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# California Mosquito and Vector Control Association, Inc.

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# SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1980

Richard W. Emmons[1], Marilyn M. Milby[2], Patricia A. Gillies[3], William C. Reeves[2], and Edmond V. Bayer[4]

At last year's meeting, we documented an increase in vector populations and viral activity in 1979 [1,2]. By Spring, 1980, water supplies and predictions of snowmelt run-off were above normal throughout the State. Those conditions, coupled with decreased funding for mosquito control as a result of Proposition 13, caused concern that epidemic levels of viral activity might occur. The State Legislature therefore approved a special appropriation of over $3,000,000 for encephalitis surveillance and vector control, allowing a level of effort similar to that carried out in recent years. This report summarizes the Department of Health Services' (DOHS) arboviral surveillance activities during 1980 in collaboration with the University of California (Berkeley) School of Public Health's Arthropod-borne Virus Research Unit (UCBSPH, AVRU) and other agencies. The increasingly important interdependence of the DOHS and the UCBSPH, AVRU in accomplishing this program is reflected in the title and authorship changes from previous reports. As usual, the program depended on the participation of local mosquito abatement districts (MADs), county health departments, the California Department of Food and Agriculture, private physicians and veterinarians, and others.

During 1980, only 264 patients had serum samples submitted to be tested for western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE), and other possible non-arbovirus causes of encephalitis/meningitis (herpes, mumps, enteroviruses, leptospirosis, etc.) by the DOHS' Viral and Rickettsial Disease Laboratory (VRDL) and county public health laboratories, the lowest number in the past decade (Tables 1 and 2). In addition to the serologic study 21 human brain samples and 2 human cerebrospinal fluid samples from suspect cases of encephalitis were tested for arboviruses in suckling mice, all with negative results. As usual, a sample of these (132 cases) will be re-tested by the UCBSPH, AVRU in the continuing study to determine if other arboviruses besides WEE and SLE cause disease in California.

No human cases of WEE or SLE in California were detected in California in 1980. However, one case of SLE from Nevada was transferred to a hospital in Oakland, Alameda County, and the VRDL assisted in confirming the preliminary diagnosis

**Table 1.** Arboviral surveillance activities and results in California, 1971-1980.

| | 1971 | 1972 | 1973 | 1974 | 1975 | 1976 | 1977 | 1978 | 1979 | 1980 |
|---|---|---|---|---|---|---|---|---|---|---|
| Suspect human cases of encephalitis/meningitis tested serologically | 620 | 729 | 1,037 | 643 | 583 | 455 | 316 | 367 | 342 | 264 |
| SLE+ | 3 | 5 | 5 | 1* | 2* | 3 | 1 | 1 | 0 | 0 |
| WEE+ | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| VEE+ | 0 | 2* | 0 | 0 | 0 | -** | - | - | - | - |
| Suspect equine cases of encephalitis tested serologically | 145 | 68 | 56 | 61 | 40 | 35 | 31 | 35 | 74 | 25 |
| WEE+ | 16 | 1 | 2 | 2 | 0 | 0 | 1 | 12 | 18 | 2 |
| Number of mosquito pools tested | 1,784 | 6,336 | 4,838 | 1,690 | 1,002 | 1,273 | 836 | 1,798 | 1,810 | 3,760 |
| WEE+ | 16 | 42 | 97 | 4 | 0 | 0 | 19 | 87 | 114 | 73 |
| SLE+ | 6 | 64 | 75 | 2 | 0 | 10 | 6 | 39 | 26 | 13 |
| Other+ | 43 | 74 | 109 | 38 | 0 | 12 | 19 | 61 | 50 | 136 |
| Total+ | 65 | 180 | 281 | 44 | 0 | 22 | 44 | 187 | 190 | 222 |

*Out-of-state contraction

**VEE not routinely tested for after 1975

**Table 2.** Humans tested serologically for mosquito-borne arboviral diseases by the Viral and Rickettsial Disease Laboratory Section, California State Department of Health Services and by County Health Department laboratories, by county and month of illness onset, California 1980.

| County | Totals | Jan | Feb | March | April | May | June | July | Aug | Sept | Oct | Nov | Dec | Unknown |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Totals | 264 | 6 | 1 | 2 | 7 | 34 | 40 | 36 | 55 | 34 | 15 | 12 | 0 | 22 |
| Alameda Co. | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| Berkeley | 7 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Butte | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Contra Costa | 7 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 1 | 0 | 1 | 0 | 0 |
| El Dorado | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Fresno* | 44 | 0 | 0 | 1 | 0 | 9 | 10 | 7 | 10 | 5 | 1 | 1 | 0 | 0 |
| Humboldt | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Imperial | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| Inyo | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Lake | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Los Angeles* | 22 | 0 | 0 | 1 | 1 | 3 | 6 | 1 | 4 | 4 | 1 | 1 | 0 | 0 |
| Madera | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Mendocino | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Merced | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| Napa | 13 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 4 | 1 | 0 | 0 | 0 | 8 |
| Nevada | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Orange* | 4 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Placer | 6 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Plumas | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Sacramento* | 24 | 0 | 0 | 0 | 1 | 3 | 3 | 5 | 6 | 2 | 1 | 3 | 0 | 0 |
| San Bernardino* | 25 | 5 | 0 | 0 | 0 | 4 | 4 | 2 | 8 | 0 | 1 | 1 | 0 | 0 |
| San Diego* | 37 | 0 | 0 | 0 | 1 | 5 | 3 | 5 | 6 | 11 | 4 | 2 | 0 | 0 |
| San Francisco | 3 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| San Joaquin | 6 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 0 | 1 | 0 | 0 | 0 |
| San Luis Obispo | 12 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 4 | 0 | 1 | 0 | 0 | 0 |
| Santa Barbara | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Santa Cruz | 6 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 2 |
| Shasta | 8 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 2 | 1 | 0 | 0 | 1 |
| Sierra | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Solano | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Stanislaus | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Sutter | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Tehama | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Tuolumne | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Yolo | 4 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 0 |
| Out of State | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| No County | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*most or all sera tested by County Health Department laboratory.

[1]Viral and Rickettsial Disease Laboratory Section, California Department of Health Services.

[2]Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley.

[3]Vector Biology and Control Section, California Department of Health Services.

[4]Veterinary Public Health Unit, Infectious Disease Section, California Department of Health Services.

which had been made by HI tests at the Center for Disease Control, Ft. Collins, Colorado. The patient was a 6½ year old girl resident of Fallon, Nevada.

Serum specimens from 25 clinically suspect equine cases were tested serologically for WEE, a record low (Table 1). In addition, 16 equine brain samples from fatal cases (April through December) were tested for arboviruses and rabies virus in suckling mice. These specimens were submitted from 23 counties in the state. The onsets of the cases ranged from February 7 to December 12. Only 2 cases of WEE were confirmed by serologic tests: (1) a 4-year old horse from Placer county, onset August 7, with complement-fixing (CF) antibody titer rise from <1:8 to >1:64 and indirect fluorescent antibody (IFA) titer rise from <1:8 to 1:64, and with full recovery; and (2) a 4-year-old horse from Kern County, onset September 10, with CF antibody titer rise from 1:32 to 1:256, and IFA antibody titer rise from 1:512 to 1:1024, and also with full recovery.

Surveillance of mosquito populations for the presence of virus was conducted at a higher level than in any of the previous 6 years, due especially to the help of the UCBSPH, ARVU in assigning Ms. Patricia Boehme to assist in the laboratory testing program. In total, 177,839 mosquitoes (3,760 pools) were tested from 37 California counties and 2 Arizona counties (Table 3). Effort was focused on the 2 most important vector species (*Culex tarsalis* and the *Culex pipiens* complex) which represented 3,404 of the pools (Table 4 and 5).

**Table 3.** Number of mosquitoes (pools) tested, by county and species, by the Viral and Rickettsial Disease Laboratory Section, California Department of Health Services, 1980.

| County | Culex tarsalis | Culex pipiens complex | Aedes melanimon | Other | Total |
|---|---|---|---|---|---|
| Alameda | 145 (8) | 22 (2) | | *Culex peus* 9 (1)<br>*Culex erythrothorax* 16 (2) | 167 (10) |
| Butte | 13,764 (289) | | 645 (18) | *Aedes vexans* 24 (1) | 14,458 (311) |
| Colusa | 6,472 (131) | | 80 (3) | | 6,552 (134) |
| Contra Costa | 285 (12) | 181 (5) | 174 (5) | | 640 (22) |
| Fresno | 1,763 (38) | 100 (2) | 16 (1) | | 1,879 (41) |
| Glenn | 6,255 (128) | | 11 (1) | | 6,266 (129) |
| Humboldt | 62 (2) | 33 (1) | | | 95 (3) |
| Imperial | 15,039 (230) | 1,308 (49) | | *Aedes dorealis* 275(13)<br>*Anopheles franciscanus* 65 (2) | 16,687 (294) |
| Inyo | 3,699 (75) | | 1,097 (24) | | 4,796 (99) |
| Kern | 21,540 (439) | 313 (8) | 9,647 (194) | *Culiseta inornata* 800 (16)<br>*Anopheles freeborni* 112 (3)<br>*Culex erythrothorax* 71 (2) | 32,483 (662) |
| Kings | 440 (13) | 12 (1) | | | 452 (14) |
| Marin | 276 (7) | | | | 276 (7) |
| Madera | 1,222 (21) | | | | 1,222 (21) |
| Mendocino | 18 (1) | | | | 18 (1) |
| Merced | 2,780 (34) | | 519 (12) | | 3,299 (46) |
| Napa | 225 (9) | 50 (2) | | *Culex peus* 43 (2) | 318 (13) |
| Orange | 160 (5) | | | *Culex erythrothorax* 150 (3) | 310 (8) |
| Placer | 5,227 (109) | | | | 5,227 (109) |
| Riverside | 12,791 (273) | 94 (4) | | *Culex erythrothorax* 200 (4) | 13,085 (281) |
| Sacramento | 6,225 (143) | | | | 6,225 (143) |
| San Bernardino | 3,922 (83) | | | | 3,922 (83) |
| San Diego | 63 (3) | | | | 63 (3) |
| San Joaquin | 1,378 (29) | 149 (4) | | | 1,527 (33) |
| San Mateo | 137 (3) | 54 (2) | | | 191 (5) |
| Santa Barbara | 4 (1) | | | *Culex erythrothorax* 331 (7) | 335 (8) |
| Santa Clara | 296 (12) | 655 (25) | | *Culex peus* 350 (7) | 1,301 (44) |
| Shasta | 3,368 (84) | 141 (9) | 25 (1) | | 3,534 (94) |
| Siskiyou | 998 (21) | | | | 998 (21) |
| Solano | 813 (19) | 12 (1) | | | 825 (20) |
| Sonoma | 25 (2) | | | *Culex peus* 73 (2) | 98 (4) |
| Stanislaus | 2,483 (60) | 530 (15) | 165 (7) | *Culex peus* 112 (3) | 3,290 (85) |
| Sutter | 16,865 (348) | 232 (5) | 866 (21) | | 17,963 (374) |
| Tehama | 2,011 (46) | | | | 2,011 (46) |
| Tulare | 9,265 (207) | 890 (23) | 16 (1) | | 10,171 (231) |
| Yuba | 7,404 (156) | 48 (1) | | | 7,452 (157) |
| Ventura | 550 (11) | | | | 550 (11) |
| Yolo | 8,556 (178) | | | | 8,556 (178) |
| Mohave, AZ | 156 (4) | | | | 156 (4) |
| Yuma, AZ | 441 (11) | | | | 441 (11) |
| Total | 157,123 (3,245) | 4,824 (159) | 13,261 (288) | 2,631 (68) | 177,839 (3760) |

**Table 4.** Summary of viral isolates from mosquitoes (and minimum infection rate per 1,000 *Culex tarsalis*) during 1980 by the Viral and Rickettsial Disease Laboratory Section, Department of Health Services.

| County | WEE | SLE | Hart Park | Turlock | California Group | Total |
|---|---|---|---|---|---|---|
| Alameda | | | 1 | | | 1 |
| Butte | 1 (0.07) | | 1 | | 1 | 3 |
| Colusa | 5 (0.77) | | 3 | 2 | 1 | 11 |
| Glenn | 1 (0.16) | | 2 | 3 | | 6 |
| Imperial | 8 (0.53) | 1 (0.07) | | 2 | | 11 |
| Inyo | | | | 2 | 2 | 4 |
| Kern | 19 (0.79) | | 41 | 5 | 6 | 71 |
| Kings | | | | 1 | | 1 |
| Madera | | | | 1 | | 1 |
| Napa | | | 1 | | | 1 |
| Orange | | | | 1 | | 1 |
| Placer | | | 1 | | | 1 |
| Riverside | 15 (1.09) | 8 (0.63) | 1 | 4 | | 28 |
| Sacramento | 1 (0.16) | | 1 | 1 | | 3 |
| San Bernardino | 10 (2.55) | 3 (0.76) | | 6 | | 19 |
| San Joaquin | | | 2 | 1 | | 3 |
| San Mateo | | | | 1 | | 1 |
| Shasta | | | 2 | 3 | | 5 |
| Stanislaus | 1 | | | 1 | | 2 |
| Sutter | 3 (0.18) | | 1 | 3 | 1 | 8 |
| Tehama | | | 2 | 3 | | 5 |
| Tulare | 5 (0.54) | | 16 | 5 | | 26 |
| Yolo | | | | 1 | | 1 |
| Yuba | 1 (0.14) | | 2 | 2 | | 5 |
| Mohava, AZ | 1 (6.41) | | | | | 1 |
| Yuma, AZ | 2 (4.54) | 1 (2.27) | | | | 3 |
| | 73 (0.44) | 10 (0.06) | 77 | 48 | 11 | 222 |

There were 222 viral isolates from mosquitoes: 73 WEE, 13 SLE, 77 Hart Park, 48 Turlock and 11 California encephalitis group. The drop in WEE viral isolation rates, compared with the previous 2 years, paralleled the apparent reduction in equine cases.

Sentinel chicken flocks were distributed to 31 sites throughout the State, from Redding to El Centro. The birds were bled each month from June through October. Overall, 15% developed antibodies to WEE virus. The infection rates for individual flocks ranged from 0 to 42% in the Sacramento Valley, 0 to 69% in Southern California and 0 to 86% in the San Joaquin Valley (Tables 6 and 7). The earliest infections occurred prior to the July sampling date in the El Centro flock. The majority of conversions occurred in the September samples. Only 1 bird, from a flock near Thermal in Riverside County, developed antibodies to SLE virus in August.

The serological conversion rate in 1980 was substantially lower than in 1979, when 38% of sentinel chickens in California became WEE positive (Table 7), and 10 Southern California birds developed SLE antibodies (Reeves and Milby 1980). We attribute this reduction in viral transmission, especially in the Sacramento Valley, to a decline in vector population levels in 1980. Below-normal temperatures prevailed in most areas in early summer, and this delayed the usual rapid increase in mosquito populations until potential breeding sites had dried up or were controlled.

The practice of providing timely surveillance reports was continued in 1980. Daily telephone reports of positive virus isolation results, human and horse cases, and chicken sero-conversions were made to appropriate MADs or other agencies. A weekly summary report was widely distributed (24 issues from May 2 to October 11), to provide instructions, test results, and other information.

The prospects for 1981 are that there will be further and drastic budget reductions for mosquito control in many of the responsible agencies. After a dry early winter, rainfall was above average in March and the Department of Water Resources now predicts about 75% of normal run-off. It is

planned to maintain a sufficient level of surveillance activities to predict build-up of viral activity in time to direct preventive efforts and to document vector population levels, viral infection and disease when and where they occur.

**Table 5.** Viral Isolates from mosquito pools tested during 1980 by the Viral and Rickettsial Disease Laboratory Section, California Department of Health Services.

| Identifying Number | County | Place | Date Collected | Species | Number in Pool | Agent Isolated |
|---|---|---|---|---|---|---|
| E08701 | Alameda | Livermore | 07-31 | *C. tarsalis* | 17 | Hart Park |
| E11173 | Butte | Gray Lodge | 06-10 | *A. melanimon* | 50 | Calif. Grp. |
| E11893 | Butte | Hamilton City | 07-24 | *C. tarsalis* | 50 | Hart Park |
| E18493 | Butte | Gray Lodge | 09-01 | " " | 50 | WEE |
| E11547 | Colusa | Colusa | 07-09 | *A. melanimon* | 50 | Calif. Grp. |
| E11541 | Colusa | Colusa | 07-09 | *C. tarsalis* | 50 | Hart Park |
| E11781 | Colusa | Colusa | 07-16 | " " | 50 | WEE |
| E11788 | Colusa | Colusa | 07-16 | " " | 50 | WEE |
| E11865 | Colusa | Colusa | 07-23 | " " | 50 | WEE |
| E11881 | Colusa | Colusa | 07-23 | " " | 50 | Hart Park |
| E11880 | Colusa | Colusa | 07-23 | " " | 50 | WEE |
| E11876 | Colusa | Colusa | 07-23 | " " | 50 | Hart Park |
| E11943 | Colusa | Colusa | 07-30 | " " | 50 | Turlock |
| E11942 | Colusa | Colusa | 07-30 | " " | 50 | Turlock |
| E11941 | Colusa | Colusa | 07-30 | " " | 50 | WEE |
| E11842 | Glenn | Willows | 07-22 | " " | 50 | Hart Park |
| E11897 | Glenn | Hamilton City | 07-24 | " " | 50 | Hart Park |
| E11908 | Glenn | Orland | 07-24 | " " | 50 | Turlock |
| E11906 | Glenn | Orland | 07-24 | " " | 50 | Turlock |
| E11998 | Glenn | Willows | 08-12 | " " | 50 | WEE |
| E18452 | Glenn | Willows | 08-19 | " " | 50 | Turlock |
| E01696 | Imperial | Rutherford Rd./New River | 07-15 | " " | 27 | WEE |
| E01690 | Imperial | Brockman Rd./New River | 07-15 | " " | 50 | Turlock |
| E01691 | Imperial | Brockman Rd./ New River | 07-15 | " " | 50 | Turlock |
| E01697 | Imperial | Brockman Rd./ Greeson Wash | 07-15 | " " | 27 | WEE |
| E01699 | Imperial | Palo Verde County Park | 07-22 | " " | 18 | WEE |
| E01710 | Imperial | Gilmore's Camp | 07-22 | " " | 50 | WEE |
| E01740 | Imperial | Salamander Trailer Park | 09-03 | " " | 50 | WEE |
| E01732 | Imperial | Senator Wash | 09-03 | " " | 50 | SLE |
| E01734 | Imperial | Senator Wash | 09-03 | " " | 50 | WEE |
| E01743 | Imperial | Mittry Lake | 09-03 | " " | 50 | WEE |
| E01758 | Imperial | Heber | 09-12 | " " | 32 | WEE |
| E02044 | Inyo | Independence | 07-30 | " " | 50 | Turlock |
| E02062 | Inyo | Lone Pine | 07-31 | " " | 49 | Turlock |
| E03137 | Inyo | Bishop | 09-09 | *A. melanimon* | 50 | Calif. Grp. |
| E03131 | Inyo | Big Pine | 09-09 | *C. tarsalis* | 50 | Calif. Grp. |

**Table 5.** continued.

| Identifying Number | County | Place | Date Collected | Species | Number in Pool | Agent Isolated |
|---|---|---|---|---|---|---|
| E16015 | Kern | Tupman | 07-29 | *C. tarsalis* | 50 | Hart Park |
| E16016 | Kern | Tupman | 07-29 | " " | 50 | Hart Park |
| E06283 | Kern | Hoseaian's Ranch | 08-05 | " " | 50 | WEE |
| E06287 | Kern | Hoseaian's Ranch | 08-05 | " " | 50 | WEE |
| E06282 | Kern | Hoseaian's Ranch | 08-05 | " " | 50 | Turlock |
| E06273 | Kern | Frank Sanders Duck Club | 08-05 | " " | 50 | Hart Park |
| E06264 | Kern | L.A. Athletic Duck Club | 08-05 | " " | 50 | Hart Park |
| E06266 | Kern | L.A. Athletic Duck Club | 08-05 | " " | 50 | WEE |
| E06303 | Kern | Lebec | 08-12 | " " | 50 | Hart Park |
| E06309 | Kern | Kern River Percolation Area | 08-12 | " " | 50 | WEE |
| E06311 | Kern | Kern River Percolation Area | 08-12 | " " | 50 | WEE |
| E06313 | Kern | Kern River Percolation Area | 08-12 | " " | 50 | Turlock |
| SV3023 | Kern | Meadowbrook | 08-19 | " " | 50 | Hart Park |
| SV3024 | Kern | Meadowbrook | 08-19 | " " | 50 | Turlock |
| SV3029 | Kern | Meadowbrook | 08-19 | " " | 50 | Hart Park |
| E06327 | Kern | Kern River | 08-26 | *A. melanimon* | 50 | Calif. Grp. |
| E06319 | Kern | Kern River | 08-26 | *C. tarsalis* | 50 | WEE |
| E06315 | Kern | Lakeside School | 08-26 | " " | 50 | WEE |
| E06316 | Kern | Lakeside School | 08-26 | " " | 50 | Hart Park |
| E06318 | Kern | Lakeside School | 08-26 | " " | 50 | WEE |
| SV3057 | Kern | Meadowbrook | 09-03 | " " | 50 | WEE |
| SV3066 | Kern | Meadowbrook | 09-03 | *A. melanimon* | 50 | WEE |
| SV3070 | Kern | Smith's Pasture | 09-03 | *C. tarsalis* | 50 | Hart Park |
| E06351 | Kern | Kern County Sewer Farm | 09-09 | " " | 50 | Hart Park |
| E06352 | Kern | Kern County Sewer Farm | 09-09 | " " | 50 | WEE |
| E06346 | Kern | Minter Field Ponds | 09-09 | " " | 50 | WEE |
| E06339 | Kern | Zerker Ponds | 09-09 | " " | 50 | WEE |
| E06341 | Kern | Zerker Ponds | 09-09 | " " | 50 | WEE |
| E06343 | Kern | Zerker Ponds | 09-09 | " " | 50 | WEE |
| E16166 | Kings | Lemoore N.A.S. | 08-13 | " " | 10 | Turlock |
| E06243 | Madera | Chowchilla | 09-04 | " " | 50 | Turlock |
| E09204 | Napa | Napa | 07-08 | " " | 19 | Hart Park |
| E04302 | Orange | Irvine | 07-29 | " " | 50 | Turlock |
| E13833 | Placer | Lincoln | 07-21 | " " | 50 | Hart Park |
| E02805 | Riverside | Mecca | 06-17 | " " | 50 | WEE |
| E02808 | Riverside | Mecca | 06-17 | " " | 50 | WEE |
| E02810 | Riverside | Mecca | 06-17 | " " | 50 | Turlock |
| E02812 | Riverside | Mecca | 06-17 | " " | 50 | WEE |
| E02813 | Riverside | Mecca | 06-17 | " " | 50 | WEE |
| E02804 | Riverside | Mecca | 06-17 | " " | 50 | WEE |
| E03004 | Riverside | Blythe | 07-01 | *C. pipiens* | 31 | WEE |
| E03005 | Riverside | Blythe | 07-01 | *C. tarsalis* | 50 | WEE |
| E03008 | Riverside | Blythe | 07-01 | " " | 50 | WEE |
| E03012 | Riverside | Blythe | 07-01 | " " | 50 | WEE |
| E02815 | Riverside | Mecca | 07-15 | " " | 50 | WEE |

**Table 5.** continued.

| Identifying Number | County | Place | Date Collected | Species | Number in Pool | Agent Isolated |
|---|---|---|---|---|---|---|
| SV2854 | Kern | Smith's Pasture | 06-01 | *C. tarsalis* | 50 | Hart Park |
| SV2857 | Kern | Smith's Pasture | 06-01 | *A. melanimon* | 50 | Calif. Grp. |
| SV2891 | Kern | Smith's Pasture | 06-09 | *C. tarsalis* | 50 | Hart Park |
| SV2897 | Kern | Meadowbrook | 06-09 | " " | 50 | WEE |
| SV2904 | Kern | Smith's Pasture | 06-15 | " " | 50 | Hart Park |
| SV2929 | Kern | Smith's Pasture | 06-22 | *A. melanimon* | 50 | Hart Park |
| SV2931 | Kern | Smith's Pasture | 06-22 | *C. tarsalis* | 50 | Hart Park |
| SV2936 | Kern | Meadowbrook | 06-22 | " " | 50 | Turlock |
| SV2937 | Kern | Meadowbrook | 06-22 | " " | 50 | Hart Park |
| SV2939 | Kern | Poso West | 06-22 | " " | 50 | Hart Park |
| SV2941 | Kern | McVan | 06-22 | " " | 50 | Hart Park |
| E16291 | Kern | Hoseaian's Ranch | 06-24 | " " | 50 | WEE, Hart Park |
| E16292 | Kern | Hoseaian's Ranch | 06-24 | " " | 50 | Hart Park |
| E16296 | Kern | Hoseaian's Ranch | 06-24 | " " | 50 | WEE, Hart Park |
| E16297 | Kern | Hoseaian's Ranch | 06-24 | " " | 50 | Hart Park |
| E16298 | Kern | Hoseaian's Ranch | 06-24 | " " | 50 | Hart Park |
| E16273 | Kern | Northfield Farms | 06-24 | *A. melanimon* | 50 | WEE |
| SV2953 | Kern | Meadowbrook | 06-29 | *C. tarsalis* | 50 | Hart Park |
| E06033 | Kern | Kern County Sewer Farm | 07-08 | " " | 50 | Hart Park |
| E06023 | Kern | Hoseaian's Ranch | 07-08 | " " | 50 | Hart Park |
| E06025 | Kern | Hoseaian's Ranch | 07-08 | " " | 50 | Hart Park |
| E06026 | Kern | Hoseaian's Ranch | 07-08 | " " | 50 | Hart Park |
| E06008 | Kern | Northfield Farms | 07-08 | *A. melanimon* | 50 | Calif. Grp. |
| E06016 | Kern | Northfield Farms | 07-08 | *C. erythrothorax* | 50 | Hart Park |
| E06003 | Kern | Torigiani Ranch | 07-08 | *C. tarsalis* | 50 | Hart Park |
| E06054 | Kern | Trach's Goode Lake Ranch | 07-15 | " " | 50 | Hart Park |
| E06044 | Kern | Kern River Percolation Area | 07-15 | " " | 50 | Hart Park |
| E06045 | Kern | Kern River Percolation Area | 07-15 | " " | 50 | Hart Park |
| E06051 | Kern | Kern River Percolation Area | 07-15 | *A. melanimon* | 50 | Calif. Grp. |
| SV2973 | Kern | Meadowbrook | 07-18 | *C. tarsalis* | 50 | Hart Park |
| SV2981 | Kern | Poso West | 07-18 | " " | 50 | Hart Park |
| SV2987 | Kern | Smith's Pasture | 07-18 | " " | 50 | Calif. Grp. |
| E06071 | Kern | Kern River Percolation Area | 07-22 | *A. melanimon* | 50 | Calif. Grp. |
| E06061 | Kern | Kern River Percolation Area | 07-22 | *C. tarsalis* | 50 | Hart Park |
| E06253 | Kern | Carmel Ranch | 07-29 | " " | 50 | Hart Park |
| E06094 | Kern | Universal Duck Club | 07-29 | " " | 50 | Hart Park |
| SV3012 | Kern | Poso West | 07-29 | " " | 50 | Hart Park |
| E16009 | Kern | Tupman | 07-29 | " " | 50 | Turlock |
| E16010 | Kern | Tupman | 07-29 | " " | 50 | Hart Park |
| E16013 | Kern | Tupman | 07-29 | " " | 50 | Hart Park |

