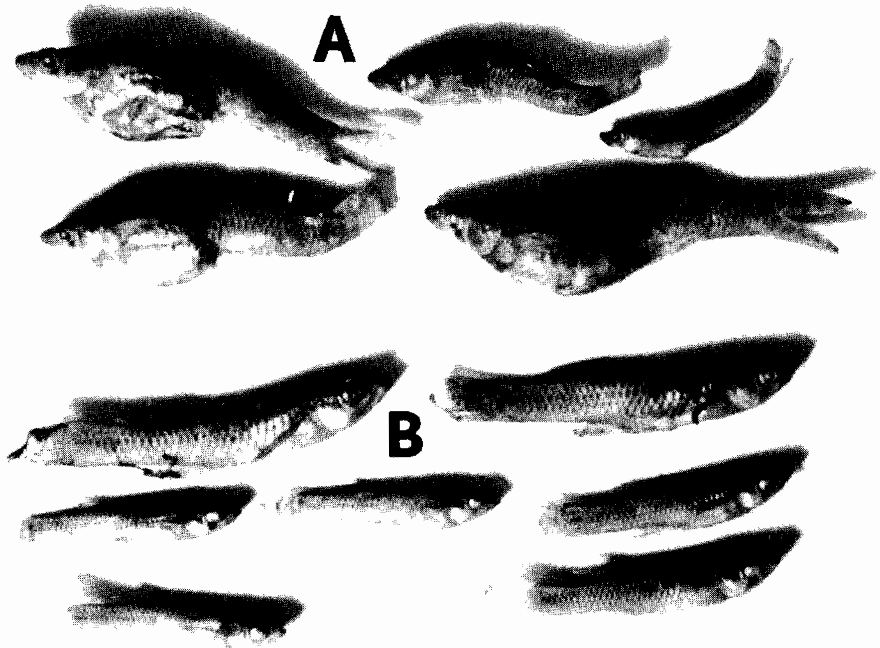

Figure 2.-Mosquitofish showing size and weight differences between drainage collected (A) and rice plot collected fish (B).

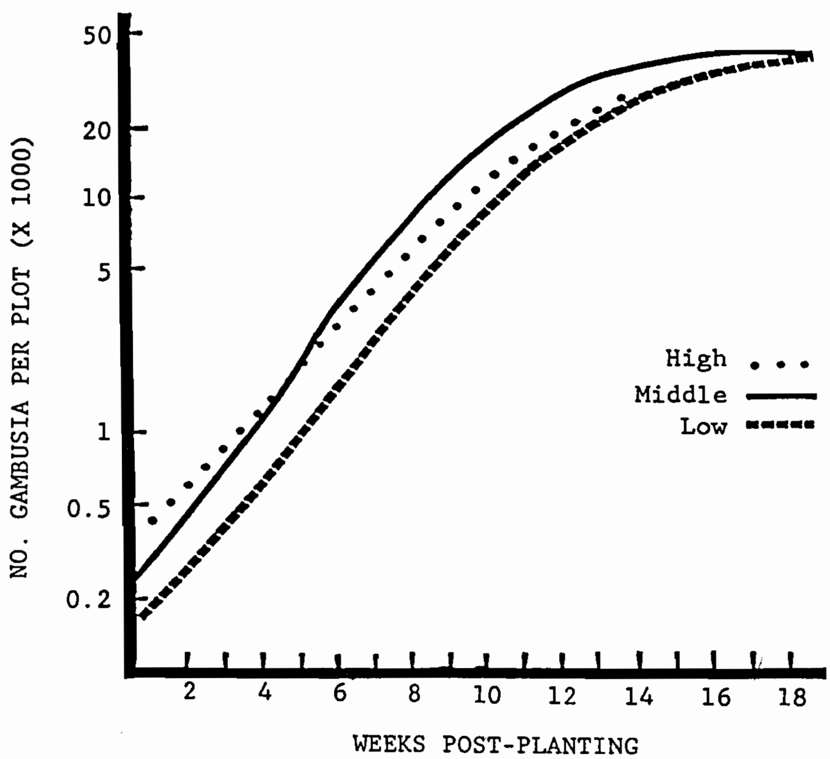

Figure 3.-Population growth curves of mosquitofish in rice plots stocked at 3 rates.

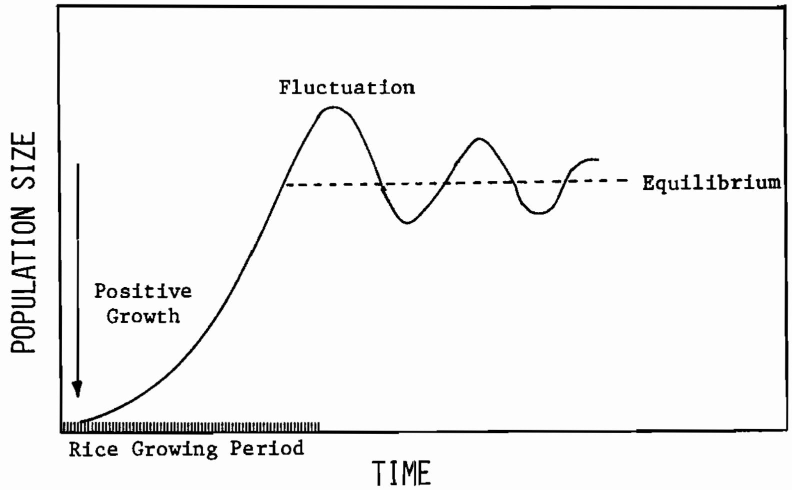

Figure 4.-An expected population growth curve of introduced mosquitofish in rice fields showing stocking time (arrow), population peaks, population equilibrium (carrying capacity) and rice growing period.

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EFFECTS OF VARIOUS FACTORS ON THE EFFICIENCY OF MINNOW TRAPS TO SAMPLE MOSQUITOFISH (*GAMBUSIA AFFINIS*) AND GREEN SUNFISH (*LEPOMIS CYANELLUS*) POPULATIONS

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ABSTRACT

Experiments were conducted in rice field enclosures and in a large (2.25 m^2) laboratory tank to assess the effects of fish size, fish density, vegetation and water depth on the efficiency of Gee minnow traps to sample populations of the mosquitofish, *Gambusia affinis*. The effects of the latter three factors on trap efficiency were also assessed for immature green sunfish, *Lepomis cyanellus*.

Larger mosquitofish ($\geq$35 mm TL) were caught at a greater rate than smaller mosquitofish in both the laboratory and field experiments. Fish density was unimportant in affecting trap efficiency in the field experiment. However, in the laboratory experiment, trap efficiency increased with increasing mosquitofish density.

Vegetation did not affect trap efficiency. The presence or absence of rice was unimportant in trapping mosquitofish in the laboratory experiment. Results of the field experiment failed to demonstrate that the type (emergent vs submergent) and the amount of vegetation affected trap efficiency for mosquitofish.

Minnow traps, lying on the substrate, were assessed for trapping efficiency of mosquitofish at three water depths - 8, 16, and 24 cm. Mosquitofish were caught at a similar rate at 8 and 16 cm. However, at 24 cm, where traps were totally submerged, the percent caught was significantly reduced probably because mosquitofish swim near the surface above the traps at this water depth.

Results of the laboratory experiment failed to demonstrate that the presence or absence of rice had an effect on trapping immature green sunfish. Green sunfish, assessed at two depths (8 and 24 cm), were trapped at a higher rate at 24 cm. Because green sunfish are generally found near the bottom, a decrease in trapping efficiency at the greater depth was not expected.