**Table 5.** continued.

| Identifying Number | County | Place | Date Collected | Species | Number in Pool | Agent Isolated |
|---|---|---|---|---|---|---|
| E02819 | Riverside | Mecca | 07-15 | *C. tarsalis* | 50 | SLE |
| E02820 | Riverside | Mecca | 07-15 | " " | 50 | WEE |
| E02822 | Riverside | Mecca | 07-15 | " " | 50 | SLE, Turlock |
| E02823 | Riverside | Mecca | 07-15 | " " | 50 | Turlock, Hart Park |
| E02825 | Riverside | Mecca | 07-15 | " " | 50 | WEE |
| E02826 | Riverside | Mecca | 07-15 | " " | 50 | SLE |
| E02827 | Riverside | Mecca | 07-15 | " " | 50 | SLE |
| E03020 | Riverside | Hemet | 07-31 | " " | 42 | Turlock |
| E03029 | Riverside | Blythe | 08-13 | " " | 50 | WEE |
| E03030 | Riverside | Blythe | 08-13 | " " | 50 | SLE |
| E03033 | Riverside | Blythe | 08-13 | " " | 50 | SLE |
| E03035 | Riverside | Blythe | 08-13 | " " | 50 | WEE |
| E03037 | Riverside | Blythe | 08-13 | " " | 50 | SLE |
| E02835 | Riverside | Mecca | 08-20 | " " | 50 | WEE |
| E02839 | Riverside | Mecca | 08-20 | " " | 50 | SLE |
| E13784 | Sacramento | Sacramento | 07-30 | " " | 50 | Hart Park |
| E13808 | Sacramento | Sacramento | 08-04 | " " | 32 | Turlock |
| E10398 | Sacramento | Galt | 08-19 | " " | 50 | WEE |
| E02267 | San Bernardino | Needles | 05-21 | *C. tarsalis* | 50 | WEE |
| E02269 | San Bernardino | Needles | 05-21 | *C. tarsalis* | 50 | WEE |
| E02271 | San Bernardino | Needles | 05-21 | *C. tarsalis* | 50 | Turlock |
| E02272 | San Bernardino | Needles | 05-21 | *C. tarsalis* | 50 | Turlock |
| E02273 | San Bernardino | Needles | 05-21 | *C. tarsalis* | 50 | Turlock |
| E02286 | San Bernardino | Parker Dam | 06-19 | *C. tarsalis* | 50 | Turlock |
| E02284 | San Bernardino | Parker Dam | 06-19 | *C. tarsalis* | 50 | Turlock |
| E02289 | San Bernardino | Needles | 06-20 | *C. tarsalis* | 50 | WEE |
| E02290 | San Bernardino | Needles | 06-20 | *C. tarsalis* | 50 | WEE |
| E02291 | San Bernardino | Needles | 06-20 | *C. tarsalis* | 50 | WEE |
| E02294 | San Bernardino | Needles | 06-20 | *C. tarsalis* | 50 | WEE |
| E02905 | San Bernardino | Needles | 07-24 | *C. tarsalis* | 50 | WEE |
| E02906 | San Bernardino | Needles | 07-24 | *C. tarsalis* | 50 | Turlock |
| E02914 | San Bernardino | Needles | 07-24 | *C. tarsalis* | 50 | WEE |
| E02900 | San Bernardino | Needles | 07-24 | *C. tarsalis* | 50 | WEE |
| E02901 | San Bernardino | Needles | 07-24 | *C. tarsalis* | 50 | WEE |
| E02920 | San Bernardino | Needles | 08-28 | *C. tarsalis* | 50 | SLE |
| E02921 | San Bernardino | Bermuda City | 08-28 | *C. tarsalis* | 47 | SLE |
| E02930 | San Bernardino | Needles | 08-28 | *C. tarsalis* | 50 | SLE |

**Table 5.** continued.

| Identifying Number | County | Place | Date Collected | Species | Number in Pool | Agent Isolated |
|---|---|---|---|---|---|---|
| E13726 | San Joaquin | Escalon | 07-16 | *C. tarsalis* | 43 | Hart Park |
| E13836 | San Joaquin | Collierville | 07-28 | " " | 46 | Turlock |
| E13868 | San Joaquin | Valley Home | 08-07 | " " | 50 | Hart Park |
| E08501 | San Mateo | East Palo Alto | 08-07 | " " | 37 | Turlock |
| E11517 | Shasta | Palo-Cedro-Bella Vista | 07-07 | *C. tarsalis* | 45 | Hart Park |
| E11277 | Shasta | Anderson | 07-21 | " " | 50 | Hart Park |
| E11280 | Shasta | Anderson | 07-29 | " " | 50 | Turlock |
| E18306 | Shasta | Anderson | 08-11 | " " | 50 | Turlock |
| E11726 | Shasta | Anderson | 08-11 | " " | 50 | Turlock |
| E13183 | Stanislaus | Patterson | 07-10 | " " | 50 | Turlock |
| E10004 | Stanislaus | Newman | 08-20 | *C. Peus* | 49 | WEE |
| E11864 | Sutter | Meridian | 07-23 | *A. melanimon* | 50 | Calif. Grp. |
| E10247 | Sutter | Pleasant Grove | 08-11 | *C. tarsalis* | 50 | Turlock |
| E10644 | Sutter | Sheppard | 08-20 | " " | 50 | WEE |
| E18468 | Sutter | Rio Oso | 08-21 | " " | 50 | WEE |
| E11504 | Sutter | Trowbridge | 06-26 | *C. tarsalis* | 50 | Hart Park |
| SY126 | Sutter | Sutter City | 08-27 | " " | 50 | Turlock |
| SY133 | Sutter | Meridian | 08-31 | " " | 50 | WEE |
| SY164 | Sutter | Robbins | 09-01 | " " | 50 | Turlock |
| E11827 | Tehama | Red Bluff | 07-21 | " " | 50 | Hart Park |
| E11916 | Tehama | Woodson Bridge State Park | 07-28 | *C. tarsalis* | 50 | Turlock |
| E18442 | Tehama | Woodson Bridge State Park | 08-18 | *C. tarsalis* | 50 | Turlock |
| E18445 | Tehama | Los Molinos | 08-18 | " " | 50 | Turlock |
| E18541 | Tehama | Red Bluff | 09-05 | " " | 39 | Hart Park |
| E18022 | Tulare | Tipton | 06-18 | " " | 33 | Hart Park |
| E18029 | Tulare | Pixley | 07-09 | " " | 50 | Hart Park |
| E18066 | Tulare | Teviston | 07-09 | " " | 24 | Hart Park |
| E18192 | Tulare | Teviston | 07-28 | " " | 48 | Hart Park |
| E18041 | Tulare | Alpaugh | 07-30 | " " | 50 | Hart Park |
| E18036 | Tulare | Alpaugh | 07-30 | " " | 50 | Hart Park |
| E18098 | Tulare | Earlimart | 08-08 | " " | 50 | WEE |
| E18096 | Tulare | Earlimart | 08-08 | " " | 46 | Turlock |
| E18084 | Tulare | Earlimart | 08-08 | " " | 50 | Turlock |
| E18085 | Tulare | Earlimart | 08-08 | " " | 50 | Hart Park |
| E18087 | Tulare | Earlimart | 08-08 | " " | 50 | Hart Park |
| E18090 | Tulare | Earlimart | 08-08 | " " | 50 | WEE |
| E18092 | Tulare | Earlimart | 08-08 | " " | 50 | Hart Park |
| E18080 | Tulare | Allensworth | 08-11 | " " | 46 | Turlock |
| E18076 | Tulare | Teviston | 08-11 | " " | 50 | Hart Park |
| E18050 | Tulare | Tipton | 08-13 | " " | 50 | Hart Park |
| E18221 | Tulare | Teviston | 08-19 | " " | 50 | Hart Park |
| E18224 | Tulare | Teviston | 08-19 | " " | 37 | Hart Park |
| E06106 | Tulare | Pixley | 08-20 | " " | 36 | Turlock |
| E18235 | Tulare | Teviston | 08-25 | " " | 50 | WEE |
| E18288 | Tulare | Earlimart | 09-02 | " " | 50 | Hart Park |
| E18289 | Tulare | Earlimart | 09-02 | " " | 50 | WEE |
| E18259 | Tulare | Teviston | 09-02 | " " | 50 | WEE |
| E18246 | Tulare | Teviston | 09-02 | " " | 50 | Turlock |
| E18069 | Tulare | Teviston | ? | " " | 50 | Hart Park |
| E18071 | Tulare | Teviston | ? | " " | 50 | Hart Park |
| E13749 | Yolo | Woodland | 07-22 | " " | 50 | Turlock |

**Table 5.** continued.

| Identifying Number | County | Place | Date Collected | Species | Number in Pool | Agent Isolated |
|---|---|---|---|---|---|---|
| E11604 | Yuba | Marysville | 07-14 | *C. tarsalis* | 50 | Hart Park |
| E10234 | Yuba | Olivehurst | 07-14 | " " | 50 | Hart Park |
| E10659 | Yuba | Olivehurst | 08-25 | " " | 50 | Turlock |
| E10656 | Yuba | Olivehurst | 08-25 | " " | 50 | WEE |
| SY154 | Yuba | Olivehurst | 09-01 | " " | 50 | Turlock |
| E02287 | Mohave, AZ | Bermuda City | 06-20 | " " | 50 | WEE |
| E02072 | Yuma, AZ | Parker | 08-06 | " " | 33 | WEE |
| E02074 | Yuma, AZ | Blythe | 08-08 | " " | 26 | WEE |
| E02126 | Yuma, AZ | Yuma | 08-28 | " " | 50 | SLE |

**Table 6.** Serological conversions to WEE virus in sentinel chickens, California, 1980.

| County | Site | No. | No. (percent) WEE positive Jul | Aug. | Sept. | Oct. |
|---|---|---|---|---|---|---|
| Shasta | MAD office | 22 | 0 | 0 | 0 | 1(5) |
| | Reading Dr. | 24 | 0 | 0 | 1(4) | 2(8) |
| Tehama | MAD office | 24 | 0 | 3(13) | 10(42) | 10(42) |
| Butte | Boswell | 22 | 0 | 0 | 0 | 0 |
| | Chico | 25 | 0 | 0 | 0 | 0 |
| | Oroville | 24 | 0 | 0 | 0 | 1(4) |
| | Grey Lodge | 23 | 0 | 0 | 1(4) | 1(4) |
| Glenn | Willows | 24 | 0 | 2(8) | 3(13) | 4(17) |
| Colusa | MAD office | 22 | 0 | 0 | 0 | 0 |
| Sutter | Dean's | 24 | 0 | 0 | 2(8) | 2(8) |
| | MAD office | 18 | 0 | 0 | 0 | 0 |
| Yuba | Marysville | 24 | 0 | 0 | 0 | 0 |
| | Krause | 19 | 0 | 0 | 2(11) | 4(21) |
| | Plumas Lake | 19 | 0 | 0 | 0 | 1(5) |
| Placer | Sheridan | 25 | 0 | 0 | 0 | 1(4) |
| Fresno | Mendota Refuge | 13 | 0 | 0 | 0 | 0 |
| Tulare | Rocky Hill | 24 | 0 | 0 | 0 | 0 |
| Kern | Oildale | 21 | 0 | 0 | 0 | 3(14) |
| | Pose West | 23 | 0 | 0 | 0 | 0 |
| | McVan | 23 | 0 | 0 | 0 | 0 |
| | Wasco | 23 | 0 | 0 | 0 | 1(4) |
| | Goose Lake | 16 | 0 | 0 | 4(25) | 10(63) |
| | Buttonwillow | 21 | 0 | 0 | 3(14) | 7(33) |
| | F.C. Tracy | 22 | 0 | 0 | 4(18) | 19(86) |
| | John Dale | 24 | 0 | 0 | 12(50) | 15(63) |
| | Arvin | 22 | 0 | 0 | 0 | 3(14) |
| | Breckenridge | 17 | 0 | 0 | 0 | 1(6) |
| Riverside | Corona | 10 | 0 | 0 | 0 | 1(10) |
| | Mecca | 23 | 0 | 0* | 0* | 1(4)* |
| San Diego | Lakeside | 23 | 0 | 0 | 0 | 0 |
| Imperial | Corda Ranch | 16 | 5(31) | 9(56) | 9(56) | 11(69) |

* 1(4%) positive for SLE virus

**Table 7.** Regional comparisons of WEE serological conversion rates in sentinel chickens, California, 1977 - 1980.

| Area | Percent infected chickens 1977 | 1978 | 1979 | 1980 |
|---|---|---|---|---|
| Sacramento Valley | 0 | 37 | 43 | 8 |
| San Joaquin Valley | 0 | 19 | 41 | 24 |
| Southern California | N.T.* | N.T.* | 9 | 18 |
| Total | 0 | 26 | 38 | 15 |

*N.T. = none tested

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# CANINE HEARTWORM: DISEASE FOCUS ALONG THE LOWER COLORADO RIVER[1]

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## ABSTRACT

A survey to establish the prevalence of canine heartworm in southern California was conducted in the fall of 1979, 1980, and winter of 1981. Initially, a telephone survey of practicing veterinarians in Riverside County was conducted. Twenty-one cases of *Dirofilaria immitis* infections in dogs native to southern California were diagnosed as having dog heartworm in the past 2 years. Subsequently, blood samples from pound dogs in western Riverside County and dogs attending heartworm clinics in eastern and central desert regions were examined for microfilaria by a modified Knott technique. *Dipetalonema reconditium* was found in 8 (2%) of the 366 pound dogs, and 2 (<1%) of the 560 dogs attending the heartworm clinics. *D. immitis* infections were not diagnosed in the pound animals, but 17 (3%) of the animals attending the dog heartworm clines were infected. Because the majority of the animals infected with *D. immitis* were native to those areas surveyed, it is concluded that *D. immitis* is enzootic in southeastern California and southwestern Arizona.

Mosquito trapping with a dog-baited trap was conducted in the summer and fall of 1980 to determine the vector potentials of local mosquito populations in Riverside County. Only one species of the mosquitoes trapped, *Culex erythrothorax* demonstrated the ability to sustain *D. immitis* infection during its development to the $L_3$ infective stage. Less than 1% of *Cx. erythrothorax* feeding on an infected dog survived the 14-day incubation period, yet 60% of the survivors harbored the infective stage larvae.

INTRODUCTION.–In 1970, a survey of 10 northern California counties revealed several cases of canine heartworm infection in dogs native to California (McGreevy et al. 1970). Ample evidence since this initial study has supported the conclusion that the disease is now enzootic in the northern part of the state (Hansen 1978, Weinmann and Garcia 1980, Walters et al. 1981). Although evidence supporting the contention that canine heartworm is a serious veterinary problem in northern California has been well documented, the status of the disease in southern California wasn't established until the completion of a preliminary study in 1980 (Corselli and Platzer 1981). In an effort to further define the problem of canine heartworm in southern California, additional surveys were conducted. Evidence provided by these surveys, as well as that acquired in our earlier efforts, indicate that *D. immitis* is enzootic in the arid regions of southeastern California and southwestern Arizona.

MATERIALS AND METHODS.–Telephone Survey - - Practicing veterinarians in Riverside County were contacted by telephone and asked as to whether or not they had diagnosed any cases of dog heartworm in dogs native to southern California during the past 2 years. Veterinarians were also asked how often and under what circumstances did they examine blood samples for the presence of circulating microfilaria.

Animal Pound Survey - - This group of dogs comprised 366 pound animals brought into the Riverside County Humane Society from various areas (Riverside, Corona, Elsinore, Hemet, Banning, Idyllwild) of western Riverside County. Blood was taken between the hours of 0800 and 1200 each day. Only animals estimated to be at least 1 year of age or older were sampled. These dogs were mostly strays, therefore, no history other than the area where they were captured was available. Observations on the dog's general physical condition and behavior were made and recorded just prior to taking blood samples.

Heartworm Clinics - - This group of animals consisted of animals brought by their owners to dog heartworm clinics held in the desert regions of central and western Riverside and San Bernardino Counties. Blood from these dogs was taken between 0800 and 1500. Dogs younger than 6 months were excluded from these surveys. A clinical history of each dog was supplied by the owner along with a complete account of the dog's past travel experience. As to whether the dog was primarily an outdoor or an indoor pet was also noted.

Blood Examinations - - Blood samples were examined for microfilaria using a modified Knott technique (Knott 1939). One milliliter of blood was drawn from the cephalic vein using a new syringe and 20-gauge needle for each blood sample. The red blood cells were lysed and the microfilaria preserved by injecting each blood sample into a 15 ml centrifuge tube containing 10 ml 2% formalin. These samples were stored at 4 C for a maximum of 2 weeks before examination. Each tube was centrifuged for approximately 7 minutes at 1500 rpm; the resultant rediment was stained with one drop of 1:000 methylene blue and examined with a compound microscope. Microfilaria identification was accomplished utilizing criteria based on measurements and morphological features reported previously (Redington et al. 1978).

[1]Supported in part by the Special Mosquito Augmentation Fund, University of California.

Mosquito Vectors - - In the summer and fall months of 1980, a dog-baited trap similar to that described by Villavaso and Steelman (1970) was used to capture mosquitoes attracted to an infected dog (Fig. 3). The dog used was a 4-year old female beagle with a microfilaremia averaging approximately 6600 mf/ml. The trap was placed in the field by 1800 each evening and retrieved by 0700 the following morning. A total of 6 different trapping sites were sampled. The trap was returned to the lab where the mosquitoes were removed, placed in 12" X 12" X 12" rearing cages and maintained for a minimum of 2 weeks at 80% RH and 28° C. Mosquitoes surviving the 2-week period were then dissected and examined for the presence of the infective larvae of the heartworm nematode. During that period the dog-bait trap was set, a $CO_2$ trap was also placed in the near vicinity. Mosquitoes caught in the $CO_2$ trap were identified and counted. This was done to determine species identity and population densities in the trapping area.

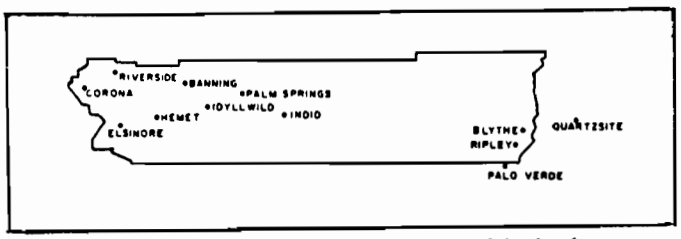


**Figure 1.** Riverside County and areas represented in dog heartworm survey.

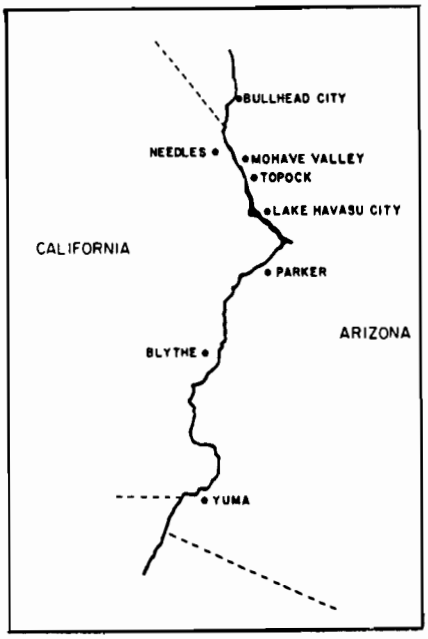


**Figure 2.** Areas along lower Colorado River represented in dog heartworm survey.

RESULTS.–Figure 1 is a map of Riverside County, listing the cities represented in our initial survey (Corselli and Platzer 1981). Figure 2 is a map of those areas along the Colorado River where subsequent heartworm clinics in Needles, California and Parker, Arizona were held.

Telephone Survey - - Twenty-two cases of dog heartworms were diagnosed in the past 2 years by the 53 veterinarians contacted by telephone (Table 1). With the exception of one animal, which had lived in the Carribean, all dogs were native to southern California. Most veterinarians questioned did not take blood samples from dogs in their practice unless the dogs had recently returned from regions where dog heartworm disease was prevalent. The two veterinarians in the Blythe area, where 15 cases of dog heartworm had been diagnosed in the past 2 years, were exceptions and frequently performed diagnostic tests for the disease on animals brought into their practice.

Pound Survey - - Microfilaria were found in 8 (2%) of the 366 animals tested (Table 2). The blunt untapered cephalic end, lengths ranging from 260 to 272$\mu$m and the relatively low number of microfilaria per milliliter of blood characterized the microfilaria as *Dipetalonema reconditum*.

Heartworm Clinics - - Three (5%) of the 63 dogs surveyed at the Indio clinic were infected with *D. immitis* (Table 3). The results of the Blythe, Needles, and Parker clinics are combined in Table 4. Five (4%) of the 162 dogs surveyed at the Blythe clinic were infected with *D. immitis.* Nine (5%) of the 203 dogs surveyed at the Needles clinic had heartworm infection. Eight of these 9 dogs were from the small community of Mohave Valley, Arizona. In Parker, Arizona, 133 dogs were surveyed and no *D. immitis* infections were diagnosed. Diagnostic features identifying the microfilaria as *D. immitis* were the tapered cephalic ends, lengths ranging from 292 to 339 $\mu$m and the higher microfilaremia (20 to 2000 microfilaria/ml). One dog, not native to California, had traveled extensively with its owner in Central America and in the southeastern part of the United States. The infected animals ranged from 1.5 to 11 years in age and were all primarily outdoor dogs. Of the 560 dogs surveyed at our heartworm clinics only 2 (<1%) were infected with *D. reconditum.* Both of these dogs came from the town of Needles.

Mosquito Vectors - - In the Santa Ana River basin, just north of the city of Riverside, California, *Culex erythrothorax* was the mosquito most frequently trapped. This species fed readily on the bait dog and considerable numbers were captured in both the dog-baited (Fig. 3) and $CO_2$ traps. The majority of the blood-fed mosquitoes died shortly after capture. Sixty percent of the blood-fed mosquitoes surviving the 14-day maintenance period did, however, harbor infective larvae. The average parasite load was 3 with a range from 2 to 5 infective larvae per mosquito.

*Psorophora columbiae* was encountered in high densities in the date groves of the Coachella Valley just east of Indio, California. Despite these high densities and several attempts at trapping, few mosquitoes of this species were attracted to the dog and none of those entering the trap fed on the dog.

The only other species of mosquito investigated was *Aedes vexans*. This species was also trapped in the date groves of Coachella Valley. All captured individuals of *Ae. vexans* fed on the dog, but after the 14-day maintenance period, there were no $L_3$ larvae of *D. immitis* found upon dissection.

**Table 1.** Telephone survey of practicing veterinarians in Riverside County for number of dog heartworm cases in 1978 and 1979.

| Area | No. of veterinarians contacted | No. dog heartworm cases in past 2 years |
|---|---|---|
| Banning | 2 | 0 |
| Blythe | 2 | 15* |
| Corona | 13 | 1 |
| Elsinore | 3 | 0 |
| Palm Springs | 4 | 5 |
| Riverside | 22 | 1 |
| Temecula | 7 | 0 |
| TOTAL | 51 | 22* |

* = estimate

**TABLE 2.** Survey for circulating microfilaria in pound dogs from Riverside County. 1978.

| Area | No. of Dogs | Dogs with microfilaria | |
|---|---|---|---|
| | | D. i. | D. r. |
| Banning | 26 | 0 | 1 |
| Elsinore | 38 | 0 | 0 |
| Hemet * | 82 | 0 | 2 |
| Riverside City | 117 | 0 | 4 |
| Riverside County | 103 | 0 | 1 |
| TOTAL | 366 | 0 | 8 |

D.i. = *Dirofilaria immitis*
D.r. = *Dipetalonema reconditum*
*includes Idyllwild

**TABLE 3.** Survey for circulating microfilaria in dogs from central Riverside County, 1979-1980.

| Area | No. of Dogs | Dogs with microfilaria | |
|---|---|---|---|
| | | D. i. | D. r. |
| Coachella | 16 | 0 | 0 |
| Indio | 26 | 2 | 0 |
| Palm Springs | 9 | 0 | 0 |
| Mountain Center | 2 | 1 | 0 |
| Riverside | 4 | 0 | 0 |
| Los Angeles | 3 | 0 | 0 |
| Hemet | 2 | 0 | 0 |
| TOTAL | 62 | 3 | 0 |

D.i. = *Dirofilaria immitis*
D.r. = *Dipetalonema reconditum*

**TABLE 4.** Survey for circulating microfilaria in dogs from southeastern California and southwestern Arizona, 1980-1981.

| Area | No. of Dogs | Dogs with microfilaria | |
|---|---|---|---|
| | | D. i. | D. r. |
| Blythe | 162 | 5 | 0 |
| Mohave Valley | 38 | 8 | 0 |
| Topock | 3 | 0 | 0 |
| Needles | 131 | 1 | 2 |
| Parker | 133 | 0 | 0 |
| Bullhead City | 31 | 0 | 0 |
| TOTAL | 498 | 14 | 2 |

D.i. = *Dirofilaria immitis*
D.r. = *Dipetalonema reconditum*

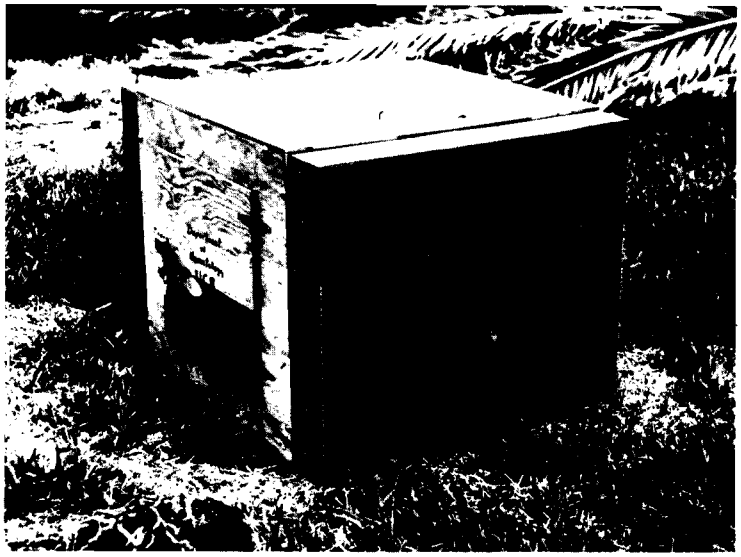

**Figure 3.** Dog-baited mosquito trap.

DISCUSSION.--The majority of the 366 pound animals tested were stray animals. They were generally in poor health and infested with fleas and ticks. These circumstances could account for the relatively high number of infections involving *D. reconditum* compared to the dogs tested at the heartworm clinics. Most of the animals brought into the clinics were in good condition and well-groomed. No external parasites were observed on these animals. Because the flea is responsible for the transmission of *D. reconditum*, one would expect to find fewer infections involving this filarial parasite (Redington et al. 1978).

Diagnosis of the 17 cases of *D. immitis* infection in our survey of the arid regions of the southeastern California and southwestern Arizona suggests a probable focus of the infection. Three factors are common to those areas where we diagnosed heartworm infection and are likely to contribute to the presence of canine heartworm in these areas: (1) A population of mosquitoes known to be potential vectors of *D. immitis* exists in those desert communities surveyed (Bemrick

and Sandholm 1966, Ludlam et al. 1970, Bohart and Washino 1978); (2) development of *D. immitis* within its arthropod host is optimal at temperatures ranging from 22.5° to 26.5°C (Christensen and Hollander 1978), and temperatures seldom fall below 22°C during the spring and summer months in the arid regions surveyed, so that development of the larval stages could easily be sustained (U.S. Environmental Data Service 1979); (3) Palm Springs and the Colorado River area are favorite recreation spots drawing vacationers from all over the United States. Infected dogs accompanying their owner from areas where canine heartworm is highly prevalent may be responsible for initiation of the disease in these recreation areas and may continue to contribute to its persistence.

Further evidence of the Blythe areas as a focus of the disease is that 66% of the total number of cases of the infection diagnosed by county veterinarians were in Blythe. It should be pointed out that the two veterinarians practicing in this area were the only practitioners surveyed who consistently took blood samples and tested for dog heartworm. The other veterinarians only performed tests for the disease when an animal had recently returned from an area where heartworm was known to be prevalent.

Telephone conversations with veterinarians in the Havasu-Needles area along the Colorado River revealed only one instance of dog heartworm disease being diagnosed in the past two years (Dr. M. Jennings, personal communication, 1980). The existence of a focus of the disease in the area was not revealed until we conducted our clinic in Needles. Eight (21%) of the 38 animals surveyed were from Mohave Valley, a farming community on the Arizona side of the Colorado River, were diagnosed as positive for the disease. Our survey results prompted a follow-up survey in Mohave Valley by the resident veterinarian who found 8 (25%) more cases out of 30 dogs sampled (Dr. D. Novak, personal communication, 1980). Increased testing by veterinarians might expose a much higher incidence of canine heartworm disease along the Colorado River and adjacent agricultural areas.

Mosquito Vectors - - The potential of *Cx. erythrothorax* as a vector of *D. immitis* in southern California was demonstrated by this study. Factors responsible for the high mortality of the blood-fed mosquitoes may have included the existence of an unusual amount of heat stress experienced by the mosquitoes as they were returned to the lab. No precaution to control humidity or temperature was taken during transport. A more likely explanation would be found in a consideration of the relatively high microfilaremia of the bait-dog. Kershaw et al. (1953) and Rosen (1954) postulated that mosquitoes which ingest large numbers of microfilariae tend to perish early and the mosquitoes which are potentially effective vectors are those which harbor fewer parasites. The few blood-fed mosquitoes that survived the 14 day maintenance period may have only experienced a limited feeding time on the dog before capture and therefore were infected with fewer parasites. Further studies on the potential of *Cx. erythrothorax* as a vector for *D. immitis* are in order.

That *Ps. columbiae* did not feed on the dog correlates with the findings of Gunstream et al. (1971), who found that this species prefers bovine and equine blood meals. Because of these observations, it is concluded that *Ps. columbiae* is an unlikely vector of *D. immitis* in southern California.

*Ae. vexans* was trapped only on one occasion. Difficulties with humidity control in the lab were believed to be responsible for the termination of larval development in these mosquitoes. This species fed readily on the dog and because of its implications in other areas as a potential vector of canine heartworm disease (Bemrick and Sandholm, 1966; Ludlam et al. 1970); it is probably the species responsible for transmission in the Indio and Coachella Valley area.

From our investigation of potential vectors of *D. immitis*, we have concluded that *Cx. erthrothorax* has proved capable of supporting the development of the parasite and should be considered a potential vector.

ACKNOWLEDGEMENTS.–The authors wish to thank David Watt, Jane Tevis, Kimberly Uchida, Drs. Thomas Jennings, Dean Novak, Thomas Porter and John Hadlock for their technical assistance and Bill Bellamy of the Riverside County Humane Society for his cooperation.

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# VECTOR POTENTIAL OF SIX MOSQUITO SPECIES IN LANDSCAPES OF HIGH HEARTWORM ENDEMICITY IN NORTHERN CALIFORNIA

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ABSTRACT

The landscape epidemiology of canine heartworm disease in northern California was investigated during 1980-81. Dog populations of 3 areas were surveyed for filariasis on a house-to-house basis. Point prevalence rates of *Dirofilaria immitis* microfilaremia in dogs older than 5 months of age were 16.7% (Palo Cedro, Shasta Co.), 24.5% (Tehama, Tehama Co.), and 24.5% (Cedar Ridge, Nevada Co.). In these areas, 114, 102 and 94 dogs respectively were tested, representing over 80% of the resident canine population. *Dipetalonema reconditum* microfilariae were detected only in dogs of the Tehama survey (4.9% prevalence).

Mosquitoes present in these biotypes and in our pilot site, Pleasants Valley (Solano Co.) were studied as potential heartworm vectors. *Aedes vexans*, *Aedes sierrensis*, *Anopheles freeborni*, *Anopheles franciscanus*, *Anopheles punctipennis*, and *Culex tarsalis* entered field-placed kennels housing a microfilaremic bait dog, and subsequently took an infective bloodmeal. Filariae became established in over 80% of engorged populations of these mosquitoes, excepting for *Cx. tarsalis*, only 9.2% of which contained worms. Filariae completed development in 12.3% ($n$= 65), 15.9% ($n$= 126), 7.9% ($n$= 25), 20.0% ($n$= 5), 1.3% ($n$= 154), and 0.8% ($n$= 141) of these six species respectively.

Wild *Dirofilaria* infections were found in 13(3.1%) of 423 *Ae. vexans* and 3 (7.1%) of 42 *An. freeborni* collected from light traps and uninfected dog bait in Tehama County. No filariae were present in 534 *Cx. tarsalis* from this site.

All species of *Aedes* and *Anopheles* in the study fed avidly on dogs and were suitable intermediate hosts of *D. immitis* in natural habitats; these species are considered of moderate to high vector potential. *Culex tarsalis* readily entered kennels and feeding on dogs was confirmed by bloodmeal precipitin tests. However, all populations of *Cx. tarsalis* studied were highly refractory to *D. immitis* infection, suggesting little or no vector potential for this species.

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# *CULEX TARSALIS* COQ. AND *CX. TRITAENIORHYNCHUS* GILES: SIMILARITIES AND DIFFERENCES IN BIONOMICS AND DISEASE RELATIONSHIPS

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Commensurate with the world's agricultural development has been an increase in water management and irrigation projects which have expanded the availability of breeding sites for ground-water mosquitoes. Abundant mosquito populations in relative proximity with man and domestic animals have lead to increases in mosquito-borne disease including arboviruses. The present brief review compares the bionomics and disease relationships of two important arbovirus vectors, *Culex tarsalis* Coquillett and *Cx. tritaeniorhynchus* Giles, which have evolved to fill similar niches in comparable agro-ecosystems on several continents.

*Cx. tarsalis* and *Cx. tritaeniorhynchus* are members of the *sitiens* group of the subgenus *Culex* and are characterized by prominent pale bands on the proboscis and tarsi (Edwards 1932). *Cx. tarsalis* is considered a single species and is widely distributed through western North America including southwest Canada, the western United States and northern Mexico (Bohart and Washino 1978) (Fig. 1). East of the Mississippi River, *Cx. tarsalis* is rare and considered a winter species (Carpenter and LaCasse 1955, Jenkins 1950).

Historically, *Cx. tritaeniorhynchus* was subdivided into two geographically disjunct subspecies: *Cx. t. tritaeniorhynchus*, distributed from India west through southern Asia and the eastern Ethiopean region, and *Cx. t. summorosus*, distributed from central India east throughout the southern and maritime portions of the Far East including its type locality in the Philippine Archepelago (Knight and Stone 1977) and the Marianna Islands (Reisen et al. 1972) (Fig. 1). However, recent taxonomic studies have considered *Cx. tritaeniorhynchus* a single species with clinal variations in some anatomical characters (Bram 1967, Sirivanakarn 1976). Crosses between colonies from Japan and Pakistan have been fertile indicating genetic compatability (Baker and Sakai 1974).

Despite widely disjunct distributions, both species have evolved relatively similar temporal life patterns in response to comparable environmental conditions. In early autumn *Cx. tarsalis* female populations bifurcate into gonotrophically active and inactive components (e.g., Nelson 1964). In the northern and colder latitudes, *Cx. tarsalis* undergoes "complete" diapause as unfed, nulliparous and inseminated fe-

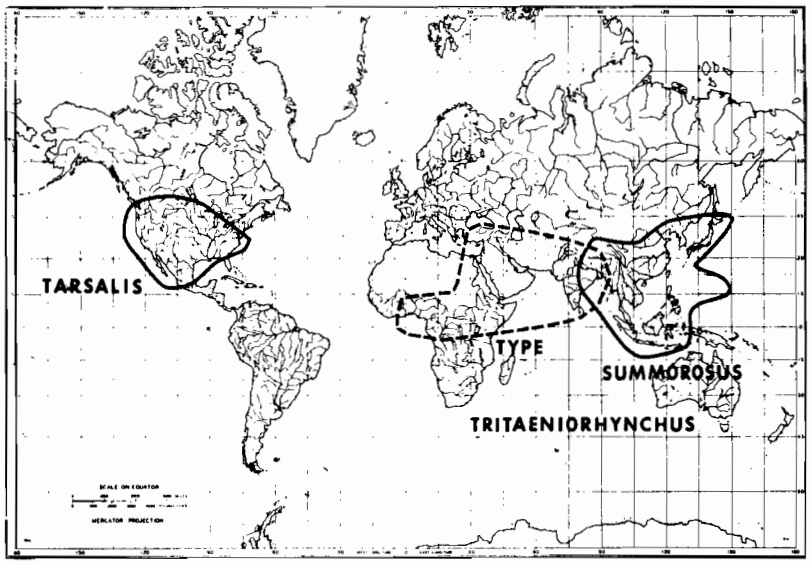


**Figure 1.** Geographic distributions of *Cx. tarsalis* and the type of *summorosus* subspecies of *Cx. tritaeniorhynchus*.

males in selected hibernacula (e.g., Mitchell 1979). In the warmer central valley of California, females exhibit facultative diapause which may be terminated by warm days and increasing photoperiod in later winter and early spring (e.g., Bellamy and Reeves 1963). In the warmer Imperial Valley, and presumeably Northern Mexico, females remain reproductively active and parous females, males and immatures can be collected throughout the year (Nelson 1971).

Similarly, female *Cx. tritaeniorhynchus* populations in temperate and subtropical areas bifurcate into gonotrophically active and inactive components in autumn (e.g., Fujimiya and Yajima 1976, Reisen et al. 1980). In the colder regions of Japan and Korea which are subjected to severe winters, the inactive component undergoes "complete" diapause (e.g., Omori et al. 1965, Jolivet et al. 1975). In Pakistan where the winters are comparatively milder, females enter facultative diapause (Reisen et al. 1977) and "complete" diapause is difficult to induce in endemic laboratory strains (Niaz and Reisen 1981). In more tropical latitudes females remain reproductively active throughout the year with population trends dictated by agricultural practices and/or annual rainfall patterns (Reisen et al. 1976).

During diapause both species seem to utilize comparable resting sites. *Cx. tarsalis* has been collected overwintering in mines, rockpiles and mammal burrows (Bohart and Washino 1978), while in Japan females may be readily collected with $CO_2$ from crevices in the rock, rice-paddy retaining walls in early spring (Wada et al. 1973). When undergoing facultative diapause, both species use more exposed resting sites; i.e., artificial resting boxes, bridges, culvert (Bellamy and Reeves 1963) and fodder crops (Reisen 1978).

With the onset of favorable conditions both species initiate reproductive activity and rapidly increase in abundance utilizing almost any form of standing ground water as breeding sites, including rice fields, irrigation channels and drains, temporary pools and hoof prints, permanent ponds, etc. (Bohart and Washino 1978, Reisen et al. 1981). Both species are tolerant of limited salinity, alkalinity and some eutrophication (Telford 1958, Jenkins 1950, Reisen et al. 1981a).

Larval development in *Cx. tarsalis* requires about 10 days with pupal development requiring an additional 2 days between 21° and 27°C (Rosay 1972, Bailey and Gieke 1968). *Cx. tritaeniorhynchus* mature faster and under similar temperature regimens in nature require 6 days for larval and 1 day for pupal development (Reisen and Siddiqui 1979). In both species males develop faster than females (Rosay 1972, Khan and Reisen 1977), but females mature sexually more rapidly than males (Reisen et al. 1979b). Female *Cx. tarsalis* are larger sized and oviposit more eggs per raft than *Cx. tritaeniorhynchus* (range of weekly means for field collected female *Cx. tarsalis*, wing length: 4.02 to 4.41 mm, raft size: 150 to 223 eggs/raft Bock and Milby 1981; *Cx. tritaeniorhynchus*, wing length: 2.5 to 3.2 mm, Reisen and Shahid, unpubl., mean raft size for 9 laboratory colonies: 112 to 181 eggs/raft, Reisen et al. 1979a). *Cx. tarsalis* populations characteristically exhibit autogeny which varies seasonally (Spadoni et al. 1974) and spatially (Hardy and Reeves 1972) in frequency of occurrence. Autogeny has yet to be reported for *Cx. tritaeniorhynchus*. Thus, *Cx. tarsalis* is larger and more fecund, but requires longer to complete immature development than *Cx. tritaeniorhynchus*, thereby slowing generation times and yielding comparable rates of increase. Both species are typical "r" strategists and able to rapidly expoit suitable habitats during peiods of favorable environmental conditions.

Both *Cx. tarsalis* and *Cx. tritaeniorhynchus* initiate flight and feeding activity in response to the exogenous cue of reducing illumination associated with dusk (e.g., Nelson and Spadoni 1972, Reisen and Aslamkhan 1978). The biting pattern is often bimodal with increased feeding activity at dusk and during early morning. The dawn increase in *Cx. tritaeniorhynchus* has been attributed to refeeding parous females which oviposited earlier in the same evening (Yajima 1974, Aslam et al. 1977).

Host selection patterns vary considerably both among and within species. As indicated by precipitin surveys, female *Cx. tarsalis* feed predominantly on birds with large domestic mammals and man being of lesser importance (Fig. 2). Host feeding patterns vary seasonally, however, with avian feeding replaced to some extent by mammals feeding during mid-summer (Tempelis et al. 1965).

Precipitin surveys of *Cx. tritaeniorhynchus* host feeding patterns have generally indicated that large domestic mammals are most frequently selected with man and avian hosts rarely utilized (Fig. 2). Results with bird-baited traps are mixed; e.g., Scherer et al. (1959) collected large numbers of females in heron-baited traps in Japan, while Reuben (1971) rarely captured *Cx. tritaeniorhynchus* in bird-baited traps in India. The mammalian hosts utilized vary geographically as a result of their relative availability which is essentially dictated by social customs. In eastern Asia where pork is eaten and piggeries are common, pig feeds predominate, while in western Islamic Asia and Africa where pork is not eaten, cattle and buffaloes become more important. The data of Pennington and Phelps (1968) indicate that if given a relatively equal choice, bovids seem to be more frequently selected. Throughout its distribution *Cx. tritaeniorhunchus* appears to rarely feed on either man or birds.

Mark-release-recapture studies have indicated that both species are highly dispersive. Marked *Cx. tarsalis* have been reported dispersing as far as 26 km downwind (Bailey et al. 1965). Similarly, *Cx. tritaeniorhynchus* have been collected 40 km from the nearest point of land in the South China Sea (Hayashi and Suzuki 1978) and 8 km downstream from a release point in the hilly areas near Nagasaki, Japan (Wada et al. 1969).

Throughout their respective distributions both species are important in the transmission of arboviruses of veterinary and public health significance (Fig. 1,3). *Cx. tarsalis* is considered to be the primary vector of Western Equine (WEE) and St. Louis (SLE) encephalitis viruses in western North America (Hammon et al. 1941, Reeves and Hammon 1962). Both SLE and WEE viruses persist in nature in passerine birds with *Cx. taraslis* functioning as the principle vector. Small and large mammals including man become involved but are essentially

dead-end hosts for the virus. *Cx. tarsalis* conforms well to the role as the implicated vector species, feeding principally on birds (the reservoir hosts) and to a lesser degree on mammals, shifting from avian to mammalian hosts seasonally and able to become readily infected and infective with both viruses at titres circulated by viremic birds.

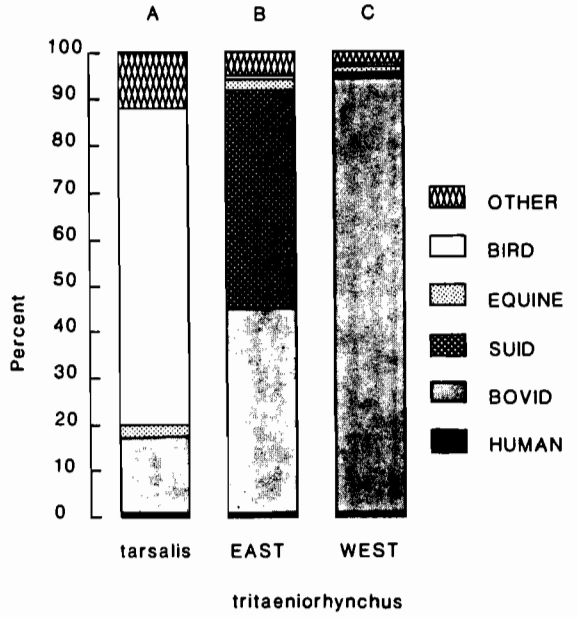


**Figure 2.** Host selection patterns of *Culex tarsalis* in North America (A) and *Culex tritaeniorhynchus* in eastern Asia (B) and western Asia and Africa (C). Data summarized from the following sources for A: Tempelis et al. 1976, 9765, Tempelis and Washino 1967, and Hayes et al. 1973. n = 16,987 females tested; B: Mitchell et al. 1973, Pennington and Phelps 1968, and Self et al. 1973, n = 21,441 females; and C: Christopher and Reuben 1971, Snow and Boreham 1978, and Reisen and Boreham 1979, n = 3,195 females.

*Cx. tritaeniorhynchus* is considered the primary vector of Japanese encephalitis virus (JEV) in Japan (Beuscher and Scherer 1959) and Korea (Self et al. 1973), but shares its vectorial role with *Cx. vishnui* (= *annulus*) , *Cx. gelidus, Cx. fuscocephala* and perhaps *Cx. pseudovishnui* in southeast Asia and India (e.g., Detels et al. 1976, Gould et al. 1973, Carey et al. 1968). JEV persists in nature in wild bird populations especially ciconiiforms, being transmitted to swine in rural piggeries where a secondary cycle is established by *Cx. triaeniorhynchus.* Pigs produce a viremia adequate to infect mosquitoes, but do not succumb to infection, although still-borne births and abortions occur. From pigs the virus spills over into equine and human populations which are considered dead end hosts for the virus (Beuscher and Scherer 1059). The geographic distribution of JEV approximates that of swine culture with its western most distribution terminating in India. West of India, JEV is replaced by another flavivirus, West Nile Virus (WNV), which has been isolated from pools of "*Cx. tritaeniorhynchus*" in Pakistan (Barnett 1967) and "*vishnui*" in India (Work 1971). Although *Cx. vishnui* occurrs commonly in India (Reuben 1969), it is rare in Punjab, Pakistan (Reisen 1978) suggesting that the reported WNV isolations were probably from *Cx. tritaeniorhynchus* in Pakistan. WNV, like antigenically similar SLE, is presumably maintained in passerine bird populations, spilling over into human and domestic mammal populations (e.g., Taylor et al. 1956). WNV antibodies are commonly recovered in bovids, but they never circulate viremias sufficient to infect mosquitoes. Further west, *vishnui* group mosquitoes are replaced by members of the *univittatus* subgroup as vectors (Taylor et al. 1956). The low frequency of avian feeds in precipitin surveys throughout its dis-

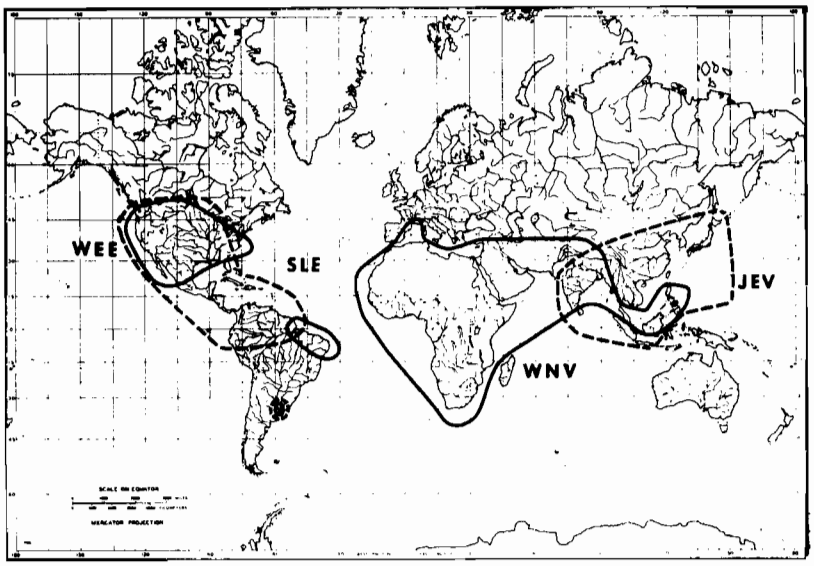


**Figure 3.** Geographic distributions of Western Equine Encephalitis (solid line) and St. Louis Encephalitis (dashed line) viruses in the New World and West Nile (solid line) and Japanese Encephalitis (dashed line) viruses in Africa and Asia.

tribution questions the role of *Cx. tritaeniorhynchus* in virus maintenance in nature among birds. Quite possibly one or more feral species which are more ornithophilic (e.g., *Cx. pseudovishnui* in India, Reuben 1971) may be important with *Cx. tritaeniorhynchus* becoming involved when the virus spills over into mammalian populations.

Due to their frequent exposure to agricultural pesticides as well as mosquito abatement efforts, multiple insecticide resistence has been reported for both *Cx. tarsalis* and *Cx. tritaeniorhynchus* (e.g., Georghiou et al. 1969, Wada 1973). The advent of resistence has prompted the initiation of trials to evaluate the feasibility of genetic control of both species. Releases of translocation-carrying males of both species have indicated that mating behavior is altered during colonization and/or maintenance, rendering altered males less competitive when released back into their parent populations (Asman et al. 1979, Baker et al. 1979, Milby et al. 1980, Reisen et al. 1980). To circumvent alterations in mating behavior, recent releases with *Cx. tarsalis* have emphasized the use of males emerging from field-collected pupae which can be readily sterilized by irradiation with little loss of competitiveness (Reisen et al. 1981b).

In summary, irrigated agro-ecosystems have lead to the proliferation of suitable habitats for both *Cx. tarsalis* and *Cx. tritaeniorhynchus* which have evolved similar life patterns and bionomics throughout their respective distributions. Both species fit well into the life cycles of several arboviruses and are important in transmitting the viruses to domestic animals and man. Problems with insecticide resistence have lead to the inception of comparable biological control studies for population suppression. Certainly, there is much information from both laboratory and field studies on both species which is readily interchangeable.

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# A REPORT ON THE PROGRESS OF *BACILLUS THURINGIENSIS ISRAELENSIS*[1]

# AND HIGHLIGHTS OF THE UC MOSQUITO RESEARCH PROGRAM

Russell E. Fontaine

I find it especially fitting that we have a panel on *Bacillus thuringiensis israelensis* here in California at this annual conference. Why? Because *Bti* is largely a product of UC research, starting with Leonard Goldberg, UC Naval Bioscience Laboratory, who brought the first isolate to California from Israel in 1977, and made it available for research and development in the University of California.

The other UC members of the panel Richard Garcia, UC Berkeley, Mir Mulla and Brian Federici, UC Riverside and Charles Schaefer, UC Berkeley, have made their own special contributions to the knowledge of this outstanding larvicide.

As you will learn from the panel, *Bti* is the most promising mosquito larvicide on the mosquito control scene for a very long time. There has been nothing quite like it in the past. The unique feature that sets it apart from all other larvicides is its selectivity for control of mosquito larvae and other filter feeding dipterous, aquatic larvae, like blackflies and chironomids. Other beneficial insects and natural enemies in the mosquito habitat are left unharmed and available to attack new mosquito generations. It is a larvicide that mosquito control operators have been looking for since mosquito control was conceived at the turn of the century. For years industry was urged to develop a selective product like it, but failed. We have it at last and EPA has approved it for registration.

Before I turn this discussion over to the panel, I would like to take a few minutes to review the progress of the University-Wide Mosquito Research Program, which has contributed so much to the development of *Bti.*

As many of you will recall, the program was organized by the University of California in 1972 under Legislative authority with an appropriation of $300,000. Let me stress that the program was not a unilateral effort of the University, but a bilateral development, urged and supported by the California Mosquito and Vector Control Association. The program emerged out of a crisis in mosquito control due to over-dependence on broad spectrum pesticides. As a result, mosquito control was undermined by widespread mosquito resistance, escalating cost of pesticides and EPA restrictions on insecticide usage. However, proven alternative controls were not readily available or well enough develped for practical use. Research had fallen behind on non-pesticide control strategies and had to be revised. It was on this premise that the University accepted the obligation to undertake an accelerated, intensified and expanded research effort.

[1]Introduction of panel on *Bacillus thuringiensis israelensis* by Russell E. Fontaine, Coordinator of UC Mosquito Research program. CMVCA Annual Conference, Redding, CA, April 27, 1981.

The research program was to include biological, chemical, physical, cultural and genetic control and also mosquito biology and ecology and public health. The UC Cooperative Extension Service was committed to work with the MAD's and growers to resolve mosquito problems associated with irrigated agriculture. It was also envisioned that MAD's would collaborate and participate with University researchers, especially in field studies. In this case, I can report that the University was richly rewarded by excellent cooperation.

After nearly ten years of research in the seventies, what can we show for the effort? On close examination of the program, it is evident that much of the effort has been expended in necessary preparatory and developmental activites required to carry the research forward to a stage of field and operational use in the eighties. However, some significant results can be reported:

(1) The use of broad spectrum synthetic pesticides has been greatly reduced and now stands at only a fourth of the level used 10 years ago. Economic factors, together with improved larvicides, selective application techniques, and biological control, instigated by California MAD's have all contributed to the reduction.

(2) Integrated mosquito control is now an accepted management practice, limited in scope only by the availability of new methods and techniques.

(3) The major mosquito-borne diseases, such as encephalitis and malaria, have been effectively suppressed, despite sources of infection within the state, and despite continuing outbreaks of encephalitis outside the state.

Much of the credit of these successes is due to the initiative and progressive attitude of California mosquito abatement districts. They have not passively waited for University research to provide new controls, but have actively participated with University researchers in field testing and evaluation of promising materials and methods. Also, they have introduced preliminary research findings in their operations whenever possible. This research cooperation has reinforced and stimulated the University program.