These results demonstrate the importance of determining the effects of environmental factors on trap efficiency before we can use direct trap counts to assess the effects of these environmental factors on fish abundance.

LARVIVOROUS PREDATORS AND ZOOPLANKTON:

INDIRECT EFFECTS ON MOSQUITOES IN RICE FIELDS

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ABSTRACT

Evidence that interactions between various predators and zooplankton have effects on mosquito survival was assessed in several studies. Such effects may take two forms: 1) zooplankton, by serving as alternative prey to generalist predators, reduce predation intensity on mosquito larvae and 2) a reduction of zooplankton by predators reduces exploitative competition between zooplankton and mosquitoes in rice fields.

In a number of experiments assessing the impact of the mosquitofish, *Gambusia affinis* and the green sunfish, *Lepomis cyanellus* on mosquitoes in rice fields, it was found that their ability to control mosquitoes was associated with the abundance of zooplankton. These results support the hypothesis that zooplankton reduces predation on mosquito larvae.

A microturbellarian flatworm, most recently described as *Mesostoma* near *lingua*, was found to be negatively correlated with *Anopheles freeborni, Culex tarsalis,* Cladocera and Ostracoda in rice field enclosures early in the season. The causal mechanism is presumed to be predation by the flatworm. Cyclopoid copepods showed no significant correlation with this predator. In the laboratory, the presence of a cladoceran species significantly reduced predation by *Mesostoma* on *Cx. tarsalis* larvae.

In another study, mosquitofish reduced cladoceran populations to low levels in rice field enclosures. First instar *Cx. tarsalis* larvae, placed in predator exclusion cages within the enclosures developed faster in the enclosures containing mosquitofish than those without mosquitofish. One explanation of this result is that, in the absence of fish, cladocerans, which have a high overlap in diet with mosquito larvae, compete with mosquito larvae in rice fields and may be an important force in mosquito population dynamics.

THE IMPORTANCE OF SINGLE SPECIES IN DETERMINING THE AVERAGE DENSITY OF PLANTS AND ANIMALS

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ABSTRACT

The presence of populations of only one or two animal species in the ecosystem can account for the low abundance of many organisms. This realization may guide biological control strategies against pests of medical and veterinary importance.

The presence of populations of only one or two animal species in the ecosystem can influence greatly the population density of certain plants and animals. The acceptance of this reality is often difficult for some modern ecologists who, through their broad experiences in measuring density dependent and density independent forces in nature, are steeped in the complexities of the ecosystem. It seems inconceivable that in the midst of all the intracting abiotic and biotic factors, that only one or two organisms could ever be responsible for the average abundance of another organism over a protracted period.

Nevertheless, proof for this "simplistic" assumption is available from any sources. Breaking down the world's biota into terrestrial plants, aquatic plants, vertebrates, phytophagous insects and insects of medical and veterinary importance, Table 1 gives selected examples to demonstrate the importance of one or two animal species in accounting for consistently low densities of other organisms. Many of the causative agents act as density dependent "regulative" forces, which bear a reciprocal density relationship to their hosts, or as "limiting" forces, which set an upper limit to the density that an organism can attain, but do not bear the close relationship of reciprocity.

If there are any doubts of the basic assumption that the presence of one or two organisms accounts for the observed low population densities of the various species listed in Table 1, the question may be asked, "What would happen to the average density of the controlled organism if the causative agent(s) were removed?" Invariably, the answer would be simply that a significant and noteworthy rise in density would follow their removal.

It is apparent that the greatest number of examples are found among phytophagous insects, which is a reflection of the greater biological control effort against this group. Insects of medical and veterinary importance are just becoming favored targets for biological control, as the desire to reduce pesticide usage against them increases. Thus, we undoubtedly will see more successful cases in years to come.

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Table 1.-Some examples showing the abundance of plants and animals dependent on the presence of one or two species of organism in the ecosystem.

Controlled Organism	Causative Agent(s)
Terrestrial Plants	
American elms in eastern N. America	*Scolytus multistriatus* (Marsham) vectoring *Ceratocystis ulmi* (Buisman) C. Moreau
Klamath weed in northwestern U.S.A.	*Chrysolina hyperici* (Forster & *C. quadrigemina* (Suffrian)
Endemic cedars in Bermuda	*Carulaspis visci* (Schrank) & *Lepidosaphes newsteadi* (Sulc.)
Opuntia cactus in Australia	*Cactoblastis cactorum* (Berg)
Opuntia cactus in Ceylon	*Dactylopius indicus* Green
Opuntia cactus on Santa Cruz Island	*Dactylopius sp.*
American chesnut in eastern N. America	*Endothia parasitica* (Murr.)
Eucalyptus at <500 meters in S. Africa	*Patasson nitens* (Girault) attacking eucalyptus snout beetle
Eucalyptus at >500 meters in S. Africa	*P. nitens*, but at higher density
Apple trees in Europe	*Prospaltella perniciosi* Tow. attacking San Jose scale
Puncturevine in coastal California	*Microlarinus lypriformis* (Woll.)
Aquatic Plants	
Potamogeton in irrigation drains of the Imperial Valley, CA	*Tilapia zillii* (Gervais)
Eichornia in Florida	*Neochetina bruchi* Hustache
Vertebrates	
European rabbits in Australia	*Myxomytosis* virus #1
European rabbits in England	*Myxomytosis* virus #2
Phytophagous Insects	
Cottony-cushion scale in >50 countries	*Rodolia cardinalis* (Mulsant) &/or *Cryptochaetum iceryae* (Williston)
California red scale in California & Europe	*Aphytis melinus* DeBach
Olive scale in California and Europe	*Aphytis maculicornis* (Masi) & *Coccophagoides utilis* Doutt
Rhodesgrass scale in south U.S.A.	*Neodusmetia sangwani* (Rao)
Florida red scale in Israel, Florida, Mexico S. Africa, Brazil & Peru	*Aphytis holoxanthus* DeBach
Walnut aphid in south California	*Trioxys pallidus* Haliday from France
Walnut aphid in central California	*Trioxys sp.* from Iran
Alfalfa weevil in coastal California	*Bathyplectes curculionis* (Thoms.)
Black scale in south California	*Metaphycus helvolus* (Compere)
Diamond-back moth in New Zealand	*Angitia cerophaga* (Grav.)
Coconut scale in Fiji	*Cryptognatha nodiceps* Mshll.
Coconut scale in Mauritius	*Chilocorus politus* Muls & *C. nigritus* Muls.
Wooly apple aphid in New Zealand	*Aphelinus mali* (Hald.)
Sugarcane borer in Barbados	*Apanteles flavipes* (Cameron)
Winter moth in Canada	*Cyzenis albicans* (Fall) & *Agrypon flaveolatum* (Grav.)
Winter moth in England	small rodents
Citrus blackfly in Cuba & Mexico	*Eretmocerus serius* Silvestri in Cuba & *Amitus hesperidum* Silvestri & *Prospaltella opulenta* Silvestri in Mexico
Oriental fruit fly in Hawaii	*Opius oophilus* Fullaway
Spiny blackfly in Japan	*Prospaltella smithi* Silvestri
Red wax scale in Japan	*Anicetus beneficus* Ishii & Yasumatsu
Blue gum scale in New Zealand	*Rhizobius ventralis* (Erichson)
Sugarcane leafhopper in Hawaii	*Cyrtorhinus mundulus* (Bredd.)
Apple mealybug in Canada	*Allotropa utilis* Muesebeck
Coffee mealybug in Kenya	*Anagyrus* nr. *kivuensis* (Compere)
Citrus mealybug in Israel	*Clausenia purpurea* Ishii
Comstock mealybug in California	*Allotropa burrelli* Muesebeck & *Pseudaphycus malinus* Gahan

continued -

(Table 1, Cont'd.)