It was Einstein who stated, "I never look into the future. It will come soon enough!" However, for purposes of the UC research program, we must ask ourselves, "What can we foresee as affecting the research program in the eighties?"

One thing we can be sure about: because of the high population growth rate in California, we can expect a continuing expansion of irrigated agriculture, industry and urbanization. This is certain to impact on mosquitoes, and I would predict as follows:

- -We should see more mosquito problems coming from the reclamation and reuse of waste water for agriculture, for in-

dustry, for recreation, and for wildlife enhancement. New sources of water are getting scarce and waste water will become more valuable and necessary with time.

- -Irrigated agriculture will continue to expand because the demand for California products will increase, limited only by the availability of water, so agricultural mosquito problems will continue. Perhaps the concern for water conservation will improve water management and help keep the problem from getting out of hand. Farm advisors working with MAD's and growers can contribute a great deal to solving water management problems.

- -If we have adequate funds in the 1980's, some important new controls for use in mosquito programs should emerge. We now have *Bti* and it's a good one- -no mistake about it, and there are many new strains being isolated that are better than the original product. Another bacterium, *B. sphaericus*, shows promise to augment *Bti* in certain mosquito breeding situations and has the added advantage of recycling.

- -Pesticides will continue to play an important role but as an adjunct to other controls in an integrated mosquito management program. The period of broad spectrum, synthetic insecticides used on a mass coverage monocontrol basis as in the 1950's and 1960's appears to have ended except in emergencies.

- -There are some promising IGR's now, as Mir Mulla and Charlie Schaefer can tell you, but registration is an obstacle.

- -The outlook for some improvement in *Gambusia affinis* mass rearing technology is promising. This is sorely needed by MAD's. If research on biology and ecology of mosquitoes and their interrelationship and interaction with predators, parasites, and pathogens is energetically pursued, biological control should be greatly strengthened in the 1980's.

- -Progress in physical and cultural control seems less optimistic than other controls because many of the things that need doing are not researchable. We know what needs to be done. The problem is getting it done. This will require MAD managers, farm advisors and growers working together to resolve many of the agriculturally associated mosquito problems.

The University mosquito research program will continue to stress collaboration and participation of MAD's and other agencies, foundations, industry and international institutions. The World Health Organization, in particular, has been an active international collaborator in biological control research.

It is our intention to continue the program along the same multi-disciplinary cooperative lines. This teamwork approach should help us achieve significant results in this decade.

Teamwork is a good note on which to end these introductory remarks. We have an important segment of the UC team on this panel today with some concrete evidence of research progress to report on. There is no research group anywhere who could provide as comprehensive and updated a story on *Bti* as you will hear today from Drs. Federici, Garcia, Mulla, and Schaefer. Unfortunately Dr. Goldberg was unable to attend.

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# THE DEVELOPMENT OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AND ITS SITE OF ACTION IN MOSQUITO LARVAE

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INTRODUCTION.–In my presentation today I will briefly review the development of the bacterium, *Bacillus thuringiensis* variety *israelensis* (H-14), also known as *Bti*, as a mosquito larvicide and discuss how we think it kills larvae, and why it is so selective in its range of activity against aquatic invertebrates, in contrast to most chemical insecticides. But before I begin, I would like to clarify some of the often confusing terminology used in reference to *Bti*. I suppose most commonly one hears *Bti* referred to as a biological control agent, and in the broadest sense, particularly when compared to chemical insecticides, it is. However, referring to *Bti* as a biological control agent has its drawbacks and is somewhat misleading in that many people have an erroneous concept of biological control agents, believing that they are inexpensive, easy to manipulate, and once introduced into an area give long term, if not permanent, control of target pests. Such biological agents do exist and are known among specialists in the field of biological control as "classical" biological control agents. In most cases, be they viruses, parasites or predators, these are organisms introduced to an area to control an introduced pest. The introduced biological control agent reestablishes a natural balance holding populations of the introduced pest below economic thresholds. California has been a pioneer in the development of classical biological control and numerous introduced agents, such as the tiny parasitic wasps which attack aphids and scale insects, save the state millions of dollars a year in insecticide costs.

In additon to classical biological control agents, there are many other types of biological control agents that give effective and specific control but differ from the classical type in that they must be periodically reintroduced to keep target organisms under control. Mosquito fish and parasitic wasps of the genus *Trichogramma* are examples of such agents.

Biological insecticide, microbial control agent, microbial insecticide, and bacterial insecticide are examples of other terms used to describe *Bti*, and the use of all of these as descriptors can be justified. Of these, I prefer the latter two terms because they most accurately infer the properties of the material. *Bti*, as we shall see as this symposium progresses, is a bacterium with insecticidal properties. However, it has little residual activity and, therefore, must be reapplied periodically, though apparently not as often as most chemical insecticides. Thus, it is not a classical biological control agent. Yet, one of its primary attributes is its high degree of selectivity, a property it shares with other biological agents. This selectivity and the fact that the active ingredient of *Bti* preparations is actually a complex proteinaceous toxin produced by the bacterium, qualify *Bti* as a biological control agent in the broadest sense, but more succinctly as a microbial or bacterial insecticide. It does not perpetuate itself in the environment at levels which yield effective mosquito control and must be reapplied like a chemical insecticide.

DISCOVERY AND DEVELOPMENT.–Historically, the search for a microbial insecticide such as *Bti* for use against mosquito larvae has been in progress for more than 25 years. The impetus for this search came from the discovery and development of strains of the bacterium *Bacillus thuringiensis* for control of larvae of such lepidopterous pests as the cabbage looper, *Trichoplusia ni*, and the corn ear worm, *Heliothis zea*. The strains developed for these pests are specific, efficacious and commercially successful, being sold under the trade names of Dipel andThuricide in the United States. Industrial estimates indicate over two million pounds of these products are sold in the U.S. annually. One of the primary reasons *B. thuringiensis* was developed successfully is that as a bacterium it was relatively easy to mass produce on artificial media and had excellent storage properties, in contrast to many other types of microbial or biological control agents which often require production in *in vivo* systems or have poor storage properties. Initial efforts to develop a bacterium for mosquito control focused on evaluation of existing strains and new isolates of *B. thuringiensis* against larvae of various mosquito species. Although many isolates possessed larvicidal activity, the rates required to obtain acceptable levels of mosquito control were too high to justify commercial development. While these evaluations were in progress isolates of another spore-forming bacterium, *B. sphaericus*, were also found to possess larvicidal activity, particularly against *Culex* species. For a time, research emphasized evaluation and development of several isolates of *B. sphaericus*, but interest in this bacterium subsided due to difficulties encountered in accurately identifying the larvicidal toxin and in preparing stable commercial formulations. During the 1970's efforts continued on the isolation and evaluation of strains of both *B. thuringiensis* and *B. sphaericus*, and I should point out, continue today.

However, a significant breakthrough occurred in 1976 with the isolation of a new serotype (H-14) of *B. thuringiensis*, designated variety *israelensis*, from a polluted pond, in the Negev desert of Israel by Dr. L. Goldberg and J. Margalit. Preliminary evaluations by these investigators, subsequently confirmed by other laboratories, demonstrated *Bti* was from 10 to 100 times as effective as previously evaluated isolates,

making it suitable for commercial development. Since these initial evaluations of *Bti*, progress on its development as a mosquito larvicide has been very rapid, and as you will see in the presentations today by Drs. Mulla and Schaefer, several commercial formulations of this bacterium are effective against important species of California mosquitoes at rates varying from .25 to 2 lbs/acre, depending on the mosquito species, formulation and habitat. We expect these formulations to be registered by the Environmental Protection Agency within a few months and to be available later this year.

MODE OF ACTION.–Like other insecticidal strains of *B. thuringiensis*, *Bti* produces a paracrystalline proteinaceous body during the process of sporulation which is toxic to insects, particularly to dipterans of the suborder Nematocera. This proteinaceous toxin, located outside the spore, is referred to technically as a delta-endotoxin and acts primarily as a stomach poison. However, as in the case of other strains of *B. thuringiensis*, its precise mode of action remains unknown. What is known is that when mosquito larvae ingest sporulated cells of *Bti* in sufficient quantities, the stomach, also known as the midgut epithelium, degenerates and is sloughed off. During this process or soon after, the larvae die. In the laboratory, early fourth-instars of *Culex tarsalis* or *Aedes aegypti* placed in water containing *Bti* at a concentration of .2 ppm will die in approximately 2 to 4 hours. Higher concentrations can bring death about in as little as 15 minutes. Thus, the toxin can act rapidly. If one examines sections of the midgut epithelium microscopically, it can be seen that the toxin acts principally on cells in the gastric caeca and posterior portion of the stomach, with cells in the anterior region of the stomach showing comparatively little degeneration. One of the functions of cells in the susceptible regions of the midgut is the absorption of sugars and starches and this may provide a clue to the mode of action of *Bti* in that the toxin is thought by several investigators to be a glycoprotein. Some believe the toxin interferes with cell permeability by binding to sites on the surface of microvilli, whereas others believe the principal site of action is within the cell, the toxin interfering with some important aspect of intermediary metabolism. There is evidence for and against each theory, but much work remains to be done before either theory is proved.

Regardless of the mode of action, what one observes in the most susceptible regions of the midgut several hours after larvae are fed *Bti* at a concentration of .2 ppm is a loss of microvillar integrity, vacuolation and hypertrophy of the cells, lateral distension of the gut, and eventually a loosening and sloughing of the cells from the basement membrane. Afflicted larvae become lethargic, floating with little activity near the water surface, cease feeding, and subsequently die. Death may be due to the larva's inability to regulate the flow of toxic substances and ions across the basement membrane, from the midgut lumen to the hemocoel.

COMPONENTS OF THE SPECIFICITY OF *BTI*.–One of the most interesting, important, and useful properties of *Bti* is its high degree of specificity for larvae of insects of the suborder Nematocera. One may well ask why *Bti* is so specific. There appear to be several reasons. First, as a stomach poison *Bti* must be eaten. Application of *Bti* formulations results in a suspension of sporulated cells on the surface, or in the water. As filter feeders that rapidly remove particulates from water, mosquito larvae will consume suspended *Bti* cells. However, most other organisms that occur in habitats with mosquitoes are not filter feeders, e.g., fish, odonata, ephemeroptera, coleoptera, crustacea, etc., and therefore will consume comparatively little toxin. Once within the gut, alkaline conditions promote dissolution of the toxin. Such conditions exist in the mosquito gut, and although they may also occur in the guts of mosquito cohorts, the small amounts of toxin ingested are either not enough to significantly affect these organisms, or they are not susceptible to the toxin.

Once dissolved, it is believed the toxin is "activated," i.e., partially digested by gut enzymes. Subsequently, the toxin binds to or is absorbed selectively by cells in specific regions of the gut. Another level of specificity enters here in that mosquito larvae are inherently more susceptible to the toxin from *Bti* than to toxins from other strains of *B. thuringiensis*. Thus, at the biochemical level, the toxin of *Bti* has unique properties which make it particularly toxic to mosquito gut cells. I should note here that other nematocerans, for example larvae of blackflies and chironomids, are also susceptible to *Bti*.

In summary, the specificity of *Bti* is due to a combination of factors including larval feeding behavior and unique physiological and biochemical properties of the larval midgut and *Bti* delta-endotoxin.

THE FUTURE FOR *BTI*.–Just how efficacious and useful *Bti* is likely to be will be determined over the next few years once commercial formulations become available for use by mosquito abatement districts and other agencies such as the World Health Organization. WHO has already scheduled large-scale field trials of *Bti* for use in the onchocerciasis control program in west Africa where it will be used against *Simulium* larvae. Operational usage of commercial formulations will determine the species and habitats against which *Bti* can be used effectively and appropriate methods and rates of application. It will be important not to overuse *Bti*, for as with many toxins, the potential for the development of resistance is always lurking in the background. *Bti*, having a different mode of action than chemical insecticides, should prove particularly useful in combating mosquitoes resistant to organophosphates and chlorinated hydrocarbons. Similarly, it is an ideal material for use in insecticide rotation programs to reduce the level of resistant genes in populations as suggested by Dr. Georghiou. *Bti* should also prove of great benefit to the development of integrated mosquito control programs.

In regard to the development of other bacterial insecticides, the discovery of *Bti* provides impetus for the search for even more effective isolates. Additionally, it provides a basis, through comparative studies, for understanding the mode of action of delta-endotoxins, which could lead to the development of new types of insecticides. The variations in activity found among delta-endotoxins for larvae of different insect

orders suggests substitutions in amino acid sequences within these toxins are responsible for differing degrees of activity. Modern biochemical technology places at hand the techniques for identifying the toxic moeity of different delta-endotoxins, thereby providing basic polypeptide sequences which perhaps can be improved upon yielding even more effective toxins. The use of recombinant DNA technology to produce such molecules would open new avenues to insect control characterized by the use of highly specific insecticides.

However, even without these developments the advent of *Bti* opens a new era in mosqutio control. It is a larvicide which acts rapidly, is highly toxic to many species of mosquitoes and has little or no effect on non-target organisms, is relatively easy to produce and has good storage properties. Its discovery is important and should greatly aid us in our efforts to control vector and annoyance mosquitoes.

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## INTEGRATION OF A SELECTIVE MOSQUITO CONTROL AGENT *BACILLUS THURINGIENSIS* SEROTYPE H. 14, WITH NATURAL PREDATOR POPULATIONS IN PESTICIDE - SENSITIVE HABITATS

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ABSTRACT

The toxin of *Bacillus thuringiensis* serotype H.14 (*B.t.* H. 14) was applied as a mosquito control agent to three pesticide-sensitive habitats. Numbers of *Culex tarsalis* immatures were reduced by 97 and 100% at dosage rates of 2.0 and 1.0 kg/ha in ponds used to breed golden shiners, *Notemigonus crysoleucas* (Mitchill); no detrimental effect to the bait fish, was observed. Complete control of *Cx. tarsalis* at a wildlife area was obtained with *B.t.* H.14 at 0.8 kg/ha. Predation by naturally-occurring aquatic beetle larvae extended the control of *Cx. tarsalis* through 22 days after treatment. *B.t.* H.14 was innocuous to the selected non-target fauna, except for an apparent 40-75% reduction in numbers of chironomid larvae. Application of *B.t.* H.14 at 1.1 kg/ha reduced *Cx. tarsalis* numbers by 93% at a duck club without affecting predacious beetle larvae. Treatment with parathion 7 days after the *B.t.* H.14 application severely reduced the numbers of the beetle larvae.

While the protein of *Bacillus thuringiensis* serotype H.14 (*B.t.* H.14) has demonstrated efficacy in control of mosquitoes and blackflies in the field (Mulligan et al. 1980 and Undeen and Colbo 1980), the effect upon associated nontarget organisms has been minimal (Miura et al. 1980 and Colbo and Undeen 1980). Also in our previous work we noted a lack of resurgence in larval mosquito numbers coupled with the increase of natural predators. Because of these characteristics, the integration of *B.t.* H. 14 with natural mosquito predators is most attractive as a control strategy, especially in pesticide-sensitive habitats. Wildlife management areas represent one such habitat. Wildlife officials are concerned not only with the toxic impact of mosquito control agents upon vertebrates but also with the quantity and diversity of invertebrates which are important in the food chain. The impacts of *B.t.* H.14 applications to three types of sensitive habitats were studied and are reported here.

MATERIALS AND METHODS.–Potencies of the three *B.t.* H.14 formulations were assayed in the laboratory with *Aedes aegypti* (L.) larvae against a standard provided by the Pasteur Institute (IPS-78 standard). These potencies are given in mean International Toxic Units (ITU/mg± S. D. At least 3 assays were combined for each mean.

A *B.t.* H. 14 formulation of 692±69 ITU/mg was applied by hand can sprayer in 9.5 liters of water to 0.04 ha ponds in Woodlake, Tulare County, CA on May 28, 1980. Dosage rates of 1.0 and 2.0 kg/ha were sprayed to a 3m perimeter band with a control pond left untreated. Composite 40 dip (300 ml water in a long handle, 450 ml enamel dipper) samples were collected daily from each pond. The samples were concentrated in alcohol in the field and immature mosquitoes, *Culex tarsalis* Coquillett, were counted in the laboratory.

An application by fixed wing aircraft to an 11 ha flooded wetlands field in the Mendota Wildlife Area (CA Dept. of Fish and Game) was made on July 16, 1980. Formulated *B.t.* H.14, assayed at 552±22 ITU/mg, was applied at 0.8 kg/ha. Surface dwelling organisms were collected with a dipper. Ten dips (3 liters of surface water) were concentrated on a 34 mesh/cm screen and placed in 95% alcohol for each of 5 daily samples. Benthic organisms were collected by means of a $0.1m^2$, plexiglass area sampler. Three benthic samples were collected daily and placed in alcohol.

On September 18, 1980 a 3 ha pond of a Kern County, CA duck club was sprayed by fixed wing aircraft with *B.t.* H.14, assayed at 576±20 ITU/mg. The dosage rate was 1.1 kg/ha. Two samples of 15 dips (4.5 liters of surface water), concentrated and placed in alcohol, were collected daily.

Organisms in the concentrated surface water samples were counted under a stereo microscope in the laboratory. Benthic samples were processed prior to counting under a microscope. These samples were separated with no. 4, 8, 10, 24 and 32 mesh Tyler sieves and washed. Organisms were separated from heavier debris by flotation in saturated salt soln.

RESULTS.–The Woodlake ponds were commercially operated for breeding and rearing of a bait fish, the golden shiner, *Notemigonus crysoleucas* (Mitchill). All sizes of the fish were present. No detrimental effects to the fish were observed through 7 days after treatment. Immature *Cx. tarsalis* were reduced by 97% at the 2 kg/ha rate, while complete control was obtained at 1 kg/ha. An increase in numbers of mosquito immatures was noted in the control pond.

Sampling of the Mendota wetlands field commenced 5 days prior to treatment and continued intermittently through 22 days after treatment. A variety of aquatic invertebrates were identified and quantified: Cladocera, *Simocephalus* sp.; Podocopa, *Cypris* sp.; Eucopepoda, *Cyclops* sp., Ephemeroptera, *Callibaetis* sp.; Zygoptera, *Enallagma* sp.; Anisoptera, *Aeshna occidentalis* Walker; Hemiptera, *Belostoma flumineum* Say, *Corisella decolor* (Uhler), *C. inscripta* (Uhler), *Notonecta unifasciata* Guerin; Coleoptera, 2 *Hygrotus* spp., *Laccophilus mexicanus mexicanus* Aube, *L. m. atristernalis* Crotch, *Thermonectus* sp., *Troposternus lateralis* (F.), *Enochrus diffusus* (LeConte), *Berosus* sp.; Chironomidae, *Chironomus stigmaterus* Say, *Goeldichiromus holoprasinus* (Goeldi), *Cricotopus* sp., *Paralauterborniella* sp., *Protanypus* sp., *Tanytarsus* sp. and *Cx. tarsalis*. Results are summarized in Table 1.

**Table 1.** Aquatic invertebrate populations of a Mendota Wildlife Area wetlands field prior to and after treatment with a mosquito control agent *Bacillus thuringiensis* serotype H. 14 (552 ITU/mg), at 0.8 kg/ha.

| | Days Post-treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | -5 | -2 | -1 | 1 | 2 | 3 | 7 | 12 | 22 |
| | (Mean no. organisms per 3 liters)[a] | | | | | | | | |
| Crustacea | | | | | | | | | |
| Cladocera | 71 | 325 | 315 | 193 | 156 | 357 | 144 | 116 | 472 |
| Eucopepoda | 145 | 342 | 83 | 114 | 112 | 200 | 154 | 70 | 72 |
| Podocopa | 246 | 38 | 17 | 73 | 145 | 122 | 65 | 11 | 254 |
| Insecta immatures[b] | | | | | | | | | |
| Ephemeroptera | | | | | | | | | |
| *Callibaetis* sp. | 111 | 374 | 236 | 270 | 241 | 349 | 179 | 126 | 239 |
| Zygoptera | 0 | 0 | 0.4 | 0.2 | 0 | 0 | 1.2 | 0.8 | 5.8 |
| Anisoptera | 0 | 0 | 0 | 0.2 | 0 | 0.2 | 0 | 0 | 3.8 |
| Hemiptera | | | | | | | | | |
| *Belostoma* sp. | 0 | 0 | 0.2 | 0.2 | 0 | 0.2 | 0.6 | 1.6 | 1.4 |
| *Corisella* spp. | 0.4 | 0.3 | 0.2 | 2.0 | 4.8 | 6.0 | 2.2 | 0.2 | 1.0 |
| *Notonecta* sp. | 0.2 | 0 | 0 | 0 | 0 | 0.2 | 0 | 0.2 | 1.0 |
| Coleoptera | | | | | | | | | |
| Dytiscidae | | | | | | | | | |
| *Hygrotus* spp. | 0.2 | 5.2 | 5.2 | 5.4 | 6.0 | 6.4 | 12.6 | 2.0 | 3.0 |
| *Laccophilus* spp. | 2.0 | 21.6 | 9.4 | 25.4 | 31.0 | 44.6 | 35.4 | 11.6 | 28.0 |
| *Thermonectus* sp. | 3.4 | 4.6 | 3.8 | 9.8 | 7.2 | 10.4 | 8.6 | 12.0 | 6.4 |
| Hydrophilidae | | | | | | | | | |
| *Tropisternus* sp. | 0 | 0.8 | 0.8 | 2.4 | 2.4 | 2.2 | 0.6 | 3.2 | 1.6 |
| *Enochrus* sp. | 1.6 | 12.6 | 15.2 | 11.0 | 12.2 | 9.6 | 17.8 | 19.8 | 4.0 |
| Mosquito | | | | | | | | | |
| instars 1, 2, | 5.8 | 2.2 | 0.6 | 0.4 | 1.6 | 0.6 | 0.2 | 0 | 0 |
| 3, 4, pupa | 2.0 | 1.4 | 0.8 | 0.2 | 0 | 0 | 0 | 0 | 0.2 |
| | (Mean no. organisms per $0.1m^2$)[c] | | | | | | | | |
| *Notonecta* sp. | | 0 | 0 | 0.7 | | 0.7 | 0.3 | 8.7 | |
| Hydrophilidae | | | | | | | | | |
| *Berosus* sp. | | 2.0 | 2.3 | 4.5 | | 2.3 | 12.0 | 26.7 | |
| Diptera | | | | | | | | | |
| Chironomidae | | 147 | 262 | 637 | | 328 | 165 | 75 | |

[a]Means derived from 5 samples of ten 300 ml dips (enamel dipper) of surface water.

[b]All insects listed were immature stages, except for *Corisella* spp. and *Notonecta* sp. which were both adults and immatures.

[c]Means derived from 3 samples taken with an $0.1m^2$ area sampler.

No deleterious effect was apparent upon the crustacean populations nor upon the mayflies (*Callibaetis* sp.). Mean numbers of these organisms fluctuated within stable ranges throughout the sampling period. Predacious insect species increased in numbers during the study. Of the predator species, the coleopteran larvae were most abundant and their increases were apparent much earlier than for hemimetabolous species (*Enallagma* sp., *A. occidentalis*, *B. flumineum* and *N. unifasciata)*. The dytiscid *Laccophilus* spp. were the most numerous planktonic insects other than *Callibaetis* sp.

There was a sharp decline in numbers of mosquito immatures *(Cx. tarsalis)* which commenced prior to the application of *B. t.* H. 14. Older stage immatures (instars 3 and 4 and pupa) disappeared after treatment, with the exception of 1 pupa collected at 1 day and I larvae at 22 days post-treatment. Young immatures (instars 1 and 2) continued to be collected through 7 days after treatment. These early instars resulted from continuing oviposition by adults in the area, however they did not mature. Chironomid larvae, from benthic samples, showed a peak in numbers 1 day after treatment with a gradual decline thereafter.

The following insect species were monitored during the Kern County duck club test: Anisoptera, *Aeschna* sp., *Pseudoleon superbus* (Hagen); Hemiptera, *N. unifasciata;* Coleoptera, *L. m. atristernalis, L. m. mexicanus, Thermonectus basillaris* (Harris), *Tropisternus* sp.; and *Cx. tarsalis*. Daily mean num-

bers for each species are given in Table 2. The *N. unifasciata* were adults, all other species were immatures. All of the predacious species showed increase or stability in numbers through 4 days after treatment. The most dramatic increase was in *Laccophilus* spp. larvae. The target organisms, *Cx.tarsalis*, showed a 93% decrease in numbers 24 h after treatment. Samples collected 7 days after treatment contained lower numbers of all organisms, except for anisopterans; the test area had inadvertently been treated earlier that same day with ethyl parathion at 0.1 kg/ha A. I. for mosquito control.

**Table 2.** Mean no. organisms per 4.5 liters of surface water from a Kern Co. duck club treated with 1.0 kg/ha *Bacillus thuringiensis* H. 14 (576 ± 20 ITU/mg) by fixed wing aircraft September 18, 1980.

| | Days after treatment | | | |
|---|---|---|---|---|
| | Pre | 1 | 4 | 7[a] |
| Anisoptera | 0.5 | 1.0 | 3.0 | 5.5 |
| *Notonecta* sp. | 1.0 | 0.5 | 1.0 | 0 |
| *Laccophilus* spp. | 0.5 | 2.5 | 15.0 | 2.5 |
| *Thermonectus* sp. | 1.0 | 1.5 | 1.0 | 0.5 |
| *Tropisternus* sp. | 1.5 | 1.5 | 2.0 | 0.5 |
| *Culex tarsalis* | | | | |
| instars 1, 2 | 99.0 | 7.5 | 22.5 | 2.5 |
| instars 3, 4 | 240.0 | 15.5 | 15.0 | 3.0 |

[a]Field treated with parathion on same day and previous to sampling.

DISCUSSION.–The decline in the numbers of mosquito immatures at Mendota was correlated to an increase in the numbers and diversity of dytiscid and hydrophilid larvae. Many species of these two predacious coleoterans have been shown to be efficient predators of mosquito larvae (James 1964, Roberts et al. 1967 and Nelson 1977). Jenkins (1964) provides an annotated list of mosquito predators, including coleopterans. In particular, species of the following genera were predacious upon mosquito larvae: *Laccophilus* (James 1964 and Roberts et al. 1967), *Hygrotus* (Jenkins 1964), *Thermonectus* (Nielsen and Nielsen 1953), *Tropisternus* (Nielsen and Nielsen 1953 and Zalom and Grigarick 1980) and *Berosus* (Nielsen and Nielsen 1953). With the exception of *Enochrus diffusus* all of the beetle species collected at Mendota were members of the preceding genera. Because the mosquito decline commenced prior to the application of *B. t.* H. 14, is is apparent that the decline was in response to predatory pressure exerted by the increasing numbers of predacious beetle larvae.

Application of the toxin effected complete control of the remaining mosquitoes, but reinfestation continued. Early instar mosquitoes did not survive to older instars, but were suppressed initially by predation from beetle larvae and later by the combination of beetle larvae and hemimetabolous predators. The predator populations were not affected by the treatment.

Except for an apparent effect upon chironomid larvae, *B.t.* H.14 was innocuous to the selected nontarget fauna at Mendota. The chironomid population at Mendota decreased after application of *B.t.* H.14. Because of the population fluctuation our data was inconclusive as to the extent of the impact upon the chironomids, although there appeared to be a 40-75% reduction. Garcia et al. (1980) found chironomids to be affected by *B.t.* H.14 but less susceptible than mosquitoes (*Cx.pipiens* L.).

There is concern among wildlife management personnel about impact upon chironomid populations. Chironomids constitute a significant portion of the diet of migratory waterfowl on early spring. In the central valley of California chironomids comprise 59% of the early spring diet of the pintail *Anas acuta* (unpublished report John Beam, CA Dept. Fish and Game 1980). Since migratory waterfowl generally depart proir to the start of the abatement season, a primary concern is depletion of the adult chironomid populations caused by continuous summer applications of mosquito control agents

Predators at the Kern duck club, though less diverse and lower in numbers than at Mendota, were likewise unaffected by the *B.t.* H.14 application and their populations increased with time. Although there was no discernible effect of predation upon the mosquito population, the potential for enchanced predatory pressure was increasing with the immature beetle numbers. Application of parathion severely impacted the larval beetle populations, most noticeably the *Laccophilus* spp. Dragonflies were apparently unaffected by the parathion treatment. A second application of *B.t.* H. 14 instead of parathion would have been more appropriate for utilization of the natural predators.

It appears that aquatic beetle larvae play a major role in natural mortality of mosquito immatures. With integration of a compatible mosquito control agent, such as *B.t.* H.14 toxin, it is possible to obtain extended control in habitats where such predators are prevalent. Also the selectivity of this toxin allows use in other pesticide-sensitive areas where more potent, non-selective toxicants would pose problems, e. g. commercial fish breeding ponds.

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# A NEW MICROBIAL INSECTICIDE FOR THE CONTROL OF STAGNANT AND FLOODWATER MOSQUITOES

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During the past 3 years, a good deal of laboratory and field studies have been conducted on the efficacy of the microbial insecticide *Bacillus thuringiensis* var. *israelensis* Serotype H-14, against mosquitoes. In general, this microbial insecticide has been found to be quite effective against culicine mosquitoes under laboratory conditions, while anopheline mosquitoes were found to be less susceptible.

To determine the activity spectrum of commercial formulations of BT (H-14) against important species of mosquitoes, we initiated extensive laboratory and field studies against stagnant and floodwater mosquitoes. All formulations of BT (H-14) were evaluated against 2nd and 4th instars of mosquitoes obtained from laboratory colonies. They were also evaluated against larvae of *Psorophora columbiae* and *Aedes nigromaculis* larvae collected in irrigated pastures. Standard bioassay procedures were used, where $LC_{50}$ and $LC_{90}$ values were determined from a dosage response line established on a probit log paper.

Field studies were conducted in a variety of biotopes characterized with no pollution to high level of organic wastes content. In the experimental ponds at Riverside, where *Culex tarsalis* prevailed, the water was clear, the ponds without vegetative growth and the water pH was 8.2. In the Coachella Valley ponds, *Cx. tarsalis* and *Culiseta inornata* were the dominant species. There the water was clear, the ponds with grass cover, and the water pH 9.5.

Both ground and aerial application of commercial formulations of BT (H-14) were evaluated against *Psorophora columbiae* and *Ae. nigromaculis* in irrigated pastures in the Palo Verde Valley and the southern San Joaquin Valley. The spray was applied at the rate of 8 gal/A in ground and 304 gal/A by the aerial method. Assessment of control was made before and 24 hrs. after treatment.

The preparations or formulations employed in these studies, with their source and potency are listed below:

| Material | Source | International Toxic Units |
|---|---|---|
| IPS-78 | Institute Pasteur | 1000 |
| R-153-78 | Roger Bellon | 3000 |
| Bactimos | Biochem | 3000-6000 |
| ABG-6108 | Abbott Laboratores | 400 |
| Sandoz 402-WDC | Sandoz, Inc. | 600 |

These comprehensive studies were carried out in cooperation with the Coachella Valley, Northwest and Tulare Mosquito Abatement Districts. A brief discussion of the results obtained under both laboratory and field conditions is as follows:

STAGNANT-WATER MOSQUITOES. The above five experimental and commercial formulations were evaluated against mosquito larvae under laboratory and field conditions. In the laboratory, the formulations were bioassayed against larvae of 8 species of mosquitoes, the test larvae obtained from laboratory colonies or from field populations when colony material was not available. In the laboratory, the culicine and aedine species essentially showed similar levels of susceptibility to a given formulation. The range of activity of various formulations against a given species was in the order of 2- to 6-fold. *Anopheles* larvae were inherently more tolerant than culicines by requiring 5- to 10-times larger quantities of the formulations for effective kill. In general, the $LC_{90}$ against 4th instars ranged from 0.13 to 0.45 ppm for the formulations having high potency (IU), to 0.45 to 0.9 ppm for the formulations having low potency (IU). Second instars were 2-8 times more susceptible to the various formulations compared to the 4th instars.

In the field, the various formulations showed different levels of activity, confirming the trend shown under laboratory conditions. In clear, shallow and nonvegetated water, the most effective formulations yielded excellent control of *Culex tarsalis* Coquillett and *Culiseta inornata* (Williston) (Table 1) larvae at rates as low as 112 to 280 g/ha. Formulations with low potency were needed at rates as high as 280-560 g/ha to yield effective control of larvae in these biotopes. In clear, shallow water with light vegetation but still open surface, slightly higher rates were necessary for effective control.

The level of organic pollution, larval density, and depth of water in dairy waste water lagoons drastically reduced the efficacy of BT (H-14) formulations. In these biotopes rates as high as 0.56 to 4.5 g/ha or higher of the various formulations were needed to produce satisfactory control of *Cx. quinquefasciatus* Say and *Cx. peus* Speiser. This increase in the rate of application as expected was partly dictated by the depth of water. Polluted and deep bodies of water, therefore, require considerably higher rates of application which might be prohibitive costwise in area-wide control programs of mosquitoes in these biotopes.

During the course of studies on the efficacy of BT (-14) formulations against mosquito larvae, observations on the impact of the various formulations on dominant macroinvertebrates were also made. None of the commercial formula-

**Table 1.** Evaluation of various formulations of *B. thuringiensis* (Serotype H-14) against *Cx. inornata* in experimental ponds.[a] (Coachella Valley Aquatic Research and Vector Control Facility)

| | Avg. no. 3-4 instar larvae/5 dips pre- and post-treat. (days) and % reduction (R) | | | | |
|---|---|---|---|---|---|
| | | 2 | | 7 | |
| Rate (g/ha) | Pretreat | No. | (%R) | No. | (%R) |
| | Bactimos/Batch 676 | | | | |
| 112 | 20 | 3 | 85 | 1 | 95 |
| 280 | 22 | 1 | 95 | 0 | 100 |
| 560 | 40 | 1 | 98 | 1 | 98 |
| | Sandoz 402-WDC BTI-1 | | | | |
| 280 | 35 | 6 | 83 | 2 | 94 |
| 560 | 29 | 3 | 90 | 1 | 97 |
| 1120 | 16 | 3 | 81 | 5 | 69 |
| | ABG-6108-D/Lot 6478-194 | | | | |
| 280 | 17 | 3 | 82 | 1 | 94 |
| 560 | 16 | 3 | 81 | 12 | 25 |
| 1120 | 14 | 1 | 93 | 2 | 86 |
| Check | 17 | 7 | 59 | 6 | 65 |

[a]Water temperature mean minimum 14°C and mean maximum 23°C.

tions at mosquitocidal and 5x these rates induced short-term effects on the dominant nontarget fauna. Mayfly, dragonfly naiads, diving beetle larvae and adults and ostracods were not affected on the rates of 0.56 to 2.24 kg/ha of the formulations employed. The data indicate that this microbial insecticide has a good margin of safety to the prevailing nontarget macroinvertebrates.

FLOODWATER MOSQUITOES. Five formulations of the microbial insecticide *Bacillus thuringiensis* Serotype H-14 were evaluated against larvae of the floodwater mosquitoes *Psorophora columbiae* and *Aedes nigromaculis* under laboratory and field conditions. Populations of the first species against which the trials were carried out were susceptible to other larvicides, while those of the later species have been characterized with high level of resistance to organophosphate larvicides.

Both species showed essentially similar levels of susceptibility to the various formulations under laboratory conditions. The 2nd instars of both species were much more susceptible than the 4th instars. The susceptibility of larvae of these two species was quite similar to that of other culicine and aedine mosquitoes.

Under field conditions, utilizing ground application methods, the Bactimos formulation yielded excellent results, producing complete control of 2-3 instars at 0.12-0.28 kg/ha. Sandoz 402 and Abbott ABG-6108 also yielded good control of early instars at the rates of 0.28-0.56 kg/ha.

The effectiveness of the various formulations applied by air was lower than that of the ground applications. Bactimos formulations produced high level of control of younger instars at 0.44 while Sandoz and ABG-6108 gave satisfactory results at 1-2 kg/ha. The latter two formulations are dilute preparations while the former is composed of higher concentration of the primary powder characterized with high potency.

Under field conditions, older instars, especially late 4ths, were hard to control with any of the formulations. Also, there was an inverse relationship between efficacy and density level of the larval populations. Higher rates of applications are needed for the control of late instars and populations prevailing at high density levels (50-100 larvae/dip). Under these conditions, treatment with BT (H-14) formulations may be economically impractical.

# STUDIES ON *BACILLUS THURINGIENSIS* var. *ISRAELENSIS* AGAINST MOSQUITO LARVAE and OTHER ORGANISMS[1]

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## ABSTRACT

A total of 23 species of aquatic organisms, other than mosquito larvae, were exposed to formulations of *Bacillus thuringiensis* var. *israelensis* (serotype H14) in this laboratory during the past year. No mortality was observed for these species with the exception of *Chironomus maturus* and a *Simulium* sp. which showed a degree of susceptibility similar to that of mosquito larvae. Field trials with various commercial formulations of Bti were successful against *Anopheles franciscanus* in fresh water ponds and streams, *Culex tarsalis* and *Culiseta inornata* in brackish water marshes and *Culex pipiens* in a stagnant water drain. Control against *Culex peus* in a primary oxidation pond was not successful.

Investigations with the mosquito and blackfly pathogen, *Bacillus thuringiensis* var. *israelensis*, (Bti), continue to be extremely encouraging both from an efficacy standpoint and from a lack of adverse impact on non-target aquatic organisms. Previous studies have indicated that many species of mosquitoes, particularly those other than *Anopheles*, and blackflies are highly susceptible to the endotoxin of this sporeforming bacterium. Thus far this pathogen seems almost entirely selective for these groups and some closely related Diptera (See refs cited). Acute toxicity tests conducted over the past 3 years against some 60 species representing 6 classes, 19 orders and 32 families of aquatic organisms have revealed no observable toxic effects (See non-target studies below and Garcia et al. 1980)

Recent investigations in our laboratory have been focusing in more detail on the impact of this pathogen on a wider variety of non-target organisms, and on the efficacy of the pathogen against mosquito larvae in different aquatic systems.

NON-TARGET STUDIES.—The non-target organisms are tested in 10-liter plastic tubs equipped with aeration devices and located in an outdoor screened enclosure. Food and aquatic vegetation are included with each species as required. Dosage of the pathogen ranges from several-fold to 100 times that used in experimental field applications against mosquito larvae. Species which have been successfully raised through their complete life cycle with 2 to 4 treatments of the pathogen include the western newt *Taricha torosa*, the tree frog *Hyla regilla*, the orb snail *Cyraulus* sp., the pouch snail *Physa* sp., the clam shrimp *Lynceus* sp. and an unidentified ostracod species. Other species which show development in the presence of the pathogen include a *Callibaetis* mayfly, the predaceous diving beetle *Dytiscus marginicollis*, the damselfly *Lestes stultus*, the dragonfly *Libellula* sp., the backswimmers *Buenoa scimitra* and *Notonecta kirbyi*, and the small water strider *Microvelia* sp. No detectable impact has been noted for any of the above species (Table 1).

At present, observations and experimentation indicate that the pathogen is only active among species in three families of nematocerous Diptera. The pathogen clearly shows strong activity among the Culicidae (mosquitoes), the Simuliidae (blackflies), and the Chironomidae (non-biting midges). However, some species within the family Chironomidae and other taxa show little, if any, effect. For example, the Clear Lake gnat, *Chaoborus astictopus*, did not show any mortality at high concentrations of the pathogen, though only small numbers were tested. Among the chironomids the species *Chironomus maturus* showed about the same susceptibility as mosquito larvae in relatively fresh clear standing water, while a member in the same genus, *Chironomus decorus*, showed no effect in sewage water at approximately 100 times the dosage applied to *C. maturus*. The sewage water (righ organically and possibly inorganically) appeared to influence the activity of the endotoxin, but this did not appear to account for the entire difference, suggesting variable species tolerance levels. One species of blackfly, *Simulium* sp. showed a susceptibility approximately equal to *Culex pipiens* larvae.

[1]This research was supported in part by Special State Funds for Mosquito Research in California and the UNDP/World Bank/WHO Special Program-proj #790197.

Table 1. NON-TARGET AQUATIC ORGANISMS EXPOSED TO *Bacillus thuringiensis* var. *israelensis*

| ORGANISM | COMMON NAME | HABITAT | NUMBER TESTED | [1] FORMUL. & CONC. (mg/ml) | OBSERVATION PERIOD | [2] NO. TX. | RESULTS |
|---|---|---|---|---|---|---|---|
| *Hyla regilla* | tree frog, tadpoles | fresh water pond | 30 | S 0.028 | 60 da | 4 | no effect; newly hatched - adult |
| *Taricha torosa* | California newt; young nymphs | " | 15 | S 0.028 | 38 da | 2 | no effect; young newts - loss of gills |
| *Taricha torosa* | " | " | " | S 0.0028 | " | " | " |
| *Taricha torosa* | eggs | " | 30 | S 0.028 | 120 da | 3 | no effect; eggs - loss of gills |
| DIPTERA | | | | | | | |
| *Simulium*, sp. (1) | blackfly | fresh water stream | 30 | B 0.002 | 24 hr | 1 | 100% mortality |
| *Simulium*, sp. (1) | " | " | " | B 0.0002 | " | 1 | 36.6% mortality |
| *Simulium*, sp. (1) | " | " | " | B 0.0001 | " | 1 | 13.3% mortality |
| *Simulium*, sp. (1) | " | " | " | B 0.00002 | " | 1 | 10.0% mortality |
| *Chironomus decorus* | midge | sewage pond | 15 | S 0.028 | 3 da | 1 | no effect |
| *Chironomus decorus* | " | " | 332 | S 0.0028 | " | 1 | " |
| *Chironomus maturus* | midge | standing water container | 60 | S 0.028 | 48 hr | 1 | 97% mortality |
| *Chironomus maturus* | " | " | 30 | S 0.0028 | " | 1 | 97% mortality |
| *Chironomus maturus* | " | " | 30 | S 0.00028 | " | 1 | 47% mortality |
| *Chaoborus astictipus* | Clear Lake gnat | fresh water pond | 30 | S 0.028 | 24 hr | 1 | no effect |
| COLEOPTERA | | | | | | | |
| *Dytiscus marginicollis* | predaceous diving beetle | fresh water pond | 12 | S 0.028 | 33 da | 2 | no effect; larva - adult |
| HEMIPTERA | | | | | | | |
| *Notonecta kirbyi* | backswimmer | " | 30 | S 0.028 | 60 da | 2 | no effect; nymph - adult |
| *Buenoa scimitra* | backswimmer | " | 15 | B 0.02 | 30 da | 1 | no effect; nymph - adult |
| *Microvelia*, sp.(1) | small water strider | " | 15 | S 0.001 | 24 hr | 1 | no effect |
| *Microvelia*, sp.(1) | " | " | " | A 0.001 | " | 1 | " |
| *Microvelia*, sp.(1) | " | " | " | B 0.001 | " | 1 | " |
| ODONATA | | | | | | | |
| *Lestes stultus* | damselfly | " | 15 | S 0.028 | 30 da | 2 | no effect; nymph - adult |
| *Argia*, sp. | damselfly | fresh water stream | 10 | B 0.02 | 14 da | 1 | no effect |
| *Libellula*, sp. | dragonfly | fresh water pond | 21 | S 0.028 | 48 da | 1 | no effect; nymph - adult |
| EPHEMEROPTERA | | | | | | | |
| *Callibaetis*, sp. | mayfly; early instar nymphs | " | 45 | S 0.028 | 33 da | 1 | no effect - molted to adult |

Table 1. NON-TARGET AQUATIC ORGANISMS EXPOSED TO *Bacillus thuringiensis* var. *israelensius*

| ORGANISM | COMMON NAME | HABITAT | NUMBER TESTED | [1]FORMUL. & CONC. (mg/ml) | OBSERVATION PERIOD | [2]NO. TX. | RESULTS |
|---|---|---|---|---|---|---|---|
| PLECOPTERA | | | | | | | |
| *Malenka*, sp. | stonefly | fresh water stream | 15 | B 0.02 | 3 da | 1 | no effect |
| HYDRACARINA | water mites | fresh water pond | 15 | B 0.02 | 30 da | 2 | no effect |
| | " | " | 20 | S 0.028 | " | 2 | " |
| OSTRACODA | ostracods | " | 30 | S 0.028 | 33 da | 1 | no effect: adult - immature - adult |
| COPEPODA | copepods | " | 75 | S 0.028 | 7 da | 1 | no effect |
| EUBRANCHIOPODA | | | | | | | |
| *Lynceus*, sp. | clam shrimp | " | 30 | S 0.028 | 95 da | 2 | no effect; adult - egg - immature |
| *Caenestheriella*, sp., (1) | large clam shrimp | " | 30 | S 0.028 | 7 da | 1 | no effect |
| *Caenestheriella*, sp., (1) | " | " | " | S 0.0028 | " | 1 | " |
| ANNELIDA | | | | | | | |
| *Helobdella stagnalis* | leech | " | 15 | S 0.028 | 10 da | 1 | no effect; adult - immature |
| GASTROPODA | | | | | | | |
| *Physa*, sp. | pouch snail | " | 60 | S 0.028 | 100 da | 4 | no effect; adult - eggs - adult |
| *Gyraulus*, sp. | orb snails | " | 60 | S 0.028 | 128 da | 2 | no effect; adult - eggs - adult |

[1]Formul. & Conc. : B = Biochem, "Bactimos" LRB No. 676; S = Sandoz, SAN 402 WDC; A = Abbott, IL 6-43-430.
[2]No. Tx. = number of treatments of Bti per observation period.

FIELD STUDIES WITH BTi[1].–Relatively small-scale field experiments have been conducted in northern California in a variety of habitats against several common species of mosquitoes: *Anopheles franciscanus, Culex tarsalis, Cx. pipiens, Cx. peus* and *Culiseta inornata.*

*Anopheles* species in general have appeared to be somewhat more tolerant to the Bti dosages than culicine and aedine species. This may be attributed to a variety of factors ranging from the feeding habits to the physiology of the anophelines. However, experiments conducted against *anopheles franciscanus* in the field this past year have shown some promising results.

*ANOPHELES FRANCISCANUS.*–Along the Russian River near Cloverdale, Sonoma Co., California, large populations of *An. franciscanus* breed in the algal mats floating on the quiet backwaters and pools alongside the main river bed. The larval population was measured with a standard aquatic dipper and averaged 8-10 larvae per dip. Fish were observed below the floating algal mats but were apparently unable to penetrate them to find the larvae. Small field plots were established and a dosage rate of 1 kg/ha was selected for both the Biochem[2] and Sandoz[3] formulations of Bti which was then sprayed over the plots from a pressurized spray can. Observations 24 hours later indicated a 100% reduction of larvae in most of the test plots (Garcia et al. 1981). Mosquito populations were not reduced significantly in 2 of the Biochem plots and in none of the Sandoz plots but this may have resulted from the more open flow of water in these areas. No detectable impact was noted in the controls nor among the non-target organisms, which were dominated by mayflies. Counts taken 72 hours later indicated that the test areas remained free of larvae.

Another field trial against *An. franciscanus* was conducted in conjunction with the Marin/Sonoma Mosquito Abatement District in Marin County, in a large, 10,000 $m^2$, pond. A heavy growth of Widgeon weed covered about 80% of the pond's surface providing the anophelines protection from the mosquito fish. Small test plots were set up and treated with the Biochem formulation of Bti at a rate of 0.5 kg/ha. Excellent control was achieved. A test was then conducted to see if control

[1]Field studies reported here were done in cooperation with Alameda and Marin/Sonoma Mosquito Abatement Districts.

[2]Biochem Products, P. O. Box 264, Montchanin, Delaware 19710. Formul: "Bactimos" LRB No. 676.

[3]Sandoz Inc., Crop Protection, 18900 S.W. 280th Street, Homestead, Florida 33031. Formul: SAN 402 WDC.

could be accomplished over a large portion of the pond. An air boat was secured to sample and treat the area which was proximately 8000 $m^2$. Pre-treatment counts with an aquatic dipper indicated a population that averaged 2 anophelines per dip. Biochem Bti was applied with the pressurized spray can at the rate of approximately .55 kg/ha. Post-treatment counts once again indicated excellent control with a reduction of larvae near 100%. No impact of the pathogen on non-target organisms was observed (Garcia et al. 1981).

*CULISETA INORNATA, CULEX TARSALIS.*–Recent field tests were conducted in salt marsh habitats in two locations in Alameda County, California. The first set of tests was conducted in the southeastern part of the San Francisco Bay and contained predominantly 3rd and 4th instar ***Cs. inornata.*** Two dosage levels of the Biochem Bti, 1.0 and 0.5 kg/ha and one dosage level of the Sandoz Bti, 1.0 kg/ha, were applied with a Solo® BackPack Sprayer 4. Six test and 2 control plots, 5m x 20m in size were marked off in one long row with 5 m separating each plot. Table 2 shows the results of these tests. In general, the Sandoz formulation at this concentration did not appear as effective as the Biochem formulations. Also, there were 2 Biochem plots where the reduction in larvae was not as complete, but this was probably due to an overnight rainstorm that contributed to an increase in the flow of water, and possibly larvae, to these two test plots.

**Table 2.** Field trials using *Bacillus thuringiensis* var. *israelensis* in brackish water against *Cs. inornata* in Alameda Co. California, 1981.

| BTI formul & dose | | No. Larvae per Dip[1] Pre | C[2] Post | C | % Reduction |
|---|---|---|---|---|---|
| Biochem:[3] | | | | | |
| 0.5 kg/ha | (1) | 16.7 | 1.1 | | 93 |
| | | 4.7 | | 3.5 | |
| | (2) | 3.8 | 0.9 | | 75 |
| 1.0 kg/ha | (1) | 3.0 | 0.7 | | 77 |
| | | 1.4 | | 1.9 | |
| | (2) | 8.3 | 0 | | 100 |
| Sandoz:[4] | | | | | |
| 1.0 kg/ha | (1) | 5.3 | 1.6 | | 70 |
| | | 1.4 | | 1.9 | |
| | (2) | 9.5 | 2.4 | | 75 |

[1]15 dips per 100 $m^2$ plots

[2]C = Control

[3]"Bactimos" LRB No. 676

[4]San 402 WDC

[4]Distributed by: Ben Meadows Company, 3589 Broad Street, Atlanta (Chamblee), Georgia 30366.

[5]Abbott Laboratories, Chemical and Agricultural Products Division, Oakwood Road, Long Grove, Illinois 60047. Formul: IL-6-43-430.

The second set of tests was conducted in a more northern section of the San Francisco Bay, adjacent to the Oakland International Airport. Early and late instars of ***Cx. tarsalis*** predominated and effective control of this mosquito was obtained with 0.1 kg/ha of Biochem Bti. Tests were also carried out in this area using the latest formulation of Abbott's Bti[5]. Effective larval control was achieved with 0.5 kg/ha of this formulation (Table 3) and future tests will determine its efficacy at lower dosages.

**Table 3.** Field trials using *Bacillus thuringiensis* var. *israelensis* in brackish water against *Cs. inornata* and *Cx. tarsalis* in Alameda Co., California, 1981.

| BTI[1] Dose | | No. Larvae per Dip[2] Pre | Post 24 hr. | % Reduction |
|---|---|---|---|---|
| 0.5 kg/ha | (1) | 1.5 | 0 | 100 |
| | (2) | 3.3 | 0.4 | 88 |
| | (3) | 4.5 | 0.2 | 96 |
| | control | 2.0 | 2.1 | -- |

[1]Bti Formul.: Abbott 1L-6-43-430.

[2]10 dips/50 $m^2$ plots.

*Cx. tarsalis* 13% total popul.

**Table 4.** Field trial using *Bacillus thuringiensis* var. *israelensis* against *Culex pipiens* in an open drainage ditch, Sonoma County, Calif., 1981.

| Abbott[1] Bti - dose | No. Larvae Per Dip[2] Pre | Post 24 hr | % Reduction | Sentinel cage Survivors |
|---|---|---|---|---|
| 0.5 kg/HA | 99 | 0 | 100 | 0/10, 0/10, 0/10 |
| Control | 17 | 6 | -- | 10/10, 10/10, 10/10 |

[1]Formul: IL 6-43-430

[2]10 dips per 50 $m^2$

*CULEX PIPIENS, CX. PEUS.*–Treatment with Bti was evaluated on two occasions against ***Cx. pipiens*** and ***Cx. peus*** in stagnant water systems. In the first experiment a large population of 3rd and 4th instar ***Cx. pipiens*** were found breeding in an open storm drain in a suburban area of Petaluma, Sonoma County. Large clumped masses of larvae and pupae were observed scattered throughout the stagnant pool which was approximately 60 $m^2$ in area. An aquatic dipper was used to assess the larval population and counts averaged nearly 100 mosquitoes per dip. The area which served as the control was about 10m away and separated from the test area by a tunnel (road overpass). It supported a lower but still substantial population of about 17 larvae per dip. Application of the *Bacillus* was with a Solo back pack sprayer at a rate of ½ kg/ha of Abbott wettable powder. Post-treatment counts conducted 24 hours later indicated a complete reduction in larvae (Table 4). Large numbers of dead floating larvae throuout the test site confirmed the success of the treatment.

Although Bti is an excellent to relatively effective control agent in a wide variety of breeding sources, oxidation ponds rich in organic matter pose a problem at practical application rates. Attempts to control *Cx. peus* in one such situation near the town of Occidental, Sonoma County, serves to illustrate this point. The primary oxidation pond, which is approximately 1000 $m^2$ in surface area, supports a dense cover of water hyacinth excepting the area around two aeration pumps near the center of the pond. Dipping with a standard dipper was conducted at mechanically cut openings through the 2 ft tall vegetation in the water. Mosquito larval counts prior to treatment ranged from 500 to 800 larvae per dip. The thickness and extent of the water lily made surface application of the *Bacillus* unfeasible. The Biochem, "Bactimos" powder, therefore, was mixed with water and poured into the head pump at the main pumping station located approximately ¼ mi. away from the primary oxidation pond. A Day-Glo dye was added in the same manner just prior to the bacteria to determine how long it would take the bacteria to reach the inlet of the pond and also to note the extent of the circulation within the pond. The dye was observed in an area surrounding the inlet but observations 24, 48 and 72 hours post-treatment indicated no reduction in mosquito larvae. It was not clear what specifically caused the failure, but lack of adequate coverage and levels of organic matter were suspected as being primary causes.