Controlled Organism	Causative Agent(s)
Citrophilus mealybug in California	*Coccophagus gurneyi* Compere
Tea tortrix in Ceylon	*Macrocentrus homonae* Nixon
Coconut moth in Fiji	*Ptychomyia remota* Aldrich
Brown-tail moth in Canada	*Compsilura concinnata* (Mg.), *Apanteles lacteicolor* Viereck & *Meteorus versicolor* (Wesm.)
Oriental beetle in Saipan	*Campsomeris annulata* Fab.
Coconut leaf mining beetle in Fiji	*Pleurotropis parvulus* Ferr.
Purple scale in California	*Aphytis lepidosaphes* Compere
Dictyospermum scale in Greece	*Aphytis melinus* DeBach
Green vegetable bug in Australia & Hawaii	*Trissolcus basalis* (Wollaston)
<u>Insects of Medical & Veterinary Importance</u>	
Aquatic midges in Los Angeles drainages	*Sarotherodon hornorum* Trewazas & *S. mossambica* (Peters)
Common housefly in S. Africa & New Zealand	*Tachinaephagus zealandicus* Ashmead
Housefly and stable fly in Puerto Rico	*Spalangia endius* Walker & *S. nigroaenea* Curtis
Black stable fly in Mauritius	*Tachinaephagus stomoxcida* Subba-Rao
Culex spp. in some Calif. rice fields	*Mesostoma* flatworms
Culex spp. in Calif. drainages	*Gambusia spp.*
Culex spp. in some Calif. ponds	*Dugesia dorotocephala* (Woodworth)
Culex spp. in Imperial Co. drainages	*Tilapia zillii* (Gervais)

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EVALUATING POND DESIGNS FOR MAXIMUM PRODUCTION OF MOSQUITOFISH IN SACRAMENTO COUNTY

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ABSTRACT

One of the objectives of the Sacramento County/Yolo County Mosquito Abatement District is to produce adequate numbers of mosquitofish for mosquito control in permanent and semi-permanent water sources.

When the District's twenty acre fish hatchery was purchased in 1977, four large existing ponds were used to raise mosquitofish. Since that time, the large ponds have been converted to 38 raceways with deep, steep levees. In 1986, the District's staff decided to evaluate the productivity of the existing raceways and to experiment with new designs.

The new pond designs were copied from productive sources found in the surrounding area. Four different types of ponds were evaluated:

1. River rock rip-rap.
2. Raceway.
3. Sloping pond bottom.
4. River rock rip-rap (without rock).

The design resulting in the highest mean yield was the "river rock rip-rap" design. These ponds produced from 243 lbs to 286 lbs each, while the most competitive pond design (river rock rip-rap design without rock), produced 192 lbs. For example, 60 lbs of fish stocked in river rock rip-rap ponds in March produced 281 lbs by June. The same amount of fish stocked in non-rip-rap ponds produced only 192 lbs by June. By combining the 38 raceway ponds into 19 river rock ponds, hatchery production could be increased from 3,021 lbs to 5,434 lbs without expanding the hatchery property.

INTRODUCTION

Historically, the Sacramento County/Yolo County Mosquito Abatement District relied on sewage oxidation ponds, dairy sumps and natural drains for their supply of mosquitofish. With the development of the Regional Sewage Treatment Facility in south Sacramento, many of these sources were eliminated.

When the District purchased the old Elk Grove sewage ponds in 1977, the four large sewage oxidation ponds were converted into 38 raceway ponds. These ponds were very productive, but still did not meet the mosquitofish requirements of the District. The objective of the District's hatchery is to supply all of the District's fish stocking requirements without having to rely on other sources.

Four pond designs were evaluated for production in the spring of 1986. These designs were copied from productive mosquitofish field sources. Production of mosquitofish is difficult to compare in the field as it is dependent not only on pond design, but a variety of other factors.

MATERIALS AND METHODS

Fertilizer (chicken manure) application rates were duplicated in all ponds and measured as gallons per acre foot of water. Dense zooplankton populations were established before fish stocks were added. The amount of fish food given daily was based on a percentage of the fish biomass present and the pond water temperature.

In this study of pond design production, four different pond designs were evaluated.

1. Raceway design:
   - 300 ft x 34 ft wide
   - 0.23 acre surface area
   - 1.17 acre ft capacity
   - 5 ft maximum pond depth
   - 1:1 slope (run over fall)

   These study ponds were grouped into 4 series. Three of the series consisted of 4 ponds while the other consisted of 2 ponds. Six-inch diameter culverts connected the ponds within each series, allowing the fish to move freely between ponds. Between the pond series, connecting culverts were screened on both ends with 1/16-inch mesh screen, so different stocking rates and habitat use could be studied. In order to obtain maximum production, ideal stocking rates needed to be determined. The series of ponds were stocked at different rates for this study.

2. River rock rip-rap design:

   - 300 ft x 68 ft
   - 0.46 acre surface area
   - 1.84 acre ft capacity
   - 5 ft maximum pond depth

- 4:1 slope (run over fall)
- 50 tons, 3 in. to 6 in. river rock cobble

The river rock cobble was used to line the sides of these ponds. This rip-rap provides spawning areas for gravid females, as well as harborage for newly spawned fry. Five ponds, stocked at 3 different rates, were used in this study.

3. River rock rip-rap design without rock:

- 300 ft x 68 ft
- 0.46 acre surface area
- 1.84 acre ft capacity
- 5 ft maximum pond depth
- 4:1 slope (run over fall)

In this pond, we used emergent grasses in place of the rock rip-rap. Nine days before the first spawn, the water level was raised 10 inches to provide a three foot margin of emergent grasses that the fish could use for spawning and harborage.

4. Sloping pond bottom design:

- 365 ft x 68 ft
- 0.57 acre surface area
- 1.99 acre ft capacity
- 7 ft maximum depth
- 50:1 slope (run over fall)

This gradual sloping bottom design provided shallow, warm areas, as well as emergent vegetation for harborage. One pond stocked at 50 lbs was used for the productivity comparison.

RESULTS AND DISCUSSION

Table 1 shows the average pounds of fish harvested and the average percent increase per

Table 1.-Fish production of raceway ponds. Pond series were stocked at 4 different rates.

Pond #	lbs of Fish Stocked	lbs of Fish Harvested	Average % Increase	Avg lbs /Group
Series 1				
3	20	88	273%	74.8 lbs
4	20	64		
5	20	65		
6	20	82		
Series 2				
7	30	69	135%	70.5 lbs
8	30	72		
Series 3				
9	10	32	460%	56 lbs
10	10	38		
11	10	74		
12	10	80		
Series 4				
13	15	88	430%	79.5 lbs
14	15	53		
15	15	87		
16	15	90		

Table 2.-Comparison of fish production among different pond designs.

Pond #	lbs of Fish Stocked	Percent Of Increase	Average lbs /Group	lbs of Fish Harvested
River Rock Rip-Rap Ponds				
1	70	306%	265.4	286
2	60	236%		256
18	60	368%		281
19	65	301%		261
20	70	247%		243
River Rock Design Without Rock				
17	60	220%	192	192
Sloping Pond Bottom Design				
31	50	94%	97	97
Raceway Design				
13	15	430%	79.5	88
14	15			53
15	15			87
16	15			90

series in the raceway ponds. Pond numbers 13-16 were the District's most productive series of raceway ponds. Although percent increase was greater in pond numbers 9-12 (series 3), total pounds of fish harvested was greater in pond numbers 13-16. Pond numbers 13-16 will be used for comparison with the production data of the other three pond designs.

Table 2 shows the production data for pond design comparisons. The river rock rip-rap design had better than a three-fold increase over the raceway design ponds in total pounds harvested.

The sloping pond bottom design produced very few fish, with only a 94% increase in biomass. The gradual sloping bottom made the mosquitofish easy targets for the herons and egrets that frequent our ponds. This design made it very difficult to establish a phytoplankton bloom. Nutrients from fertilizers were quickly used by emergent and submergent vegetation.

The design resulting in the highest mean yield was the river rock rip-rap design. These ponds produced from 243 lbs to 286 lbs each, while the most competitive pond design (river rock rip-rap design without rock), produced 192 lbs. By combining the 38 raceway ponds into 19 river rock ponds, hatchery production could be increased from 3,021 lbs to 5,434 lbs without expanding the hatchery property.

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FURTHER INSIGHTS INTO EXTRANUCLEAR INFLUENCES ON BEHAVIOR ELICITED BY MALES IN THE GENUS *MUSCIDIFURAX* (HYMENOPTERA: PTEROMALIDAE)

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ABSTRACT

Continuing studies into the inheritance of behavior in the parasitoid genus *Muscidifurax* point to dual extranuclear influences and genic changes for such traits as gregarious and solitary oviposition and fecundity. However, plausible explanations for inheritance of thelytoky need not include genic change. Whether the nuclear genome or a genome associated with cytoplasmic elements changes is not known, but further attention naturally focuses on the role of the male's seminal fluid as a conveyor of substances capable of modifying behavior and possible subsequent genomic alteration.

The discovery that hymenopterous parasitoid males caused behavioral changes in females with whom they mated, and that such changes were subsequently inherited by resulting progeny (Legner 1986), prompted further detailed studies into apparent dual biochemical and genic phenomena in inheritance (Legner 1987b). For example, inheritance of traits for gregarious or solitary oviposition in *Muscidifurax raptorellus* Kogan & Legner included two major phases; (1) the female parasitoid expressed either trait shortly after mating, its intensity determined by the male's genome; and (2) the trait was then apparently fixed into a genome of her offspring who demonstrated it in the virgin state (Legner 1987b). In the first phase, <1/2 the magnitude of behavioral expression was shown in the mated female, full expression occurring in the F_1 diploid virgin hybrid.

Matings with hybrids from subsequent backcrosses showed that >1/2 of the behavior was expressed by the female after mating (Legner 1987b). Related traits such as the number of parasitoids developed per host and total progeny, were also similarly expressed and inherited. A quantitative or polygenic mode of inheritance was apparent, similar to that found in human skin color (Harrison & Owen 1964), with ≥3-22 genes estimated using Lande's (1981) formulae (Legner 1987b).

The involvement of microorganisms and/or chemicals (enzymes) present in hymenopteran seminal fluid was postulated for the extranuclear phase. Because males appeared capable of activating a part of the species' genetic make-up within their own generation, by causing immediate expressions of some unique traits in the females with whom they mated, it was thought that natural selection in this system should be accelerated, not having to wait to act on inherited traits expressed only in the progeny. Thus, not only may functional *Muscidifurax* haploid males provide a means for the rapid elimination of unfavorable genes as suggested for such organisms by Dobzhansky (1941), but they may serve to quicken the pace of natural selection for both nonlethal undesirable and desirable characteristics (Legner 1987b).

Investigations of a related species, *Muscidifurax raptor* Girault & Sanders, showed that thelytoky (females produced from unfertilized eggs) could be acquired through mating hybrid females with naturally occurring males of a thelytokous species, *Muscidifurax uniraptor* Kogan & Legner (Legner 1987a). Changes observed during the transfer process also suggested the involvement of extranuclear phenomena (Legner 1987c). Studies with newly acquired field cultures of *M. uniraptor* further supported the involvement of extranuclear steps in the acquisition of thelytoky (Legner 1988).

Various hypotheses could explain the startling sequence of events in inheritance observed within this genus.

Inheritance of Gregarious & Solitary Oviposition in *Muscidifurax raptorellus*

A relationship between extranuclear influences and genic changes in studies with Peruvian and Chilean strains of *M. raptorellus* (Legner 1987b), was made obvious by the behavioral changes observed in parental females shortly after mating and by their subsequent apparent incorporation in a genome of the progeny.

Various extranuclear influences on behavior involving cytoplasmic entities are well known among both prokaryotes and eukaryotes (Beale & Knowles 1978, Cosmides & Tooby 1981, Goodenough 1984, Levine 1973, Sager & Ramanis 1963, Sonneborn 1959), but the subsequent incorporation of an extranuclear expression into the nuclear genome apparently has not been found. Extranuclear factors in the form of microorganisms (e.g., viruses, bacteria, spirochaetes) can alter sex ratios in parasitoids by selectively killing developing males or females (Skinner 1982, 1985; Werren et al. 1981, 1986), may confer resistance to host encapsulation (Krell & Stoltz 1979; Stoltz & Vinson 1977; Stoltz et al. 1976; Vinson & Stoltz 1986), and affect sex ratios in *Drosophila* (Poulson & Sakaguchi 1961), and are passed on to succeeding generations. Cosmides & Tooby (1981) recently reviewed how cytoplasmic genes control such characters as allocation of reproductive effort in hermaphrodites, sex ratios of offspring (Williamson & Poulson 1979), organism size (Faulker & Arlett 1964), growth rate, colony size, rate of senescence (Smith & Rubenstein 1973), competitive ability (Preer et al. 1974), drug resistance in bacteria, protozoans, fungi and mammals (Beale & Knowles 1978), and rates of recombination among nuclear genes (Thoday & Boam 1956). Oishi et al. (1984) explained how two kinds of microorganisms (spiroplasm and virus) may interact to modify expressions of the sex-ratio factor in *Drosophila*.

The direct effects of the cytoplasmic genome on the nuclear genome has been hypothesized (Cosmides & Tooby 1981), but not demonstrated. But the extrachromosomal genetic system can be influenced by the chromosomal system (Levine 1973). The microorganisms implicated in inheritance have been known to cause illness and death in male *Drosophila* (Leventhal 1968).

However, the observation that behavior in *M. raptorellus* was influenced in a way that depends on the nature of the male could also be explained by a behavior-modifying chemical. If at mating, a male transfers to the female a compound which influences her behavior, or induces enzymes that influence her behavior by lowering her sensitivity to a chemical marker that tells a female that she has already deposited one egg in a puparium, she might respond as observed in the *M. raptorellus* study (Legner 1987b). The material or the enducing enzymes might be transferred from the male's seminal fluid onto the chorion (or another part) of the next-to-be oviposited ova, and from here influence the chemosensory responses of the next generation. One generation may then bequeath a chemical legacy to its progeny. Because mating with Peruvian males causes a decrease in the gregarious oviposition expression of Chilean females and their hybrids (Legner 1986, 1987b), a second kind of chemical substance would have to be postulated, one which increases sensitivity to the presence of already oviposited eggs.