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# HATCHING IN THE MERMITHID NEMATODE *OCTOMYOMERMIS MUSPRATTI*[1]

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## ABSTRACT

1. Eggs of *Octomyomermis muspratti* hatched slowly and sporadically, thus hindering attempts at mass rearing of this mermithid.
2. Ingestion of *O. muspratti* eggs by *Aedes taeniorhynchus* induced hatching.
3. Brief exposure of the eggs of *O. muspratti* to full-strength bleach enhanced the hatching response.
4. Secondary exposure of bleach-treated eggs to a solution simulating mosquito digestive juices (surfactant, high $CO_2$, isosomotic solution) augmented the hatching response.

The mermithid nematode *Octomyomermis muspratti* was discovered in Zambia by Muspratt as a parasite of treehole mosquitoes (Poinar 1979). The life cycle as established by Obiamiwe and Macdonald (1973) is similar to that of *Romanomermis culicivorax*, but differs in timing and effects on host development. Recently, Petersen (1977a, b 1978) has developed preliminary techniques for mass production of *O. muspratti* and has shown that this mermithid has substantial tolerance for organic pollution and salinity (Petersen, personal communication).

Three cultures of *Octomyomermis muspratti* were obtained from Dr. Petersen (ARS, USDA, Lake Charles, Louisiana) in June, 1980 . Cultures were flooded in July, 1980 and very few (less than 50) preparasitic larvae were recovered from each culture. However, numerous eggs were present and the enclosed larvae appeared to be fully developed and mature. Re-evaluation of Petersen's (1977a, b, 1978) publications revealed that low hatching occurred and he was able to propagate *O. muspratti* because he had diligently accumulated numerous cultures.

Subsequently, a method to stimulate hatching of the eggs was sought. Since tree holes usually contain considerable organic detritus, anaerobiosis was investigated as a possible hatching stimulus. A multitude of anaerobic and/or organic rich regimens were tried as hatching stimuli: degassed water, cysteine and ascorbic acid solutions, sodium bisulfite and sodium dithionite solutions, putrified water from mosquito cultures, water extract of live oak leaves and bark, aqueous extract of peat, tea and coffee. All these conditions were ineffective in stimulating hatching in *O. muspratti*.

Some resolution was supplied by observing that *Culex pipiens* was infected when eggs of *O. muspratti* were added to actively growing cultures of *Cx. pipiens*. However, the level of infection couldn't be controlled - either the mosquitoes were overinfected or underinfected. In addition, this technique wasted eggs because most eggs remained unhatched in the mosquito rearing pans and were lost during the collection of mosquito larvae. However, the fact that some increase in hatching appeared to take place encouraged the following investigations.

Individual larvae of *Cx. pipiens* were placed in Analo-Cups with 0.5 ml water, some larval food and about 10 *O. muspratti* eggs. Each container (replicated 10 times) was observed daily for one week but no hatching was observed nor were infected larvae recovered. This experiment was repeated with 3rd instar *Aedes taeniorhynchus* with the following changes: five mosquito larvae were placed in each Analo-Cup with 50 eggs of *O. muspratti*. Observations after 1 hr showed that all the eggs had disappeared. Presumably, the larvae had ingested the eggs. After 24 hours, a number of larvae were dissected with the expectations that the nematode eggs had hatched within the mosquito gut. However, after dissection of five larvae it was apparent that none of the mosquito larvae were infected and all the eggs were within the larval gut. Upon removal of the eggs from the gut, changes in the eggs were apparent. *O. muspratti* eggs recovered from sand cultures are 85 microns in diameter and the internal contents are obscured by adherent clay and other small detritus (Fig. 1). The eggs removed from the mosquito gut were unchanged in size but all the detritus had been removed from the egg surface presumably through the action of mastication and/or digestion by the mosquito larvae. Twelve recovered eggs were set aside in another Analo Cup. The container was observed several days later and several eggs had hatched and after one week all eggs had hatched.

The above observations suggested the possibility of an interesting and unique interaction between the host and parasite. In the absence of mosquito larvae, the nematode eggs hatch sporadically and at a very low frequency, less than 0.1%. When the tree hole is populated by mosquito larvae, the older

[1]Supported in part by the Special Mosquito Augmentation Fund, University of California.

**Figure 1.** Eggs of *Octomyomermis muspratti* recovered from sand cultures are heavily coated with detritus. Egg diameter is 85 microns.

larvae browse in the bottom detritus and thereby ingest the nematode eggs. Passage through the larval gut triggers the hatching mechanism in the quiescent infectious larvae. The larvae emerge and seek out the host and gain entrance to the host hemocoel by means of cuticular penetration. Such an unique interaction is unknown amongst other insect parasitic nematodes, but occurs occasionally in plant parasitic nematodes and is common in parasites of vertebrates. If the above hypothesis is valid then possible hatching stimuli may be sought from the physiology of the digestive tract in larval mosquitoes. Figure 2 depicts schematically this phenomenon in the cycle of *O. muspratti.*

In subsequent hatching attempts, I tried to duplicate some of the conditions found within the mosquito gut. *O. muspratti* eggs were exposed to trypsin, chymotrypsin, and chitinase enzymes, but hatching was not increased compared to controls in tap water. Since the one readily apparent change in mermithid eggs removed from the mosquito gut was the clean egg surface, I decided to try several conditions well known for removing residues from nematode eggs. *O. muspratti* eggs were treated with commercial bleach (5 to 100%) for several minutes or left in sodium hydroxide overnight. All chemical agents were removed by centrifuging the eggs in 1.5 ml centrifuge tubes in a Beckman Microfuge for 30 sec at 8300 x g. Eggs were resuspended in tap water and washed 4 to 6 times by this method. Washed eggs were transferred to Analo-Cups containing 0.5 ml tap water and observed. Bleach and sodium hydroxide remove the detritus coating the eggs and the physical appearance was the same as eggs passed through the gut of *Ae. taeniorhynchus* larvae (Fig. 3). Eggs treated with bleach started hatching within several days and a substantial proportion (10-20%) had hatched within 10 days. The best hatching occurred in eggs treated with full strength bleach. However, all eggs did not hatch and alkali-treated eggs hatched at a rate equivalent to that found in the weak bleach (5%) treatment group. All treated eggs had the same physical appearance and it appeared that the strong oxidizing action of

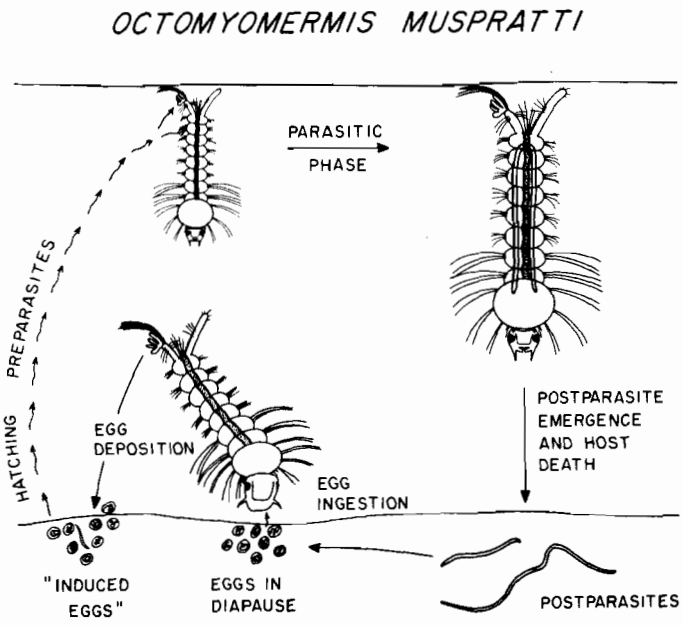


**Figure 2.** Life cycle of *Octomyomermis muspratti.* The preparasitic (infective) larvae are free-swimming and penetrate into the body cavity of mosquito larvae through the cuticle or siphon. The parasitic stage is usually located in the abdomen. Mosquito larvae die when the nematodes emerge about 14 days postinfection at 27°C. The postparasitic larvae complete their development in the detritus in tree-holes. Here the mermithids mate and the females lay their eggs. After completion of egg development, egg hatching is induced after passage through the digestive tract of mosquito larvae.

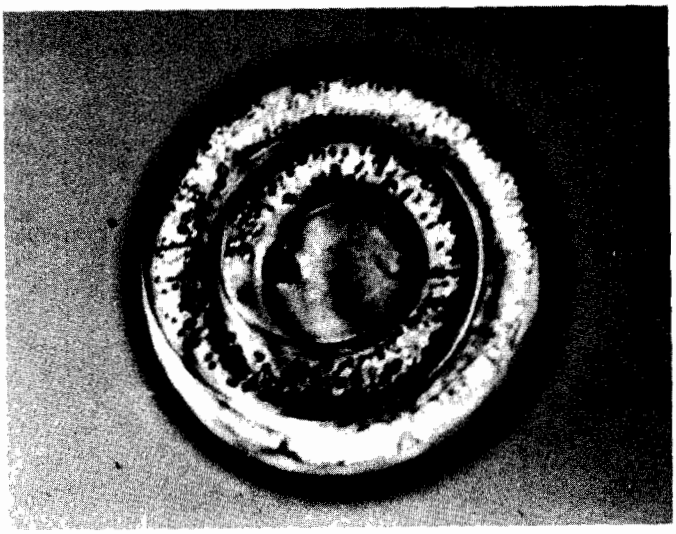

**Figure 3.** Appearance of eggs of *Octomyomermis mustratti* after treatment with bleach. The external appearance of the egg shell is equivalent to that observed for eggs recovered from the gut of mosquito larvae. Egg diameter is 85 microns.

the full strength bleach had modified the properties of the egg in some unknown way.

It is well known that arthropods require a dietary source of cholesterol. Although the uptake mechanism for cholesterol is unknown in mosquitoes, it has been established that choles-

terol uptake in the crustacean intestinal tract involves the surfactant action of an acyl sarcosine-taurine detergent (Vonk 1960). An attempt to simulate this possible effector mechanism was made. The surfactant from the crustacean digestive tract is not commercially available and the common paraffin chain detergent sodium dodecyl sulfate (SDS) was used to model its possible effects. Bleach-treated eggs were exposed to 43 $\mu$M SDS in *Aedes* saline saturated with $CO_2$ for 5 to 30 min. The SDS solution was removed by washing and the eggs were placed in Analo-Cups (***supra vide***). Egg hatching started within 6 hr post-exposure but continued to completion over the following 6 days.

Although these findings are encouraging substantially more refinement of the hatching stimulus is required. The synthesis of the crustacean intestinal detergent, N-(N-dodecanoylsarcosyl) taurine is simple (Dowd and Little 1976). Another source is intestinal fluid from freshwater crayfish (Vonk 1960). In addition, acyl-taurine detergents common in industrial applications are commercially available and may be suitable substitutes for the naturally occurring detergent.

These findings lend further support to the hypothesis that host ingestion stimulus is a major factor in the hatching of *O. muspratti* eggs (Fig. 2).

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# MASS REARING OF *ROMANOMERMIS CULICIVORAX*[1]

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The purpose of this project is to mass rear the mermithid nematode *Romanomermis culicivorax* on an operational level. Mass rearing, as opposed to laboratory or research colonies, is defined by Dr. Eugene J. Gerberg (Gerberg et al. 1969) as the synchronous production, on a regular schedule, of a standardized or specified mosquito (or other organism) in large quantities. Arbitrarily, "large quantities" are considered as over 10,000 individuals per rearing unit or tray, or weekly production of at least 100,000 mosquito larvae per rearing cycle.

"Operational", as opposed to "research", implies the production of sufficient numbers of the organism to be used as a practical part of the control program.

Many papers have been published on mass rearing of *Romanomermis culicivorax* (Petersen 1972, Petersen and Willis, 1972, Petersen 1973, Petersen 1978, Petersen et al. 1978, Platzer and Stirling 1978 and Brown 1978), as well as on mass rearing of the mosquito *Culex pipiens* (Gerberg et al. 1969 and Gerberg 1970). The basic techniques may be obtained from these papers; however, only problems encountered and solutions worked out by Sutter-Yuba M.A.D. will be covered in this paper. The Sutter-Yuba M.A.D. conducted a mass rearing project in 1972, utilizing these organisms in cooperation with Dr. James J. Petersen and Dr. James B. Hoy, U.S.D.A. This experience is being used as a basis for this project.

The Sutter-Yuba M.A.D. is comprised of 660 square miles and is primarily agricultural. The principal crop is rice, 121,500 acres in 1980 and the largest single producer of mosquitoes. The District must also deal with extensive drainage systems and seepage associated with agriculture.

Fish are presently used in these sources, supplemented with chemical control and source reduction. The District felt that with limited availability of fish and the peculiar nature of some mosquito sources, another bio-control agent was desirable. The District has cooperated in research on many different biological control agents in the past several years and felt that the mermithid nematode *Romanomermis culicivorax* held the most promise under our conditions. Therefore, in May 1980, in cooperation with Dr. Robert K. Washino, U.C. Davis, this project was started with the goal of cycling 1,000,000 mosquito larvae per week by the spring of 1981. This goal has not been realized because numerous problems have been encountered. Their solutions are being painfully worked out.

An existing wet laboratory was redesigned for the rearing of the nematode, the rearing of mosquito larvae for the stock adult mosquito colony, and to house the adult mosquito colony. This necessitated maintaining the room's temperature near 80° and the humidity at 80% or greater. Exterior doors had to be sealed, as well as all electrical outlets. All seams had to be caulked and a large metal roll-up door extending into the second floor had to be sealed inside and out. Humidity, in addition to that produced by evaporation from the surfaces on the rearing trays, was provided by a large pan of water on an electrical hot plate. The electric hot plate in this system proved to be an undesirable source of heat and was replaced by a system utilizing evaporation from a water soaked burlap surface. The system consists of a water reservoir, recirculating pump, and a metal frame covered with burlap. Air circulation was at first provided by two floor fans but is now accomplished with a small twin squirrel cage ventilation fan placed on a wall at ceiling level. Heat is provided by two small (1500 watt & 1600 watt) electrical heaters. The adult mosquito cages used in this project are 2ft.x 2ft.x 2ft. Two were purchased from a biological supply firm, two obtained from other sources, and the rest constructed by District personnel from surplus window screens. The mosquito larvae are reared in three different kinds of containers -- plastic food-handling pans approx. 17in.x 13in.x 5in. provided by Dr. Robert K.Washino, galvanized metal pans (painted with a non-lead base paint) approx. 54in.x 30in.x 2in. left over from the 1972 project, and new fiberglass pans approx. 48in.x 20in.x 2in. purchased for this project. Pan racks are made from expanded metal (Dexion/Acme), with each rack holding ten large pans or twenty-four of the food handling pans. Japanese quail (*Coturnix*) are used for blood hosts and are housed in animal cages, kept in a portable screened room to prevent access by wild mosquitoes, thus preventing the possible introduction of disease organisms into the stock mosquito colony. Nematode eggs are stored in an area separate from the rearing room. Heavy plastic was used to section off a corner of a room and a small heater added to provide a temperature of approximately 70°F.

In the spring of 1980, autogenous *Culex pipiens* mosquitoes were obtained from the Vector Biology and Control Section, California Department of Health Services, and from Dr. Robert K. Washino, (originally from VB&CS colony). A mixed colony from these two sources was started and converted to an anautogenous colony. Initially, standard laboratory enamel pans were used to rear the mosquito larvae, then when the adult mosquito colony was large enough, larvae production was begun in the plastic food-handling pans. Dr. James J. Petersen (Petersen 1972) reared *Culex pipiens* larvae in metal

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pans, approx. 54in.x 20in.x 2in., at a concentration of 18.5 larvae per square inch. The U.S.D.A. is no longer using this high concentration of larvae (Dr. Donald L. Bailey, personal communication). Initially, the plastic food-handling pans were used at a concentration of 1200 to 1500 larvae per pan (5.4 per $in^2$ to 6.8 per $in^2$); and then increased to 2000 larvae per pan (9.1 per $in^2$) without any major problems and probably could be increased further.

We decided that the food-handling pans were too labor-intensive and took up too much space to be practical for this project. Therefore, we put back into service those metal pans left over from the 1972 project until sufficient fiberglass pans could be obtained. We are presently using the fiberglass pans at a concentration of 10,000 larvae per pan (10.4 per $in^2$) and 14,000 larvae per pan (14.6 per $in^2$) depending on the number of eggs available.

The diet and feeding regimen used in this project was provided by Dr. Robert K. Washino and consists of Tetra Min, Guinea Pig Chow, and yeast. The mixture is made once a week and is stored in an air-tight container and refrigerated. The District obtains its water from a deep well irrigation pump. The water is very hard and is passed through a water softener. The literature states that the pre-parasites of *Romanomermis culicivorax* are sensitive to sodium salts (Petersen & Willis 1970, Dhillon et al. 1980); therefore, untreated water was piped into the rearing room. Initially, mosquito larvae reared in this untreated water did quite well, but when the pre-parasitic nematodes were introduced in January, 1981, problems became apparent. This was manifested in erratic rates of parasitism from as low as 5% to as high as 100% with multiple parasitism of 50% or greater from pan to pan and/or cycle to cycle at the same inoculation rates. At this time, new procedures were introduced.

Each adult cage population now consists of pupae taken only from one cycle. When the number of egg rafts falls below 50 per cage and the percentage of hatch is less than 50% the adults in that cage are discarded. Egg rafts produced by each cage are kept separate and the number of rafts per blooding are recorded. The average number of eggs per raft and the average percent of hatch are determined for each cage. All egg rafts and first instar larvae put into each rearing pan are from the same cage where possible. Problems still persisted and in early March 1981, large numbers of mosquito larvae were found to be dying in the fourth instar, and it was found that small amounts of turbine oil from the well were being introduced into the pans with the water. The District then switched to city water and this problem seems to have been solved. Another possible reason for erratic rates of parasitism with the same inoculation rates is poor survival of the mosquito larvae in the first and second instar and poor infectiveness of the pre-parasitic nematodes. Tests are presently being conducted to determine the percentage of survival to the fourth instar of the mosquito larvae. A record search is being made to try and determine if one or more pans of nematode eggs have produced live, but non-infective pre-parasites.

The procedures in handling both the pre-parasitic and the post-parasitic forms of the nematode, as well as the nematode egg holding pans have been described in the literature (Petersen & Willis 1972). We are following those procedures with one variation: Petersen and Willis placed 10-15 gm of parasites per egg holding pan; we are using 5gms. per pan as recommended by Dr. Harold C. Chapman (personal communications). The adult mosquito colony is maintained with mosquitoes reared exclusively for it while those pupae produced in the nematode operation are discarded. This is done to preclude any possible development of resistance by the mosquito to infection by the nematode (Petersen 1978). The District has found that it is necessary to keep the post-parasites in as clean an environment as possible, as a fungus (unidentified) will form on food debris, fecal material, and the bodies of dead mosquito larvae. The nematode may then become entangled in the fungus and subsequently infected and killed.

One step that we use as a preventive measure in this regard is a final separation through cheesecloth. The cheesecloth is placed on the screen of an emergence pan and water added just to cover. This allows the post-parasite to pass through the material and leaves any excess debris on the cheesecloth. The District has not yet had any problem with the fungus *Catenaria anguillulae* as experienced by Dr. Edward G. Platzer (Platzer & Stirling 1978).

In summary, the District makes the following recommendations to any agency who wishes to mass rear the nematode *Romanomermis culicivorax* operationally: Secure and maintain a healthy mosquito colony; secure a reliable source of healthy, viable, and infective nematodes; determine with the highest possible accuracy, the actual number of mosquito larvae being reared in each pan; evaluate each step in the cycle, with every cycle and keep detailed records; realize that protocols may have to be modified to meet individual needs and conditions. When changes in equipment or procedures become necessary, whether from problems or otherwise, try to change only one thing per rearing cycle so that it's impact can be measured. Both the mosquito and the nematode are sensitive organisms and even small changes may have an accumulative effect on their environment which may not be readily traced to any one change. Nothing has yet been said about labor requirements or costs. Some preliminary figures on the man-hours required to do the various phases of a cycle are available and anyone interested may contact the District at a later time. It should be remembered that though it takes a certain number of hours per cycle to maintain minimum production, as production per cycle is increased, the man-hours required do not necessarily increase proportionally. The foregoing has been only a brief description of the equipment and procedures used by the Sutter-Yuba Mosquito Abatement District, the problems it has encountered and some it has solved. The District feels that this project will be a success and that any district with the will to succeed can mass rear *Romanomermis culicivorax* on an operational level.

ACKNOWLEDGMENTS.—The district would like to acknowledge Dr. Robert K. Washino and his staff and Dr. Edward G. Platzer for their assistance with this project.

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# GEOTHERMAL AQUACULTURE: A PILOT PROJECT FOR INTENSIVE CULTURE OF THE MOSQUITOFISH, *GAMBUSIA AFFINIS*

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## ABSTRACT

A pilot project for mass rearing mosquitofish, *Gambusia affinis*, using facilities heated with 170° F Geothermal water has been undertaken by the Klamath Vector Control District. Our purpose is to provide the district with sufficient numbers of fish for its biological control program. Year-round reproduction was noted, without manipulation of photoperiod, by maintaining a temperature range of 70° - 85°F. Initial harvest yielded a projected 2,500 lb. per acre of *Gambusia* with a minimum of management.

INTRODUCTION.--Any mosquito control agency, regardless of size or scope of mosquito problems, eventually reaches the point where biological control must assume an increasing role in the control program. The mosquitofish, *Gambusia affinis*, has been the primary biological mosquito control agent; and even with advances in new biological agents, the mosquitofish will likely remain as an important biological weapon.

The major stumbling block in expansion of the use of the mosquitofish is supply. Natural reproduction is not sufficient to supply needed amounts of fish in early spring. This lack of adequate supply also hinders mosquito control use in another way. Mosquito control people often do not know the extent of control they can achieve with mosquitofish, because most have never had enough fish to establish the percentage of control achievable.

Most efforts at rearing mosquitofish have been hampered by lack of gravid females during winter months (Coykendall 1977, Reynolds 1977). Johnson and Gieke in two separate projects have worked on pond designs to maximize overwintering of *Gambusia affinis* (1977a) and a heat exchanger for warm water use (1977b). Current work by Drazba and Gall (1980) is developing a culture system in a greenhouse environment.

Oregon's Klamath Basin has an abundant supply of geothermal water. This can range as high as 200° F and is generally free of mineral contaminants often associated with hot water.

The Klamath Vector Control District has used this resource in an opportunistic manner for several years. We have areas heated by geothermal heating effluents which will support

high populations of *Gambusia affinis* on a year-round basis. Harvest of fish from these areas is hampered by various combinations of several factors: other species of animals, lack of access, excessive vegetation, or very soft muddy bottoms that make proper operation of a siene nearly impossible. While these problems are not totally insurmountable, solutions require extra labor, which serves to decrease cost efficiency. In addition, extra labor is beyond our financial ability at present.

CONSTRUCTION OF REARING FACILITY.–In January of 1979, I began the establishment of a mass rearing facility for production of *Gambusia affinis*. I am currently operating a single unlined, earthen raceway 200′ in length, 15′ wide, and 4′ deep. This is a surface area of .064 acres and a volume of 72,000 gallons. Pond temperature is kept in 70° - 80° F temperature range. Heating is accomplished by injecting 170° F effluent water from a commercial greenhouse. The hot water is injected directly into the rearing pond through a perforated iron pipe on the pond bottom. A remote bulb thermostat opens the hot water valve, in this case fed under pressure from the greenhouse, at the lower limit and then closes the valve when upper limit is reached.

Any temperature range to an upper limit of 90° F can be used with the thermostat. Fig. 1 shows the temperature fluctuations under different weather conditions.

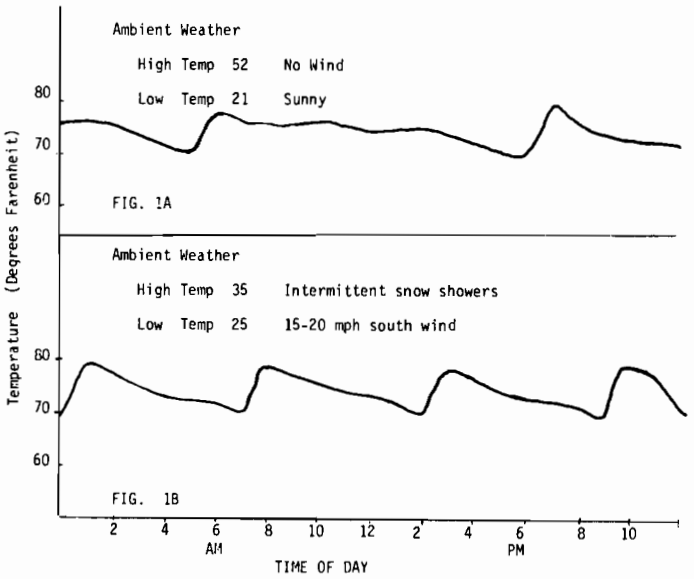


**FIGURE 1.** Examples of Temperature Patterns in Rearing Facility.

RESULTS AND DISCUSSION.–Water of this temperature can be both blessing and curse. Ineffective water control systems will produce lethal temperatures if either valves or pumps fail to close when they should. We are currently working on a computer controlled operating system that will read temperatures, open and close appropriate valves, provide a timer function for operation of automatic feeders, and provide a telephone warning in case of any failure of the system. This system will operate several ponds, allowing for anticipated expansion.

The first year harvest from the .064 acre raceway was 150 lb. of *Gambusia affinis* from an initial stocking of 6 lb. By extrapolating this upward to terms of one acre, this was 100 lb. per acre stocking rate a 2500 lb. per acre yield. Harvest was begun 12 months after stocking. The second year results are not yet complete. A very dry winter resulted in an exceptionally high number of dry sources, and early spring rains delayed the full force of early irrigation.

When dealing with only one pond, experimental designs for maximum production are limited. In order to keep labor costs at a minimum, the management level was kept low. The fish were contained in the raceway for harvest purposes, provided with 70° - 85° F temperature range, and fed once per day.

While working with *Gambusia affinis* and hot water, whether dealing with the rearing pond described here, or in other sources heated by hot water effluents, I have noted gravid females in every month of the year. I have also found fry during the month of December, when photoperiod reaches its shortest length. Research by Sawara (1974) indicated day length to be more critical than water temperature. Drazba and Gall (1980) in their work manipulated photoperiod as well as temperature in order to obtain winter fry. However, these have been laboratory experiments, so perhaps the influence of photoperiod in a more natural environment is less critical. This district's stock of *Gambusia affinis* have been in a heated environment since 1967. It may be that the more productive aquatic environment offered by year-long warm water has created some positive selection pressure for increased reproductive ability in these mosquitofish. However, fry in our facility are quite obviously more abundant beginning in February - March than they are from November - January. It is quite probable that photoperiod enhancement may prove to be very important in any management philosophy for rearing mosquitofish. *Gambusia affinis* is an easy species to raise and can be produced quite inexpensively, so I feel that quite substantial numbers can be raised in a system of this nature.

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# A LABORATORY SYSTEM FOR SMALL SCALE MASS PRODUCTION OF *GAMBUSIA AFFINIS*

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## ABSTRACT

INTRODUCTION.—In recent years Contra Costa Mosquito Abatement District has needed a greater in-house supply of mosquitofish as a result of a reduced labor force, increased public demand and a continuing depletion of natural sources due to urban development. As a result, a laboratory was constructed which contained two temperature controlled, recirculating systems to facilitate spawning, production and rearing of fry.