In one possible scenario, solitary parasitoids may inject a chemical with the first egg that is laid that signals the female to refrain from further oviposition; gregarious parasitoids may inject another chemical which inhibits the expression of the first chemical. If in the solitary line both sexes have marking-chemical-enzyme, it could be transferred from the male to the female at mating, with the female then transferring it to the egg. In the egg (or chorion) it would induce in the hatched larva a chemical, so that the next generation too has the chemical and can make a response to it. Such enzymes may confer the gregarious or solitary oviposition tendency, and it is possible to envision quantitative variation in enzyme, and therefore chemical contact.

Chemical substances affect behavior of insects following mating. Reports include a lepiopteron (Webster & Carde 1984), an ichneumon wasp *Venturia (Nemeritis) canescens* (Gravenhorst) (where heneicosane was involved) (Mudd et al. 1982); and the diptern *Drosophila*, involving enzymes (Mane et al. 1983, Richmond & Senior 1981). Prostaglandins, derivatives of certain polyunsaturated fatty acids, alter egg laying behavior in crickets (Stanley-Samuelson & Loher 1986). It has been suggested that an influence on the chemosensory responsiveness of an individual by chemical cues derived from it parents would be hard to distinguish from a genetic effect (Corbet 1985).

The particular chemical substance or substances would necessarily affect behavior in *M. raptorellus* only after the ovipositor is inserted and not during the host selection process, because both the Peruvian and Chilean strain equally avoid already parasitized hosts (Legner 1987b).

However, the chemical legacy hypothesis of expression is not fully acceptable, primarily because of the linear additivity of the inherited trait and the uniformity of expression witnessed in all hybrids (Legner 1987b). A more plausible explanation might involve behavioral chemicals in the first phase of inheritance at mating, followed by a DNA (genomic) change in the progeny.

Thelytoky in *Muscidifurax uniraptor* and *M. raptor*

During the transfer of thelytoky from *M. uniraptor* males to *M. raptor* females, there was a significant reduction in the proportion of female eggs laid following mating (Legner 1987c). Matings of thelytokous females with males produced naturally in such cultures caused significant reductions in both total progeny and the proportion of females (Legner 1988).

One hypothesis to explain these results relates to the genic mechanism of sex determination. If diploid males were produced, either uni- or biparentally, their mating might cause zygotic death because diploid males usually produce diploid or aneupliod sperm, conditions that could lead to high zygotic inviability (Bull 1983). The high host destruction shown by *M. uniraptor* cohorts of diapause origin and maintained by mated females (Legner 1988) supports the probability that hosts were parasitized but that eggs did not hatch. However, dissections were unable to verify the existence of such unhatched eggs. Diploid males could also affect the sex ratio since they often arise from fertilized eggs that would normally produce diploid females.

The microorganismal extranuclear factor and chemical legacy hypotheses which were implied in the inheritance of oviposition behavior in *M. raptorellus* may also apply with thelytoky (Legner 1986, 1987a,b) as well as the chemical legacy hypothesis. Both hypotheses assume that at insemination females may receive certain microorganisms and/or chemicals which modify reproduction.

There is also an apparent relationship to the titre of the causative factor in thelytoky. For example, production of thelytokous females in *M. uniraptor* is greatest when oviposition is interrupted for 24 h by scheduling host presentation on alternate days (Legner 1985a) or by slowing oviposition rates during early adult life (Legner &

Gerling 1967). Such interferences may allow the titre of the factor to rise. Higher concentrations of microorganisms or chemicals may thus guarantee a greater proportion of thelytokous female offspring. It could reasonably be assumed that microorganisms and certain chemicals produced by them are involved, with the latter inducing endomitosis (Legner 1985b) which results in thelytokous offspring.

Heat treatment (32.°C. for >24 h) beginning at a critical stage in oocyte formation blocks endomitosis and male progeny result (Legner 1985b). If any enzymes, microorganisms or both were involved directly or indirectly in promoting endomitosis, the prolonged exposure to higher temperature could kill or inactivate them. This work (Legner 1985b) also points to their probable residence in or near oocytes which are in later developmental stages. Malogolowkin (1959) was able to apparently "kill" the sex-ratio factor in *Drosophila* by heat treatment which he suspected to be a virus. Stoltz & Vinson (1979) have found viruses in the calyx epithelial cells of endoparasitoids, and Fleming & Summers (1986) found them also in the lumen of the oviduct. Such viruses were passed from parent to offspring (Stoltz et al. 1986), males being able to transmit viral DNA to females with whom they mated.

The last two hypotheses tend to preclude a genetic aspect to thelytoky. If, for example, microorganisms and accompanying chemicals or enducing enzymes which they produce are transferred to the ova as suggested previously in *M. raptorellus*, endomitosis might be influenced in the next generation, and thelytoky would be passed on without genetic change. With such a system it is also possible to envision quantitative variation in microorganisms and enzymes and hence the number of thelytokous females produced. Because the titre appears to build up during host-free periods (Legner 1985a, Legner & Gerling 1967), microorganismal multiplication and/or elaboration of the chemical substance(s) would have to proceed relatively slowly.

Thus, integrating all three hypotheses, the low proportion of female progeny from cohorts of diapausing *M. uniraptor* and following early matings (Legner 1988) might be related to a gradual mortality of microorganisms during diapause and a lower titre among original males. Matings with males that did not possess microorganisms may have resulted in the zygotic death referred to earlier. Then, several generations in culture could have favored microorganismal multiplication, and hence the rise in proportion of females.

Some Basic Genetic Considerations

Only extranuclear factors resident in the cytoplasm have been implicated in eliciting behavior that was not under control of nuclear genes. Therefore, in *Muscidifurax*, attention is logically directed to the seminal fluid which conveys the sperm, because the latter possess very little cytoplasm. The factor or factors transferred at mating would likely be associated with seminal fluid as, e.g., metagons and kappa particles are associated with cytoplasm in *Paramecium* and mitochondria with cytoplasm in *Neurospora* (Levine 1973). The existence of complete genetic systems in mitochondria is known (Ayala & Kiger 1984), and the direct effects of a cytoplasmic genome on the nuclear genome has been hypothesized (Cosmides & Tooby 1981). Could a similar extranuclear genome have been changed following mating in *Muscidifurax*?

Significance For Biological Control

The ability of male parasitoids to change the behavior of females with whom they mate has practical significance in biological control. Greater importance is now placed on liberated males during mass release strategies to seasonally accelerate and increase the magnitude of parasitism, because it may be possible to convey certain desirable strainal characteristics directly to unmated females already resident in the environment.

Significance For Other Animal Systems

The ability to trace inheritance of behavior from an immediate expression after mating to the possible subsequent fixation into a genome was facilitated here by development of hymenopteran unfertilized eggs into male offspring. In other organisms where only fertilized eggs produce viable offspring, it would be impossible to compare mated with unmated individuals in the same way. However, this does not exclude the possibility that similar processes could apply elsewhere.

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FEEDING ACTIVITY OF *CULEX PEUS* AND *CULEX TARSALIS* ON HUMAN SUBJECTS UNDER LABORATORY CONDITIONS

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ABSTRACT

Culex peus females were allowed to take a blood meal from human subjects under laboratory conditions. Results are compared to those obtained from *Culex tarsalis* exposed to comparable conditions.

INTRODUCTION

Light trap collections indicate that *Culex peus* Speiser is an abundant domestic species in Butte County. It breeds prolifically in log decks, sewage leaks, dairy drains, road drains, and many other sources created by domestic and agricultural activities (Carpenter and LaCasse 1955). There seems to be some question as to whether or not *Cx. peus* will take a blood meal from humans (Bohart and Washino 1978, Carpenter and LaCasse 1955, Tempelis 1970). In the past, a consensus that *Cx. peus* does not feed on humans has guided work conducted on this species. However, recent studies have shown *Cx. peus* to be a very competent vector of St. Louis encephalitis (SLE) (Hardy et al. 1985). This stimulated a reconsideration of the issue of human feeding. Analysis of engorged female *Cx. peus* collected from rural areas of the Sacramento valley have indicated blood meals were taken from humans (Hardy 1986). Unpublished data have shown that blood meals identified from *Cx. peus* collected at monthly intervals throughout the breeding season indicate a low but consistent mammalian feeding rate. Observations by field operators have also indicated human feedings.

Culex tarsalis Coquillett has been found to be the primary vector of both SLE and Western Equine Encephalomyelitis (WEE) (Reeves and Hammon 1962). With this in mind, a brief study was conducted to compare human feeding activity between *Cx. peus* and *Cx. tarsalis* under controlled conditions.

MATERIALS AND METHODS

Late instar *Cx. peus* larvae were collected in the field and allowed to emerge. Adults were fed 10% sucrose for four days post-emergence. On day five, the sucrose-water was removed. At dusk, one person placed his arm into a cage of mixed male and female adults for one hour, allowing females to feed freely. This was repeated with three different people and different mosquitoes in order to avoid the possibility of personal repellency. Females were aspirated following the feeding period, killed, and then identified and counted under a dissecting scope, looking for evidence of blood feeding.

Late instar *Cx. tarsalis* larvae were obtained from Debbie Lemenager, entomologist at the Sutter/Yuba MAD. The larvae were an F1 generation from a colonized field collection. The adults were again allowed to emerge and fed 10% sucrose for four days post-emergence. On day five, the sucrose-water was removed. At dusk one person placed his arm into the cage for one hour, allowing females to feed freely. This procedure was repeated with a different person and different mosquitoes. Females were aspirated following the feeding period, killed, and counted under a dissecting scope, again looking for evidence that the mosquitoes had taken a blood meal.

RESULTS AND DISCUSSION

The results show that almost twice as many *Cx. tarsalis* fed on humans as compared to *Cx. peus* (Table 1). If it can be assumed that the actual field feeding frequency can be expected to be less than the rate experienced in the lab, then *Cx. peus* is at best an incidental human feeder. However, the rate at which *Cx. tarsalis* fed is also not exceptionally high for an effective vector.

There appears to be a host factor shown by the data. Person A was more attractive to both species of mosquitoes than person C. This, if it is experienced in the field, would provide an explanation for a physiological barrier for virus transmission. The variability in feeding behavior by different mosquitoes or lack of attractiveness to a certain individual is not unusual (Curtis 1986). Humans have been shown to differ markedly from one another in their attractiveness to mosquitoes (Clements 1963). Host attractiveness is certainly a factor which must be considered in feeding studies. While not recorded as to a specific time, one of the test persons observed a change in feeding activity with changes in time. This suggests a feeding periodicity which could further influence observed feeding preferences. Based upon our observations, it would seem more accurate to report feeding preferences and attractiveness as a comparison between two or more kinds of mosquitoes in place of observations on a single population and the numbers which have fed.

The data also raises some questions as to what role (if any) *Cx. peus* may play in the cycle of SLE. If *Cx. peus* is a passive or opportunistic human feeder with a high preference for passerine birds which may act as a reservoir for the virus, then the role of *Cx. peus* may be more

Table 1.

Species	Person	Number Blooded	Total Number	Percentage
Cx. peus	A	38	457	8.3
Cx. peus	B	1	56	1.8
Cx. peus	C	0	55	0.0
	Total	39	568	6.8
Cx. tarsalis	A	63	569	12.1
Cx. tarsalis	C	9	101	8.2
	Total	72	670	10.7

than that of an enzootic vector. It is possible that SLE is being transmitted by a vector with a high bird-low human attack rate.

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TEMPERATURE-RELATED GROWTH AND MORTALITY RATES OF FOUR MOSQUITO SPECIES

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INTRODUCTION

The purpose of this research was to determine growth rates of common species of mosquitoes in Alameda County. *Culex pipiens* L., *Culex tarsalis* Coquillett, *Culiseta incidens* (Thomson), and *Culiseta inornata* (Williston) were reared at temperatures normally found in the field. The results are used in a computer simulation model that will enable technicians to schedule treatments by simulating the growth of the larvae since the last inspection.

Many researchers have studied temperature-related growth rates of mosquitoes (Pritchard and Mutch 1985, Shelton 1973, Bailey and Gieke 1968, and others). However, we felt it necessary to rear the local species rather than use published data, since variation in the species may exist due to climate or locality.

The following was needed for the model: the time required for the immature period from the first instar to the adult stage at various temperatures, and the average time required for each instar and pupal period. A rate summation method, as described by Wagner et al. (1985), is used in the computer model because this method avoids the need to determine maximum and minimum threshold temperatures in larval development. We have assumed that the growth rates obtained in the laboratory will represent those in the field.

MATERIALS AND METHODS

Egg rafts were collected in the field and hatched at room temperature. Larvae were transferred to four or five white enamel pans (41 x 25 x 6 cm) partially filled with aged tap water (about 3 liters); then reared at 49 ±1.5°F (in the refrigerator), 66 ±3.3°F (room temperature) and several temperatures ranging from 72-90 ±0.7°F, depending on that normally encountered by the species. These pans were each heated with a 25 or 50 watt submersible aquarium heater ("Dansea" and "Thermal Compact"). Thirty to 100 larvae were reared per pan. One gram of dry bakers yeast was dissolved in 50 mls of water, and 2 mls of this solution were given per 10 larvae each day. The pans were covered with a plexiglass lid and a small net was attached over an opening in the center to collect the adults. Black plastic was used to cover the lid during the adult emergence stage. For each species, this procedure was repeated up to six times depending on the availability of egg rafts. The number of larvae in each growth stage, time of day, and temperature were recorded twice daily.

The time for the immature period was calculated from the start of the recording period to 50% adult emergence. Linear regression was used to determine the cumulative days to the nearest tenth at 50% emergence.

Percent survival was that which survived to the adult stage from the number of larvae at the start of the recording period. Mortality rate per day was calculated using the compound interest formula $M = [(A/L)^{1/n} - 1]$ where M is the mortality rate, A is the number surviving to the adult stage, L is the beginning number of larvae, and n is the number of days to 50% adult emergence or complete mortality. When a sample did not survive to the adult stage, 0.1 was used as the value for A. Instar boundaries were determined to be when 50% of an immature stage reached the next stage. The first instar began with the start of the recording period. Linear regression was used to determine the time when the population was composed of 50% of each stage.

RESULTS AND DISCUSSION

Linear regression equations were developed to represent the growth rate at various temperatures for each species (Figure 1). The results showed a similarity of growth rates between species of the same genera. The growth rates of the two *Culiseta* species were found to be less responsive to temperature than the two *Culex* species as indicated by the slope of the equations. The *Culiseta* species tolerated the lower temperatures while the *Culex* species tolerated higher temperatures (see also Table 1). Overall, *Culex pipiens* exhibited the highest growth rate at temperatures above 65°F, as indicated by its position on the graph.

The growth rates obtained at temperatures higher than indicated by the regression lines in Figure 1 were not included in the calculations since plotting the inverse of time results in a straight line for the middle range temperatures only (Clement 1963). The developmental time for *Cs. incidens* at 84°F increased rather than decreased (Table 1), indicating this to be an extreme temperature for the species. This increase in developmental time at high temperatures occurred for *Cx. pipiens* at 90°F. Other mathematical formulas have been developed by researchers that include the temperature extremes in a nonlinear prediction equation (McHugh and Olson 1982).