Each system is independent of each other and an individual system is comprised of four tanks (7'x3'x18") connected to a biofilter reservoir (22"x22"x30"), a 10-gallon water heater which is in turn controlled by a thermoregulator. Water is pumped from the biofilter to the tanks and returns to the filter by gravity flow. All piping and valves are of PVC material, pipes being connected to the tanks with radiator hose secured with clamps to provide some flexibility at these connection points. Within each system, water flow is regulated by valves between the pump and the tanks and by the size of spouts into each tank. A gravity return flow was chosen as a fail safe system to offset electrical blackouts and limited presence of employees. A biofilter has two layers and sizes of gravel, a 6" top layer of 15 mm angular gravel above a 14" layer of 10 mm pea size gravel. The remote sensor of the thermoregulator is inserted in water within a glass container partially submerged in the biofilter. The thermoregulator is then connected to the heater and in turn overrides the heater sensing unit. Screens are inserted over the outflow and return lines of the filter to prevent any movement of gravel into the pipes and allow the water heater to be flushed out by opening and closing appropriate valves.

Studies will be conducted at different temperatures to determine optimal growth densities of fry, holding populations of females for conditioning and ultimately, a continuous production system.

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# EFFECT OF TEMPERATURE AND RATION SIZE ON THE FOOD CONSUMPTION AND GROWTH RATES OF MOSQUITOFISH, *GAMBUSIA AFFINIS*

J. J. Cech, W. A. Wurtsbaugh, and B. C. Vondracek

## ABSTRACT

As part of a larger research program to construct a bioenergetic model for *Gambusia affinis*, the present laboratory study investigated the relationships among environmental temperature, ration size, growth rate, and food consumption rate in newborn *Gambusia* fry. Approximately two-hundred fry were raised per separate aquarium (ration treatment group). Four aquaria were situated in each temperature-controlled water bath. On a diet of chopped *Tubifex* worms, food consumption increased from 7% dry body weight/day at 10°C to 82%/day at 35°C under *ad lib.* rations. Growth increased from 0% dry body weight/day (no growth) at 10°C to 22%/day at 30°C and declined slightly at 35°C under *ad lib.* rations. Gross efficiencies increased from 0 at 10°C to a peak of 28% at 30°C and declined at 35°C.

Under reduced rations (20% of body weight/day) growth rates are greatly reduced with the maximum rate (6%/day) shifting downward to 25°C. Weight loss under starvation varied from 1.5%/day at 10°C to 9%/day at 35°C, presumably from the higher respiratory metabolic energy demands at the warmer temperatures.

A separate experiment investigating comparative ration type showed that *Gambusia* fed *Artemia* grew slightly faster than those fed *Tubifex* worms. After 25 days, the mean weight of *Artemia*-fed fish was 22% greater than fish fed *Tubifex* rations.

Taken together, these data will provide laboratory baselines for computer models of *Gambusia* in the field. Using these relationships, it will be possible to estimate food consumption rates of field populations from their growth rates and field temperature regimes. With a known diet composition, the impact of *Gambusia* on particular prey species, such as mosquitoes can be estimated and *Gambusia* stocking levels can be calculated.

# A RECOMMENDED STRATEGY FOR THE ALLOCATION OF AGENTS (PARTICULARLY *GAMBUSIA AFFINIS)* USED TO CONTROL *CULEX TARSALIS* IN THE RICE FIELDS OF CALIFORNIA[1]

F. H. Collins[2] and R. K. Washino[3]

## ABSTRACT

A four year study of the factors which affect the among-fields distribution of *Culex tarsalis* in the rice fields of northern California has revealed that cultivational practices play an important role in restricting the majority of mosquito production to a small and readily identifiable subset of rice fields. Because of the expense entailed in preparing fields for rice cultivation, rotation of fields to an alternate crop rarely occurs more frequently than every five or six years. This practice appears to foster a temporal process of change in the rice field aquatic community which is initiated when a field is returned to rice after a period in an alternate crop. The populations of a number of invertebrate mosquito predators, particularly the flatworm *Mesostoma lingua*, are significantly lower in fields newly returned to rice cultivation than in fields which have been in rice for two or more consecutive years. Because of this process, predator induced mosquito mortality in these first year fields is significantly lower than in older fields, thus resulting in markedly higher *Cx. tarsalis* production by such fields.

Additionally, particularly during years of limited irrigation-water availability, a portion of rice fields are irrigated with water from deep wells. This well-water, with its higher level of dissolved salts, has been found in laboratory ovipositional choice experiments to be preferred by female *Cx. tarsalis* over the lower conductivity water originating from river impoundments. Furthermore, well-water-irrigated rice fields have been found to contain significantly higher densities of *Cx. tarsalis* egg rafts than river-water-irrigated fields. The net effect of this process is that well-water-irrigated fields generally support densities of preimaginal *Cx. tarsalis* which are between 10 and 20 times higher than fields irrigated with river water.

These two cultivational practices appear to interact multiplicatively in their effect on *Cx. tarsalis* production. In the 1979 survey of 30 rice fields, the four well-water-irrigated-fields in the study accounted for 72.3% of all preimaginal *Cx. tarsalis* sampled during the season, the six first-year fields accounted for 54.8%, and the one first-year/well-water field produced 43.3% of the total. Thus the nine fields identified by these two characteristics were responsible for 83.8% of the total mosquito production as compared with only 16.2% from the remaining 21 older/river-water-irrigated fields.

Clearly, because of limits on the availability of *Gambusia*, only about 20% of rice fields are generally stocked with fish. We believe that allocation of control resources on the basis of these two readily identifiable characteristics (irrigation water source and rice field age) can greatly enhance the effectiveness of current control efforts.

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# DISTRIBUTION AND MIGRATORY MOVEMENTS OF MOSQUITOFISH IN A SACRAMENTO VALLEY RICE FIELD

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ABSTRACT

Results showed that substantial numbers of nearly or newly mature mosquitofish emigrated from a stocked Sacramento Valley rice field between July 17 and September 10, 1980. Downstream trapping confirmed that the fish emigrating from the last paddy probably represented a net downstream movement of the offspring population of the entire field and not merely a limited movement of the fish residing in the last paddy. Distributional trapping conducted in one paddy indicated that most mosquitofish preferred the relatively open water habitats adjacent to the border levees; however, fish were distributed throughout the rice stand as well. Fish emigration, downstream movements and distribution were all found to be positively correlated with water temperature. Dissolved oxygen measurements collected in a single 24-hour test showed a maximum of 17.8 ppm (mg/l) at 1300 PDT and minimum of 0.2 ppm (mg/l) at 0500 PDT.

Recent field studies by Farley and Younce (1978 and 1979) have revealed that substantial numbers of nearly or newly mature, unfertilized female mosquitofish, *Cambusia affinis* emigrate from Fresno County (San Joaquin Valley) rice fields approximately 40 to 50 days after stocking; the exodus continues for about 25 to 35 days and then slowly wanes. This emigration behavior may adversely affect mosquito control efficacy in certain fields during peak emigration periods. In addition, these studies have also demonstrated that mosquitofish activity peaks between 1200 and 1400 hours and cycles downward to practically zero between dusk and dawn.

While the basics of rice culture are virtually identical anywhere in California's central valley, many farmers of Sutter County (Sacramento Valley) utilize irrigation and drainage methods that do differ somewhat from those employed by farmers of Fresno County. The present study was conducted, therefore, to determine if this pattern of fish emigration was a localized phenomenon unique to the fish stocked in Fresno County rice and associated with the rice farming practices there or typical behavior for the fish stocked in other rice growing regions of California as well. Our study also examined the nature and patterns of mosquitofish movement and distribution within the rice field itself. In support of the foregoing experimentation, other measurements and monitoring of physical, chemical and biological factors, which could possibly influence behavioral patterns in the mosquitofish, were collected and recorded for correlation purposes. If significant correlations could be made, perhaps a better understanding of mosquitofish behavior in the rice habitat could be gained.

MATERIALS AND METHODS.–The contour-levelled rice field selected for this study, located on the Jopson Ranch near East Nicolaus, California, encompassed 75 acres and contained fifteen paddies of unequal area and configuration. Both surface (ditch) and wellwater sources that were used to irrigate the field flowed through serially-arranged rice boxes which were all installed near the west side of the field. The individual paddies ranged in area from approximately 1.6 to 13.5 acres (0.6-5.5 ha); however, only the lower thirteen paddies were used in the evaluation because irrigation water was retained in the second field paddy and merely seeped into the third through accidental openings in the levee. The lower thirteen paddies were, therefore, surface-water irrigated while the first two paddies received a mixture of both source waters. This rice field was prepared and seeded somewhat later in the spring than usual, so was not ready for fish stocking until July 1st. For biocontrol purposes, a rice field in our area is deemed 'stockable' when the farmer has made his final adjustment on water levels and plans no further significant drawdowns for weed control or cold weather. By this time the rice has usually grown well above the water surface and has a dense sturdy appearance.

The adult, mixed-sex stock of mosquitofish introduced into the field on July 1st, were seined from an oxidation/stabilization impoundment at the Wheatland Wastewater Treatment facility and were then transported directly to the study field by tank truck. The fish were stocked into the field at an overall treatment rate of 0.2 lbs per acre (0.22 kg/ha); therefore each of the fifteen paddies received one pound (0.45 kg) of fish, despite differences in individual paddy acreage. The Sutter-Yuba MAD generally follows the practice of stocking all paddies within a field to ensure that fish are not excluded from physically isolated paddies, which could occur if the fish were stocked using single or multiple drop application methods.

Fish samplings were conducted by means of two trapping techniques. The fish emigration and movement within the

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field studies employed a downstream box trap virtually identical to the trap designed and used by Farley and Younce (1978). These traps were utilized for four-hour sets (1100-1500 PDT) one day at weekly intervals. With the trap installed below each rice box, practically all fish moving downstream or exiting the field were captured during each of the eleven samplings periods. Preliminary trapping studies conducted here indicated that approximately 57% of those fish moving downstream would be captured between the hours of 1100 and 1500 PDT. It was not feasible to trap for full 24-hour sets as red swamp crawfish, *Procambarus clarki* and larvae of several species of predaceous beetles were also captured in large numbers at night commencing several weeks after the field was stocked with fish. These organisms preyed extensively upon captured fish, thus making it difficult to enumerate and measure the fish catch. Consequently, estimates of the 24-hour and 7-day catch were extrapolated from each week's 4-hour (1100-1500 PDT) set. In all samplings, fish were returned to the paddy from which they were captured.

The second type of trap utilized in this study was the Gee® minnow trap, custom ordered with 8-mesh hardware cloth screening. On two dates (August 21 and September 9, 1980), eleven of these traps were used to assess fish distribution in paddy #11. The trapping sites were situated on an east-west transect, with the first site 3 feet (0.9m) eastward from the western border levee. The remaining sites were spaced at even 10-foot (3.1m) intervals across paddy #11 in an easterly direction. Thus the minnow traps were dispersed for a total distance of 103 feet (31.4m), which was over halfway to the nearest edge of the eastern border levee of this paddy. The trapping site transect line was placed at a point equidistant from both north and south paddy levees at their juncture with the western border levee.

Water depths in this paddy were fairly uniform for all trapping sites with each trap only partially submerged in about 8 inches (20.3cm) of water. All traps were installed with their entry portals on a north-south alignment. Foot access to each of the trap sites was varied to prevent creation of openwater pathways that could influence trapping success.

Data collected on the trapped fish during the course of the study (July 1 to September 10, 1980) included simple enumeration, individual length measurements and sexual determinations. Water temperatures were measured twice daily (1100, 1500 PDT) at each trapping site with an Electromedics Model TC100 digital thermometer. All water temperatures were taken at a probe depth of 3 inches (7.6cm). Air temperatures were also recorded at the beginning and ending of each trapping session. Dissolved oxygen (D.O.) measurements were made with a Yellow Springs Instruments, Model 57, complete with a remote stirring probe. This instrument's output was coupled to a Linear Instruments Corporation, Model 142 portable strip chart recorder. Statistical analyses included the use of arithmetic means, Pearson's coefficient of correlation and the Student's T test (LaMont, M.D. et al. 1977).

RESULTS AND DISCUSSION.–Fish Emigration.--Outfall trapping conducted below the last rice box in the field (Paddy #15) resulted in the data presented in Table 1. Water outflows from this box were fairly uniform during the study so trapping was never hindered by inadequate flow rates. No fish were captured in this trap during the first two 4-hour sets (July 2 and July 9); however, some emigration may have occurred on these two dates, but the fish may have been so small that they passed through the 8-mesh screening of the trap. Offspring of the stocked adults were first observed in several paddies on July 9 and averaged about 0.43 inches (11mm) in total length at that time. The smallest offspring ever caught in any of the traps measured 0.75 inches (19mm) total length; which may be quite close to the smallest fish length that can be retained with our particular hardware cloth screening. Therefore, it was not possible to determine at what size or age the fish began their downstream movement.

Only ten stocked adults were captured during the course of this study in the outfall trap and no stocked females were trapped after August 13. Through extrapolation, seasonal estimates of fish emigration were calculated to range betwen 3,779 and 5,064 with a mean value of 4,093 fish. Emigration of young mosquitofish continued throughout the season and slowly waned near the end of the rice growing season. In the outfall trap, a positive correlation ($P<0.05$) was found to exist between the daily mean temperature measured in the trap and the cumulative catch. (That is, the higher the outfall water temperature the greater the fish emigration.) The slight increase in fish numbers captured the last day of the experiment was most likely influenced by the decreased field water depths, which concentrated the fish nearer the rice boxes.

Young male fish first appeared in the outfall trap on August 20 (51 days post-stocking) and were captured regularly thereafter. The mean length for young female fish progressively increased until August 6, when it dropped over 0.24 inches (6mm) to indicate that a younger age group of fish were probably being recruited into the population of young fish moving downstream. Thereafter, the mean length for all female offspring steadily increased until the end of the study. Although the estimated numbers of fish emigrating daily did not approach the 'several hundred to several thousand fish' reported by Farley and Younce (1979); it does appear that the emigration of nearly mature or mature mosquitofish is a phenomenon common to the fish populations of both rice growing regions. Further tests involving many more Sacramento Valley rice fields would serve to either support or refute this tentative conclusion.

Downstream Fish Movement Within the Rice Field–In addition to outfall trapping, identical traps were installed below the rice boxes of the individual paddies within the study field. These twelve traps in Paddies #3 through #14 were set and retrieved on the same schedule as the outfall trap in Paddy #15. Table 2 presents the results of both downstream and emigration trapping. Cumulative catches from the traps placed below each rice box within the field were found to be positively correlated ($P<0.001$) with the outfall trap catches. Almost twice as many fish were captured in the lower paddies than in the upper paddies, which most likely indicates a net downstream movement trend. The outfall trap also had a seasonal cumulative catch of more than twice that of any other trap within the field.

**Table 1.** Catch results for mosquitofish trapped at the field outfall (paddy #15), between the hours of 1100-1500 PDT.

| Date | Mean Temp (F/C) | Stocked Adults | Young Females | Female Mean Length (in./mm) | Young Males | Total Catch/Set |
|---|---|---|---|---|---|---|
| 7/2/80 | - | 0 | 0 | - | 0 | 0 |
| 7/9 | - | 0 | 0 | - | 0 | 0 |
| 7/17 | - | 5 | 53 | 0.8/20.0 | 0 | 58 |
| 7/23 | 83.0/28.3 | 3 | 82 | 1.06/26.9 | 0 | 85 |
| 7/30 | 76.6/24.8 | 0 | 42 | 1.14/29.1 | 0 | 42 |
| 8/6 | 70.6/21.4 | 1 | 70 | 0.90/22.9 | 0 | 71 |
| 8/13 | 70.0/21.1 | 1 | 26 | 1.02/26.0 | 0 | 27 |
| 8/20 | 67.4/19.7 | 0 | 14 | 1.07/27.2 | 8 | 22 |
| 8/27 | 65.9/18.8 | 0 | 14 | 1.09/27.8 | 3 | 17 |
| 9/3 | 67.5/19.7 | 0 | 0 | - | 1 | 1 |
| 9/10 | 67.0/19.4 | 0 | 16 | 1.31/33.4 | 5 | 21 |
| Season Total: | | 10 | 317 | | 17 | 344 |
| Mean: | 71.0/21.7 | 0.9 | 28.8 | 1.06/26.8 | 1.6 | 31.3 |

**Table 2.** Catch results for mosquitofish captured in downstream traps installed below each rice box between the hours of 1100 & 1500 PDT one day per week.

| Date: | 7/2 | 7/9 | 7/17 | 7/23 | 7/30 | 8/6 | 8/13 | 8/20 | 8/27 | 9/3 | 9/10 | Sum Fish/ Paddy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Paddy #3 | 0 | 0 | 11 | 3 | 7 | 9 | 5 | 5 | 12 | 10 | 3 | 65 |
| Paddy #4 | 0 | 0 | 7 | 14 | 16 | 2 | 8 | 20 | 7 | 7 | 13 | 94 |
| Paddy #5 | 0 | 0 | 1 | 4 | 0 | 9 | 1 | 10 | 5 | 8 | 3 | 41 |
| Paddy #6 | 0 | 0 | 11 | 22 | 19 | 23 | 16 | 19 | 7 | 7 | 6 | 130 |
| Paddy #7 | 0 | 0 | 6 | 13 | 7 | 26 | 13 | 11 | 2 | 1 | 10 | 89 |
| Paddy #8 | 0 | 0 | 6 | 29 | 15 | 17 | 10 | 6 | 2 | 1 | 9 | 95 |
| Paddy #9 | 0 | 0 | 11 | 21 | 3 | 23 | 22 | 14 | 6 | 10 | 7 | 117 |
| Paddy #10 | 0 | 0 | 34 | 34 | 11 | 27 | 17 | 4 | 4 | 2 | 2 | 135 |
| Paddy #11 | 0 | 0 | 4 | 21 | 10 | 12 | 16 | 8 | 1 | 0 | 4 | 76 |
| Paddy #12 | 0 | 0 | 26 | 28 | 20 | 22 | 16 | 15 | 26 | 4 | 5 | 162 |
| Paddy #13 | 0 | 0 | 23 | 33 | 12 | 13 | 3 | 5 | 12 | 0 | 16 | 117 |
| Paddy #14 | 0 | 0 | 49 | 12 | 16 | 19 | 8 | 0 | 10 | 0 | 27 | 141 |
| Paddy #15 (Outfall) | 0 | 0 | 58 | 85 | 42 | 71 | 27 | 22 | 17 | 1 | 21 | 344 |
| Total Fish/ Trap Set | 0 | 0 | 247 | 319 | 178 | 273 | 162 | 139 | 111 | 51 | 126 | 1606 |

Water temperatures sampled in each downstream trap throughout the study followed two fairly distinct patterns. During the first two or possibly three sampling dates (July 23 through August 6), mean temperatures (shown in Table 3) increased as the water passed downstream through the field. From the fourth sampling date (August 13) onward the upper

to September 10), the percentage of males in the catches steadily increased (14% to 30%, respectively). According to Krumholz (1948), male mosquitofish may reach maturity at about the same or a slightly greater age than do females. This slowness in male maturation may have caused the differences in observed sex ratios over the latter part of this study.

**Table 3.** Mean water temperatures. Samplings collected inside the traps while they were installed below each paddy box. (1100 PDT temp + 1500 PDT temp)/2 = mean temp (F/C)

| Date: | 7/23 | 7/30 | 8/6 | 8/13 | 8/20 | 8/27 | 9/3 | 9/10 |
|---|---|---|---|---|---|---|---|---|
| Paddy #3 | 72.5/22.5 | 71.2/21.8 | 68.9/20.5 | 69.3/20.7 | 70.6/21.4 | 70.4/21.3 | 70.9/21.6 | 71.8/22.1 |
| Paddy #4 | 72.0/22.2 | 71.8/22.1 | 69.1/20.6 | 69.4/20.8 | 70.6/21.4 | 70.6/21.4 | 73.0/22.8 | 71.2/21.8 |
| Paddy #5 | 75.0/23.9 | 72.8/22.7 | 70.3/21.3 | 70.0/21.1 | 69.6/20.9 | 68.6/20.3 | 69.9/21.1 | 69.4/20.8 |
| Paddy #6 | 74.0/23.3 | 72.6/22.6 | 69.8/21. | 69.8/21. | 69.8/21. | 69.0/20.6 | 69.8/21. | 69.4/20.8 |
| Paddy #7 | 74.0/23.3 | 72.4/22.4 | 70.0/21.1 | 70.2/21.2 | 70.2/21.2 | 69.2/20.7 | 70.5/21.4 | 69.4/20.8 |
| Paddy #8 | 74.5/23.6 | 73.6/23.1 | 70.2/21.2 | 70.5/21.4 | 70.8/21.6 | 69.4/20.8 | 70.7/21.5 | 69.5/20.8 |
| Paddy #9 | 75.0/23.9 | 74.0/23.3 | 69.8/21. | 70.4/21.3 | 70.0/21.1 | 69.0/20.6 | 69.7/20.9 | 68.8/20.4 |
| Paddy #10 | 76.5/24.7 | 74.8/23.8 | 70.2/21.2 | 70.4/21.3 | 69.4/20.8 | 68.2/20.1 | 68.4/20.2 | 68.4/20.2 |
| Paddy #11 | 81.8/27.7 | 76.4/24.7 | 72.0/22.2 | 70.4/21.3 | 68.8/20.4 | 67.8/19.9 | 67.4/19.7 | 67.3/19.6 |
| Paddy #12 | 82.0/27.8 | 77.2/25.1 | 70.8/21.6 | 70.2/21.2 | 69.4/20.8 | 68.3/20.2 | 67.1/19.5 | 67.8/19.9 |
| Paddy #13 | 82.2/27.9 | 77.6/25.3 | 71.6/22. | 70.4/21.3 | 68.6/20.3 | 67.8/19.9 | 67.4/19.7 | 67.8/19.9 |
| Paddy #14 | 82.0/27.8 | 77.8/25.4 | 71.0/21.7 | 70.4/21.3 | 68.6/20.3 | 67.8/19.9 | 66.7/19.3 | 67.8/19.9 |
| Paddy #15 | 83.0/28.3 | 76.6/24.8 | 70.6/21.4 | 70.0/21.1 | 67.4/19.7 | 65.9/18.8 | 67.5/19.7 | 67.0/19.4 |
| Mean: | 77.3/25.2 | 74.5/23.6 | 70.3/21.3 | 70.1/21.2 | 69.5/20.8 | 68.6/20.3 | 69.2/20.7 | 68.9/20.5 |

Temperatures not recorded on 7/2, 7/9 and 7/17.

paddies were warmer than those further downstream. The catch data seemed to parallel the temperature measurements for the period in which recordings were made (July 23 through September 10). Large trap catches were recorded during the first half of the study, but catches gradually decreased during the last half of the study period.

In contrast to the findings of Farley and Younce (1979), positive correlations were found to exist between water temperature measured in the traps and the magnitude of fish either moving downstream ($P<0.001$) or emigrating from the field ($P<0.05$). No irrefutable explanation can be forwarded relating to the basic motivation for the migratory behavior observed in these young fish. It could be simply an inherent distribution mechanism, as suggested by Farley and Younce (1979), or possibly a positive thermotactic response to warmer areas within a rice paddy, as indicated by the results of this study.

The sex ratio of captured fish was recorded once young males began appearing in the trap catches. The females always dominated the catch; but as the season progressed (August 20

Fish Distribution Within A Rice Paddy.--Periodic observations made in the rice paddies from many different sites revealed that fish usually seemed to congregate in the borrow pits adjacent to southern and western paddy levees. However, fish were observed to be very well distributed in all relatively open water areas of a paddy. The distribution trapping conducted on two separate dates (August 21 and September 9) resulted in data that may allow a suggestion as to why fish were congregated in these open water areas adjacent to the shoreline.

From the data presented in Table 4, it can be seen that on both dates most fish were collected in Site #1, which was the closest trap to the western border levee. Combining the almost identical catch data from both sampling dates resulted in 89.4% of the total catch being trapped at Site #1, while trapping at Sites #2-11 amounted to only 10.6%. Water temperatures were found to decrease as one penetrated further into each paddy. Again the combined catch for each trap site was found to be positively correlated ($P<0.001$) with the mean water temperature measured at that trapping station. This data also seems to support the contention that the fish may be

**Table 4.** Mosquitofish distribution trapping results. (Trapping dates: 8/21, 9/9; Location: Paddy #11; Trap Used: Gee Minnow Trap (8-mesh).

| Date: 8/21/80 | Stocked Adults | Young Females | Female Mean Length(in./mm) | Young Males | Male Mean Length(in/mm) | Total Catch/Trap | % of Catch |
|---|---|---|---|---|---|---|---|
| Site #1 | 0 | 79 | 1.10/27.6 | 8 | 1.06/27.0 | 87 | 79.8 |
| Site #2 | 0 | 3 | 1.19/30.3 | 2 | 0.96/24.5 | 5 | 4.6 |
| Site #3 | 0 | 2 | 1.20/30.5 | 2 | 1.04/26.5 | 4 | 3.7 |
| Site #4 | 0 | 0 | - | 0 | - | 0 | 0 |
| Site #5 | 0 | 0 | - | 0 | - | 0 | 0 |
| Site #6 | 0 | 0 | - | 0 | - | 0 | 0 |
| Site #7 | 0 | 0 | - | 1 | 0.98/25.0 | 1 | 0.9 |
| Site #8 | 1 | 0 | - | 1 | 1.10/28.0 | 2 | 1.8 |
| Site #9 | 0 | 1 | 0.87/22.0 | 1 | 0.94/24.0 | 2 | 1.8 |
| Site #10 | 0 | 3 | 0.91/23.0 | 0 | - | 3 | 2.8 |
| Site #11 | 0 | 4 | 0.86/21.8 | 1 | 1.02/26.0 | 5 | 4.6 |
| **Total:** | 1 | 92 | | 16 | | 109 | |

**Table 4.** continued

| Date: 9/9/80 Mean Temp (F/C) | Stocked Adults | Young Females | Female Mean Length (in/mm) | Young Males | Male Mean Length (in./mm) | Total Catch/Trap | % of Catch |
|---|---|---|---|---|---|---|---|
| Site #1 72.5/22.5 | 1 | 154 | 1.34/34.0 | 138 | 1.06/27.0 | 293 | 92.7 |
| Site #2 70.8/21.6 | 0 | 3 | 0.96/24.3 | 1 | 0.94/24.0 | 4 | 1.3 |
| Site #3 69.5/20.8 | 0 | 1 | 1.38/35.0 | 0 | - | 1 | 0.3 |
| Site #4 68.8/20.4 | 0 | 0 | - | 1 | 0.94/24.0 | 1 | 0.3 |
| Site #5 68.8/20.4 | 0 | 1 | 1.02/26.0 | 0 | - | 1 | 0.3 |
| Site #6 68.8/20.4 | 0 | 0 | - | 0 | - | 0 | 0 |
| Site #7 68.8/20.4 | 0 | 0 | - | 0 | - | 0 | 0 |
| Site #8 68.7/20.4 | 0 | 4 | 0.81/20.5 | 0 | - | 4 | 1.3 |
| Site #9 68.8/20.4 | 0 | 1 | 0.75/19.0 | 0 | - | 1 | 0.3 |
| Site #10 68.8/20.4 | 0 | 9 | 0.82/20.8 | 0 | - | 9 | 2.9 |
| Site #11 68.7/20.4 | 0 | 2 | 0.73/18.5 | 0 | - | 2 | 0.6 |
| Total: | 1 | 175 | | 140 | | 316 | |

showing preference for warmer (Site #1) waters within a rice field.