Many of the results obtained in this study compared favorably with results of other work. The total development times obtained for *Cs. inornata* at 69° and 74°F (Table 1) were similar to the results of Shelton (1973). The larval period obtained for *Cx. tarsalis* at all temperatures up to

Table 1.-Mean duration per stage and percent survival at various temperatures.

Species	Mean Temp (°F)	n+	Days per Stage 1	2	3	4	P	Total Days	% Surv.
Culex pipiens	49	2	8.5	30	38	...	...	101*	0
	64	1	2.5	1.9	2.2	4.0	3.7	14.3	84
	67	3	2.8	2.6	1.6	5.4	3.2	15.6	80
	73	2	1.9	1.5	1.6	3.0	1.9	9.8	91
	78	3	1.3	1.3	1.2	2.7	2.3	8.8	78
	87	1	1.3	0.8	++	++	1.5	6.1	48
	90	1	++	++	++	++	++	9.3	65
Culex tarsalis	50	2	15	12	23	...	...	55*	0
	67	3	3.3	2.6	2.3	6.6	3.9	18.7	65
	73	1	2.7	2.0	1.9	5.5	2.5	14.6	44
	81	2	1.4	1.1	1.9	++	++	11.5	74
	85	2	1.6	1.3	1.3	++	++	9.3	68
	88	1	1.1	1.0	1.2	2.6	1.5	7.4	76
Culiseta incidens	49	2	10	15	19	30	15	89	46
	64	1	3.5	4.0	3.3	6.4	5.4	22.6	95
	67	4	2.3	2.7	3.1	5.3	4.5	17.9	86
	74	4	1.5	2.2	2.1	5.2	3.3	14.3	83
	79	2	1.4	1.8	2.0	5.1	2.3	12.5	52
	83	2	0.7	1.8	1.6	3.9	2.7	10.7	47
	84	3	2.0	2.4	3.0	++	++	16.8	8
Culiseta inornata	51	1	8.2	7.2	7.7	14.5	10.6	48.2	88
	62	1	3.2	2.4	2.6	5.5	5.1	18.8	91
	69	2	3.2	1.7	2.4	4.0	3.9	15.2	89
	74	3	2.3	1.8	1.8	4.2	3.2	13.3	87
	78	1	1.7	1.6	...	...	...	8.0*	0

*Total days to complete mortality

++Instar boundaries could not be determined

+number of samples

85°F were similar to both lab and field results of Bailey and Gieke (1968).

The original experimental objectives did not include obtaining survival data. However, the information is considered useful because it will provide mortality due to temperature in the computer model. Percent survival to the adult stage at each temperature is given in Table 1 and mortality rates per day are shown in Figure 2. The mortality rates of the species did not appear to vary in relation to temperature unless the temperatures were in the high or low extremes for the species (Figure 2). Rates for *Cx. tarsalis* were variable and appeared to be caused by factors other than temperature. Analysis of the data, however, indicated that the larval densities used in this study did not affect mortality rates.

The mortality rate curves also show a similarity between genera of the species studied. The mortality rates of the *Culiseta* species increased rapidly at temperatures above 75°F. *Cs. inornata* showed the most sensitivity to temperature increase and the most tolerance to low temperatures. It appears the high temperatures selected for this study approached the lethal threshold of the *Culiseta* species, while the low temperatures remained at optimal levels. The *Culex* species showed higher mortality rates at the low temperature range and variable mortality at the higher temperatures. The highest temperatures used for the *Culex* species did not reach lethal levels.

The computer simulation of larval development required knowledge of the duration of each immature stage (Table 1). For this purpose, the time for each stage was converted to a percentage of the total developmental time and averaged for each species (Figure 3). The times calculated for

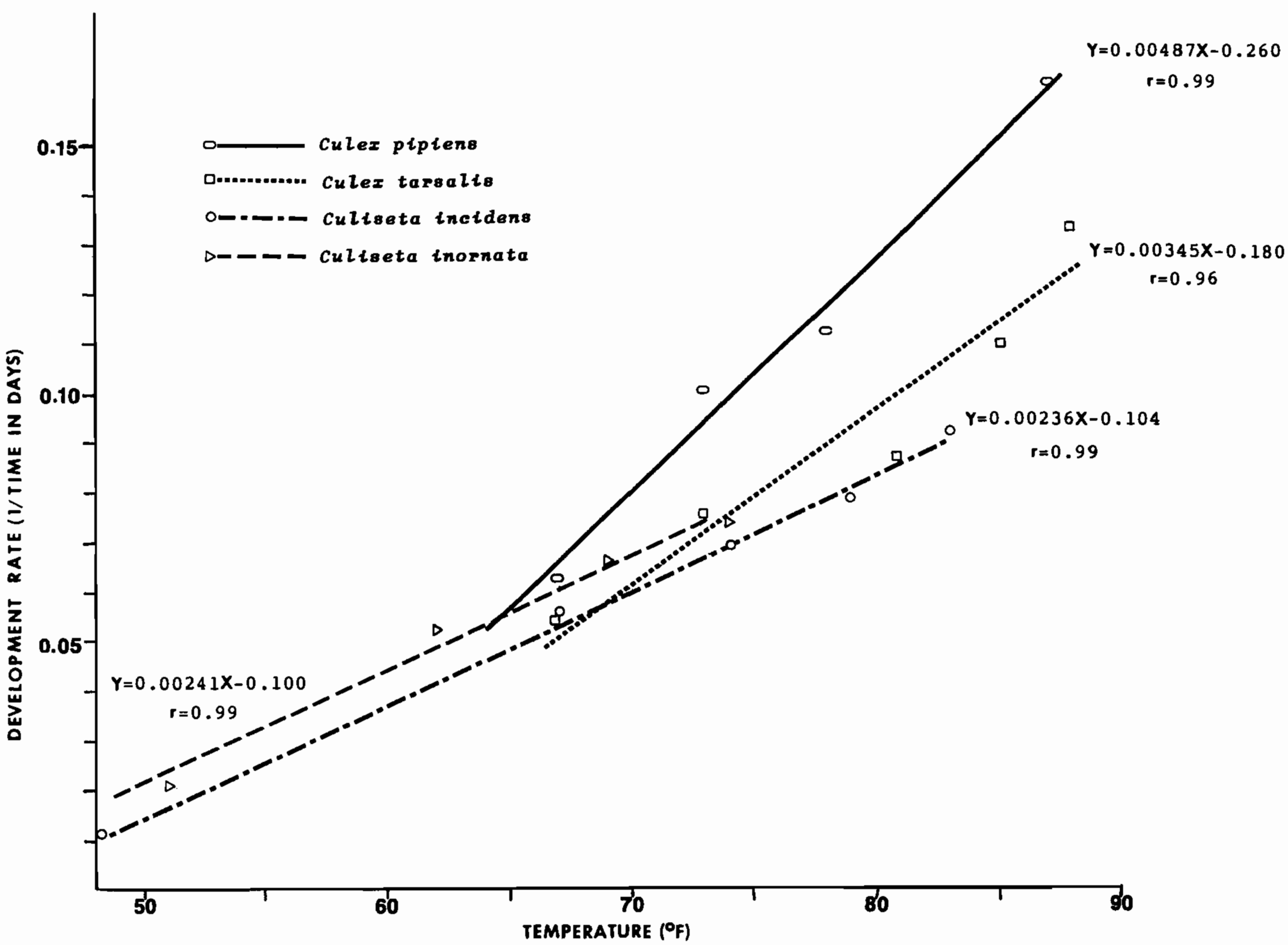

Figure 1.-Growth rates from early first instar to adult at various temperatures. Lines are a least-squared fit to points of mean development time.

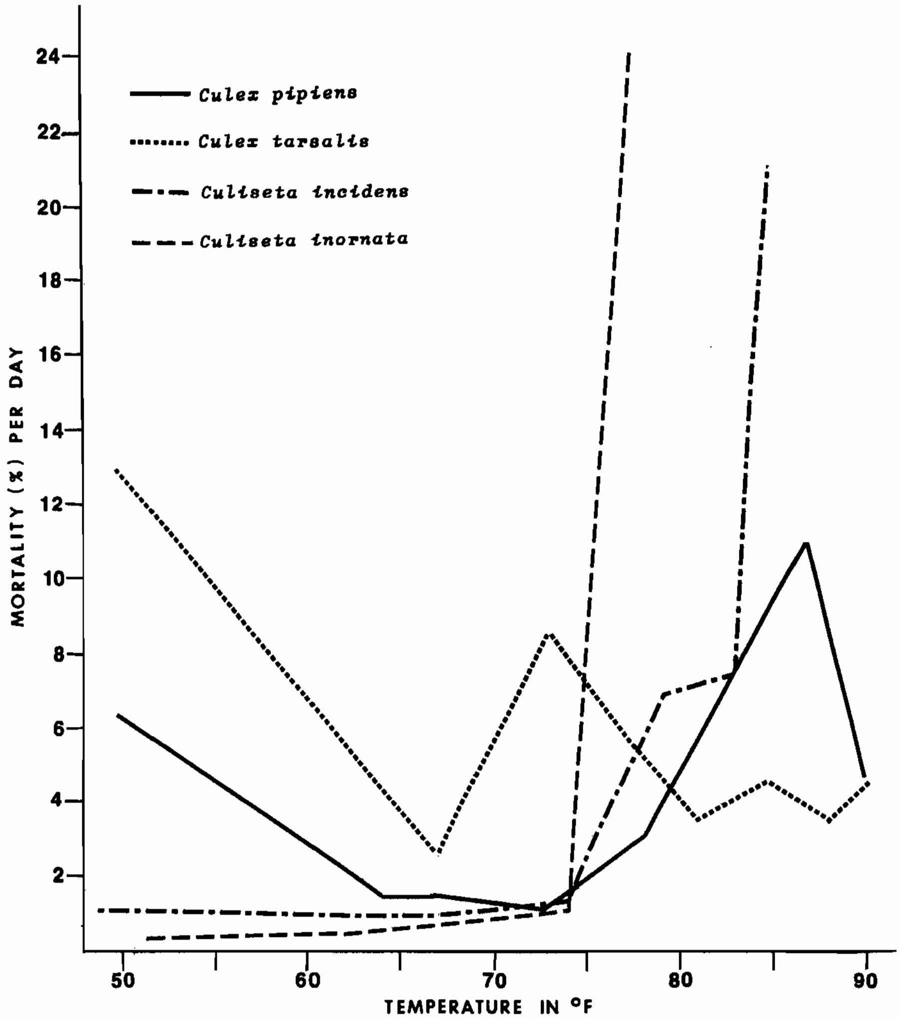

Figure 2.-Mean mortality rate per day at various temperatures.

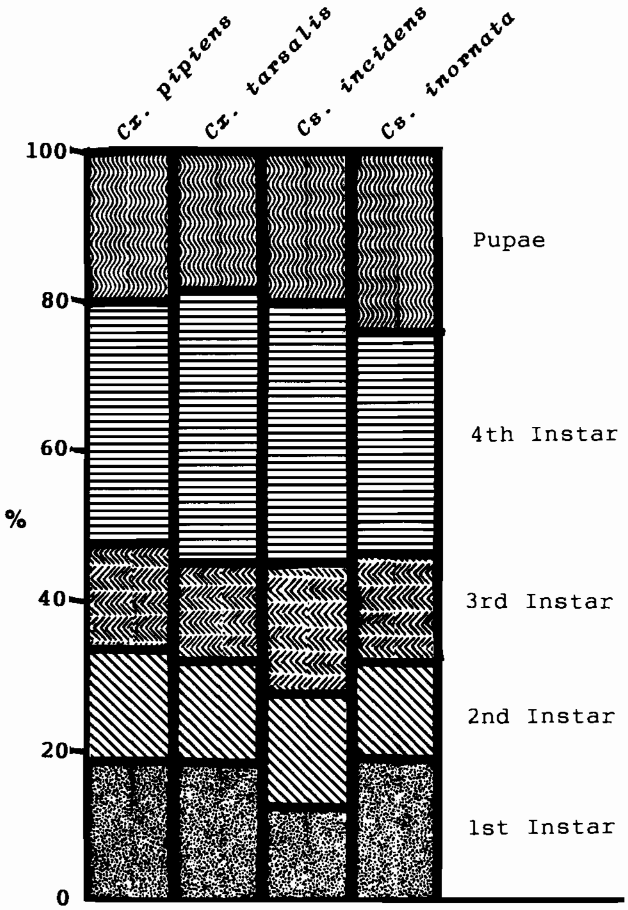

Figure 3.-Duration (%) of each immature stage for *Cx. pipiens* at 64-78°F, *Cx. tarsalis* at 67-73°F, *Cs. incidens* at 49-79°F, and *Cs. inornata* at 51-74°F.

high temperatures were not accurate because sampling could not occur often enough; therefore, only the percentages below 80°F were used. The differences between species appear to be insignificant but will be used in the simulation model to represent the appropriate species.

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DR. ERNEST R. TINKHAM
1905-1987

Dr. Ernest R. Tinkham

Ernest Tinkham received his degrees in entomology as follows: BS from the University of Alberta, MS from the University of Montana and PHD from the University of Minnesota. He taught at the Universities of Montana and Arizona, besides the Ling Nan University in Canton, China before the Communist take over.

Ernest was a medical officer in the Army Medical Corps from 1942 to 1946. He taught jungle survival, insect-borne diseases and other related subjects and worked on a typhus problem in Korea during the Occupation. After his military service, he had a two-year Guggenheim grant to work on desert ecology at the University of Arizona.

In September of 1948, Ernest came to Coachella Valley Mosquito Abatement District as District Entomologist to work on the severe eye gnat problem and related "Pinkeye" epidemic. In April, 1951, he became the District Manager-Entomologist and continued his research on eye gnat biology, ecology and control. Until he resigned from the District in October of 1954, he conducted research on mosquitoes and other medically important insects. Although a portion of his work was on the chemical control of these public health pests, Ernest stressed the use of biological and physical control methods wherever possible.

During his six years with the District, he published papers on his research, gave talks to the scientific community and Valley citizenry, and worked hard to further District and CMVCA goals. After leaving the District, Ernest had four National Science Foundation grants to study various aspects of the desert. He taught classes in high school and various colleges about the desert and other aspects of the sciences.

During his life, Ernest related his love and knowledge of nature to everyone. He was an outstanding scientific illustrator and had over 90 scientific papers published. Dr. Ernest R. Tinkham passed away on February 26, 1987. He will be missed by whose who knew him. On July 14, 1987, the Board of Trustees of the Coachella Valley Mosquito Abatement District dedicated its newly finished laboratory building in his memory.
-Michael J. Wargo-Manager, Coachella Valley MAD

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