In addition to the trapping aspects of this study, dissolved oxygen levels were monitored over a single 24-hour period (July 16-17). The probe was located in Paddy #15 about 16.4 feet (5m) eastward of the western border levee at a depth of 1.6-2.0 inches (4-5 cm). Dissolved oxygen was monitored continuously while temperatures were sampled with the same unit periodically over the 24-hour period. The results of this monitoring are presented in Figure 1. The highest levels of dissolved oxygen occurred between 1200 and 1500 PDT and the lowest occurred between 0400 and 0600 PDT. A maximum level of dissolved oxygen of approximately 17.8 ppm (mg/l) was measured at 1300 PDT, while a minimum value of only

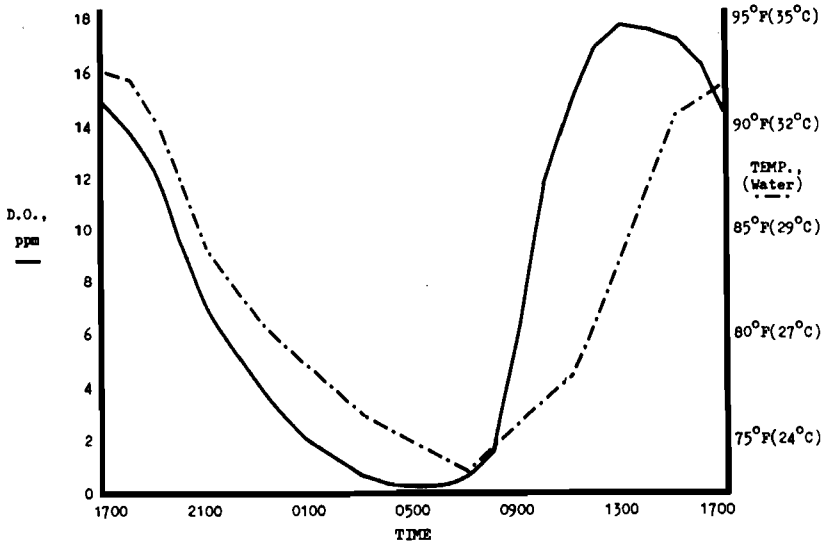


**Figure 1.** Dissolved oxygen and water temperature data collected in Paddy #15 between 1700 PDT(7/16/80) and 1700 PDT (7/17/80).

0.2 ppm was measured at 0500 PDT. The maximum recorded value (17.8 ppm) was over 2.3 times the saturation value (7.69 ppm) with the water temperature of 84°F(29°C). Supersaturation of dissolved oxygen does not appear to adversely affect mosquitofish, as measurements of over 35 ppm have been collected in wastewater oxidation impoundments having very healthy populations of mosquitofish. However, mortalities involving caged mosquitofish have been observed to occur in Sacramento Valley rice fields due to inadequate levels of dissolved oxygen. The fish may survive if they are able to reach the air-water interface as they can withstand short-term D. O. levels of less than 1 ppm if they can surface-gulp atmospheric air or take in dissolved oxygen in the very thin surface film. Caged or trapped fish in rice fields must be allowed free access to the water surface to prevent suffocation.

The results of this study and those related studies by Farley and Younce (1978, 1979) demonstrate that substantial numbers of nearly or newly mature mosquitofish do emigrate from the rice fields of both areas. Fish emigration, movements and distribution appear in at least this study to be positively correlated with water temperature. It is not possible at this time to conclude that the emigration plays an adverse role in the overall biocontrol efficacy of the fish in a particular rice field. The downstream trapping portion of this study shows that the fish emigrating from the last paddy most likely represent a net downstream movement by offspring produced in all the paddies and not merely a limited movement exclusively associated with the young fish produced in the last paddy of a serially-irrigated rice field. Results from distributional trapping within the rice paddy indicate that most of the mosquitofish prefer the open water habitats adjacent to the border levees. It also shows that fish can be found in lesser numbers throughout the rice stand as well.

It would be difficult and probably impractical to screen individual rice boxes of a field to prevent downstream fish movement or emigration. Screens used for such a purpose have in actual use been too easily plugged with debris and vegetative matter and thus required too much maintenance to keep clean. If rice farmers were to install parallel, deadend paddies fed from a common manifold ditch, this behavior might be averted entirely. However, this could prove to be expensive and impractical for the rice farmer and perhaps even detrimental to the rice production as well. It may be advisable at this time to accept this fish behavior and work to minimize the potentially negative effects. Fish traps similar to the ones employed in this study could be routinely placed below the last paddy's rice box to capture the emigrants. These fish could be reintroduced into the first paddy. Further studies might be conducted to determine if this fish redistribution results in improved biocontrol efficacy. If not, trapped fish could be moved to new mosquito sources or held for future stockings.

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# IMPACT OF THE USE OF CANDIDATE BACTERIAL MOSQUITO LARVICIDES ON SOME SELECTED AQUATIC ORGANISMS

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## ABSTRACT

The bacterial mosquito larvicides, *Bacillus sphaericus* Neide and *Bacillus thuringiensis* Berliner serotype H-14 deBarjac, when applied at rates used for mosquito control, are very safe to organisms associated with mosquito breeding habitats including natural enemies of mosquito larvae. When various aquatic organisms were exposed to the bacteria under laboratory, simulated or field conditions, no adverse effect was noted on the organisms with the exceptions of Chironomid and psychodid larvae. Chironomid larvae were slightly affected by *B. t.* H-14 treatment at a rate used for mosquito control but pyschodid larvae were only affected at higher concentration (50 ppm).

Bacterial pesticides, *Bacillus thuringiensis* Berliner serotype H-14 deBarjac, and *Bacillus sphaericus* Neide have recently been successfully tested against aquatic insect pests including mosquito and black fly larvae under laboratory and field conditions (Garcia and Desrochers 1979, Goldberg and Margalit 1977, Kellen et al. 1965, Mulla et al. 1980, Ramoska et al. 1977, Undeen and Nagel 1978). For mosquito control, they have been evaluated in natural breeding habitats such as irrigated pastures, dairy discharge water holding ponds, roadside ditches and flooded field depressions (Mulligan et al. 1978, 1980, Ramoska and Burgess 1978). However, more study on the impact of the bacterial pesticides on non-target organisms is needed (Miura et al. 1980).

The present study was carried out to elucidate the impact of *B. thuringiensis* serotype H-14 (*B. t.* H-14) and *B. sphaericus* on nontarget organisms when applied to mosquito breeding habitats.

MATERIALS AND METHODS. -Bacterial Larvicides - - *B. sphaericus* strain 1593-4 used in this study was produced and provided by Stauffer Chemical Company. It was supplied as a fermentation broth containing ca. $1 \times 10^9$ viable bacterial cells/ml. Later the company supplied a WP formulation of the bacterium; this formulation (Stauffer WP4920-34-3 Lot no. 0002613) contained ca. $2.4 \times 10^{10}$ spores/g.

*B. thuringiensis* serotype H-14 (*B. t.* H-14) was supplied by Sandoz Inc., as a flowable formulation (SAN 402 I WDC) and it contained ca. $1.5 \times 10^{10}$ viable spores/ml (692 ± 69 International Toxic Units/mg).

Simulated Field Tests - - A simulated field test against crustaceans was conducted outdoors in 20-liter capacity aquaria. A mixture of *Ceriodaphnia* sp., *Simocephalus* sp., *Moina* sp., *Alona* sp., *Cyclops* sp., *Cyprois* sp., and *Hyalella azteca* (Saussure) were maintained in aquaria containing 16 liters of water each. One aquarium was treated with the broth suspension of *B. sphaericus* ( $1 \times 10^4$ cells/ml) and one was left as a treatment check.

The mosquitofish, *Gambusia affinis* (Baird and Girard) was used in a simulated field test with *B. t.* H-14 ($5.4 \times 10^3$ spores/ml) in a metal tank (112 x 76cm surface area, 200-liter capacity) containing 160 liters of water, an identical tank was used as a treatment check.

Pretreatment and posttreatment population census for crustaceans were examined twice a week for 25 days using a method described previously by Miura and Takahashi (1973). Population counts of mosquitofish were made with minnow traps (Miura and Takahashi 1975).

Field Tests - - Each test was assigned a field test no. (F. T. no.). Since it is impractical to include all the details recorded for each test, e.g., air and water temperatures, wind velocity and direction, sky-cover, water depth, vegetation canopy, etc., this information was omitted from this report but is available to interested persons upon request.

The broth suspension of *B. sphaericus* was evaluated against a mixed population of *Aedes melanimon* Dyar and *A. nigromaculis* (Ludlow) in 0.2 ha plots (F. T. 76-4) in the Tracy Experimental plot (Miura et al. 1976) 4.75 liters of bacterial broth determined to yield final concn of $1 \times 10^4$ cells/ml, was mixed with 5.75 liters of water in a hand sprayer and applied to the surface of the water. *B. t.* H-14 was evaluated against a mixture of *A. melanimon* and *A. nigromaculis* at a rate of 1 kg/ha ($5.4 \times 10^3$ spores/ml) (F. T. 79-6).

A total of 12 ha of El Dato Loco Duck Club ponds in Kern County, California was treated with *B. t.* H-14 (F. T. 79-8). A suspension of bacterial spores was sprayed by fixed wing aircraft at a rate of 1.12 kg/ha against *Culex tarsalis* Coquillett.

A population census of planktonic organisms was taken by

dipping with a long handled dipper (450ml) from 10 semi-fixed stations. The samples were brought back to the laboratory and examined under the stereo microscopes. Pretreatment samples and samples collected immediately after treatments were held in their original water and population changes were monitored daily (Miura and Takahashi 1975).

Free-moving aquatic insect populations were sampled by trapping with modified minnow traps (Miura and Takahashi 1975). Pretreatment and posttreatment chironomid larval populations in the duck ponds (F.T. 79-8) were estimated by using a modified area sampler (Roberts and Sanlon 1974).

Laboratory Tests - - Colonies of hydra, planaria [*Dugesia dorotocephala* (Woodworth)] chironomid larvae [*Goeldichironomus holoprasinus* (Goeldi)] and psychodid larvae (*Telmatoscopus* sp.) were exposed to *B. t.* H-14 and *B. sphaericus* at rates recommended to use for practical mosquito control ($5.4 \times 10^3$ spores/ml and $2.4 \times 10^4$ cells/ml respectively). Percentages of mortality were determined at the end of 48h posttreatment, if no mortality occurred the tests were extended another 2 to 3 days.

RESULTS AND DISCUSSION.–Simulated Field Tests - - Results of the *B.sphaericus* treatment against crustaceans are shown in Fig. 1. Generally, crustaceans, especially cladocerans and amphipods, are very sensitive to most mosquito larvicides. Even insect growth inhibitor type larvicides have suppressed the populations (Miura and Takahashi 1973, 1975). However, *B. sphaericus* did not deleteriously affect the population of the crustaceans tested in the aquaria. A similar result was reported with *B. thuringiensis* H-14 (Miura et al. 1980).

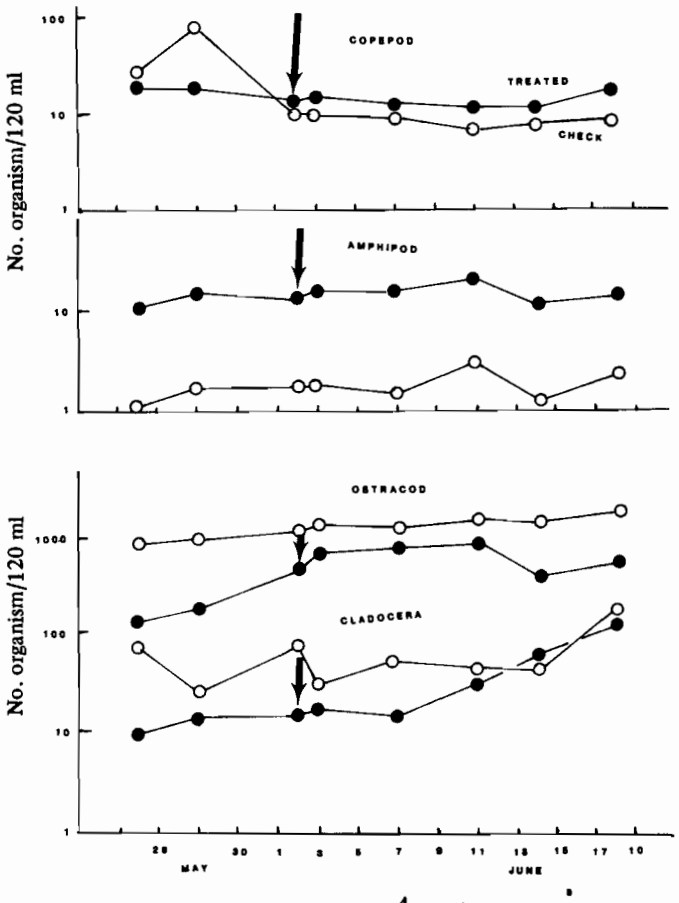


**Figure 1.** Effects of *B. sphaericus* ($1 \times 10^4$ cells/ml) against zooplankton. Arrows indicate application days.

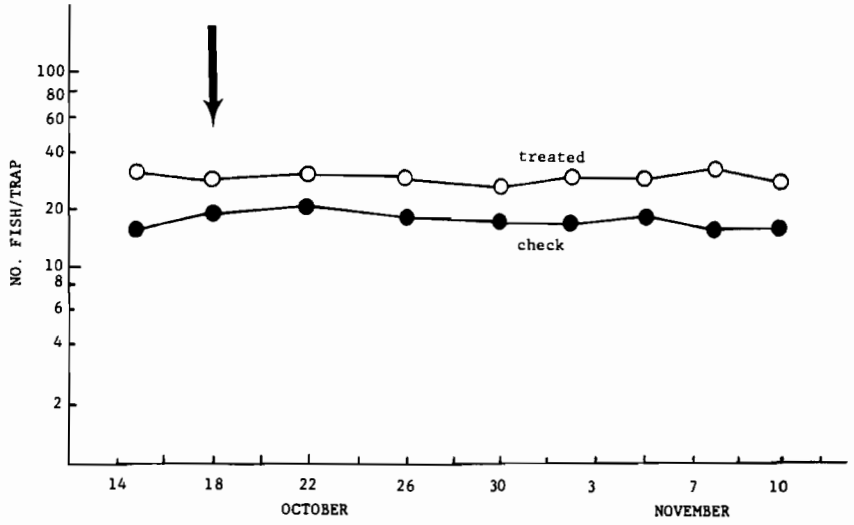


**Figure 2.** Effects of *B. t.* H-14 ($5.4 \times 10^3$ spores/ml) against mosquitofish. Arrows indicate application.

Results of the *B. t.* H-14 treatment against mosquitofish is shown in Fig. 2. No adverse effect from *B. t.* H-14 was detected by monitoring the population. This is important because mosquitofish, at present, is the only introduced biological agent widely used for mosquito control in semi-and permanent water bodies (Hoy et al. 1972). Mulligan et al. (1978) reported that there was no adverse effect on mosquitofish in a static test using *B. sphaericus*.

Field Tests - - Initially, impacts of the bacterial treatments on nontarget organisms were to be evaluated by each species. However, the number of each species collected was so sporadic they are grouped into higher taxonomic categories and impacts were examined.

Non-target organism collections pertaining to the F.T. 76-4 *(B. sphaericus*, 1 x $10^4$ cells/ml) are shown in Table 1 and 2. No adverse effect was detected by monitoring the populations. Similar results were obtained from the *B. t.* H-14 treatments (F. T. 79-6) with the exception that 2 chironomid larvae in the sample water collected immediately after treatment and kept in the laboratory for daily observations were dead (Table 3). Although, it is known that *B. t.* H-14 does affect chironomid larvae (Garcia and Goldberg 1976, Miura et al. 1980) our larval collection number is insufficient to draw any conclusion.

In order to confirm whether or not *B.t.* H-14 does affect chironomid larvae, an area sampler was used to monitor pretreatment and posttreatment population densities at the duck club pond test (F. T. 79-8); prior to the treatment, 232 larger larvae (ca. 3-4 instars) were collected from 10 semi-fixed stations and 4 days posttreatment, 56 larvae were collected from the same stations, thus, the *B. t.* H-14 treatment reduced ca. 76% of the larval population.

No deleterious effect of the *B. t.* H-14 treatment (F. T. No. 79-8) on planktonic organisms and aquatic insects was detected by sampling their populations (Table 4).

**Table 1.** Effects of *B. sphaericus* (1593-4) on nontarget organisms. Applied at 1 x $10^4$ cells/ml against *Aedes* spp. (F.T. No. 76-4).

| Organism | No. organisms in the pretreatment water (4,500ml) held in the laboratory | | | | | |
|---|---|---|---|---|---|---|
| July | 22 | 23 | 24 | 25 | 26 | 27 |
| Cladocera | 33 | 41 | 39 | 44 | 85 | 182 |
| Copepod | 9 | 11 | 18 | 25 | 39 | 201 |
| Ostracod | 3 | 3 | 2 | 2 | 2 | 2 |
| Mayfly N | 32 | 32 | 32 | 32 | 32 | 32 |
| Chironomid L | 12 | 12 | 14 | 14 | 14 | 14 |
| Beetle L | 14 | 10 | 8 | 7 | 7 | 7 |
| | No. organismsin water (4,500ml) collected immediately after treatment and held in the laboratory | | | | | |
| Cladocera | 30 | 26 | 26 | 29 | 29 | 36 |
| Copepod | 8 | 11 | 11 | 17 | 83 | 181 |
| Ostracod | 3 | 3 | 3 | 3 | 3 | 3 |
| Mayfly N | 30 | 30 | 30 | 30 | 30 | 30 |
| Chironomid L | 9 | 8 | 8 | 8 | 8 | 8 |
| Beetle L | 8 | 8 | 8 | 7 | 7 | 7 |
| | No. organisms in water (4,500ml) daily collection | | | | | |
| Cladocera | 31 | 33 | 62 | 152 | 135 | 1740 |
| Copepod | 8 | 9 | 6 | 86 | 34 | 125 |
| Ostracod | 3 | 3 | 5 | 3 | 3 | 4 |
| Mayfly N | 31 | 1150 | 1017 | 865 | 206 | 595 |
| Chironomid L | 10 | 12 | 18 | 25 | 29 | 43 |
| Beetle L | 11 | 14 | 5 | 12 | 4 | 11 |

L = Larvae
N = Nymphs
Beetle larvae = mostly *T. lateralis, Laccophilus spp. H. triangularis.*

**Table 2.** Effects of *B. sphaericus* (1593-4) on nontarget nektonic organisms (F.T. No. 76-4).

| Organism | Mean no. organisms/trap | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | check field | | | | treated field | | | |
| July | 22 | 23 | 24 | 25 | 22 | 23 | 24 | 25 |
| Notonectid | 3 | 14 | 17 | 8 | 10 | 12 | 18 | 14 |
| Corixid | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| Belostomatid | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Dytiscid A | 12 | 15 | 13 | 12 | 10 | 6 | 13 | 8 |
| Hydrophilid A | 2 | 1 | 0 | 2 | 1 | 1 | 0 | 0 |

A = Adults. Hydrophilid = *H. triangularis, T. lateralis.*
Dytiscid = *Laccophilus spp. Thermonectus spp.*

*Treatment applied on July 22, 1981.

**Table 3.** Effects of *B.t.* H-14 (San 402 IWDC) on nontargets: Applied at 1.0 kg/ha against *Aedes spp.*, (F.T. No. 79-6).

| Organism | No. organisms in the pre-treatment water (4,500ml) held in the laboratory | | | | | |
|---|---|---|---|---|---|---|
| Aug. | 8 | 9 | 10 | 11 | 12 | 13 |
| Cladocerans | 12 | 12 | 16 | 16 | 14 | 16 |
| Copepods | 2 | 2 | 2 | 2 | 2 | 2 |
| Mayfly N | 6 | 9 | 13 | 13 | 13 | 13 |
| *Copelatus sp. L* | 42 | 40 | 36 | 36 | 36 | 36 |
| Chironomid L | 2 | 2 | 3 | 3 | 3 | 3 |
| | No. organisms in water (4,500ml) collected immediately after treatment and held in the laboratory | | | | | |
| Cladocerans | 16 | 16 | 14 | 15 | 14 | 26 |
| Copepods | 2 | 2 | 2 | 2 | 2 | 2 |
| Mayfly L | 6 | 7 | 11 | 11 | 11 | 11 |
| *Copelatus sp. L* | 22 | 22 | 22 | 20 | 19 | 19 |
| *Laccophilus sp. L* | 1 | 1 | 1 | 1 | 1 | 1 |
| Chironomids L | 2 | 2 | 0 | 0 | 0 | 0 |
| | No. organisms in water (4,500ml) from daily collections | | | | | |
| Cladocerans | 8 | 12 | 15 | 25 | 320 | 250 |
| Copepods | 1 | 1 | 2 | 10 | 115 | 380 |
| Mayfly N | 6 | 121 | 53 | 153 | 466 | 380 |
| *Copelatus sp. L* | 31 | 8 | 8 | 10 | 8 | 9 |
| *Laccophilus sp. L* | 0 | 0 | 2 | 3 | 2 | 3 |
| *H. triangularis L* | 0 | 0 | 0 | 0 | 3 | 2 |
| Chironomid L | 2 | 12 | 38 | 80 | 153 | 233 |

L = Larvae
N = Nymphs

**Table 4.** Effects of *B. t.* H-14 on nontarget organisms over a 7 day period. Applied at 1.12 kg/ha against *C. tarsalis* (F.T. No. 79-8).

| Organism | No. organisms/4,500ml of sample water | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pretreatment | | Posttreatment | | | | |
| | 2 | 1 | 1 | 2 | 3 | 4 | 7 day |
| Cladocera | 60 | 110 | 115 | 185 | 150 | 185 | 148 |
| Copepod | 390 | 725 | 920 | 675 | 850 | 675 | 935 |
| Ostracod | 90 | 60 | 105 | 50 | 49 | 140 | 125 |
| Mayfly N | 20 | 6 | 12 | 16 | 46 | 38 | 34 |
| Corixid | 0 | 2 | 1 | 4 | 3 | 8 | 4 |
| Notonectid | 0 | 1 | 0 | 2 | 1 | 2 | 0 |
| *Tropistermus spp. L* | 0 | 1 | 0 | 1 | 3 | 2 | 1 |
| *Laccophilus spp. L* | 25 | 23 | 24 | 53 | 32 | 28 | 22 |
| Dragonfly N | 4 | 3 | 0 | 2 | 0 | 0 | 3 |
| Chironomid L | 3 | 4 | 0 | 4 | 2 | 2 | 16 |
| Damselfly N | 0 | 1 | 0 | 7 | 1 | 1 | 3 |

N = Nymphs
L = Larvae

**Table 5.** Effects of bacterial larvicides (*B. t.* H-14 and *B. sphaericus*) on Chironomid midge larvae (*Goeldichironomus holoprasinus*) in the laboratory.

| concn (ppm) | *B. t.* H-14 | | *B. sphaericus* | |
|---|---|---|---|---|
| | No. larvae/test | mean mortality(%)* | No. larvae/test | mean mortality(%)** |
| 0 | 24 | 0 | 16 | 0 |
| 0.5 | 22 | 77.3 | --- | --- |
| 0.5 | 44 | 84.1 | --- | --- |
| 1.0 | 69 | 75.4 | 46 | 0 |
| 1.0 | 49 | 89.8 | 42 | 0 |

1 ppm *B. t.* H-14 = 1.5 x $10^4$ spores/ml.
1 ppm *B. sphaericus* - 2.4 x $10^4$ cells/ml.

*48 hr. exposure time.
**96 hr. exposure time.

Laboratory Test - - The effect of *B. t.* H-14 and *B. sphaericus* treatments on the chironomid larvae, *C. holoprasinus*, are shown in Table 5. Only the *B.t.* H-14 treatments affected the larvae, ca. 83% of the larvae died when exposed to a 1 ppm concn of bacterium ($1.5 \times 10^4$ spores/ml). No mortality was obtained by exposure to the same concentration of *B. sphaericus*; on a later occasion, a colony of chironomid larvae (*C. stigmaterus*) was exposed to a 100-fold concn of *B. sphaericus* with no mortality.

Hydra and planaria (*D. dorotocephala*) are not seen in the treated area, but they are well-known mosquito predators (Jenkins 1964, Legner and Ya 1975, Yu et al. 1974). Therefore, laboratory colonies of these predators were treated to both bacterial larvicides at rates (1 ppm) recommended to control mosquito larvae. Neither bacterial treatments produced deleterious effects on these predators.

When psychodid larvae (*Telmatoscopus* sp.) were exposed to *B. t.* H-14 only high concn (50 ppm) produced ca. 84% mortality.

In summary, the data obtained from these studies in the laboratory and field clearly indicate that these bacteria could be used safely without causing any damage to many nontarget organisms, including important natural enemies of mosquitoes. Therefore they can be safely utilized in an integrated control program against mosquitoes. The primary objective of integrated control of pests is to use all possible means of control agents to enhance overall reduction of pest populations.

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# *LEPTOCONOPS* BITING MIDGES IN SOUTHERN CALIFORNIA AND ARIZONA

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Among the ceratopogonid biting midges, members of the genus *Leptoconops* are hematophagous, being zoophilic and ornithroprophilic in their feeding behavior. Although, not much is known on the nature and scope of autogeny in *Leptoconops* species, it is fairly safe to say that probably all species are anautogenous. These midges are vicious biters attacking humans on the exposed portions of the body, along the hairline on the head. On large animals, they feed on the underside of the belly, head region and inside ears.

In humans, the bite is not too painful initially, but within a few hours a red spot develops at the puncture site, itching and burning sensation follows. These symptoms persist for about a day or so. Many bites which are not uncommon in the infested areas can cause malaise, weakness and feverish conditions. In sensitive and hypersensitive individuals, severe allergic manifestations ensue, and these syndromes are discussed elsewhere in this symposium.

The breeding habits of *Leptoconops* midges are quite varied; they propagate in different types of semi-aquatic and terrestrial biotypes. The well known species in California are those breeding in heavy clay soils in the Sacramento and San Joaquin Valley. Another species breed in the rolling hills area of San Mateo and Santa Clara Counties where the soils develop cracks after drying in the late spring or early summer months. The well known "Bodega Black Gnat", which has a wide distribution, breeds in mud flats along the shoreline of the Bodega Bay.

In southern California coastal and inland regions, there is yet a different species which propagate in wet sandy areas of the Santa Ana River, and a closely related species breed in salt flats and mud flats in the Salton Sea Basin. Most of these species belong to the *kerteszi* complex.

In recent years, *Leptoconops* midges have become a serious pest and public health problem in the cove cities area of the Coachella Valley, in the City of Desert Hot Springs, in Sky Valley and the eastern portion of Riverside County. The emergence and production of this midge is geared to the summer rains falling on the foothills and canyons in this region. With a precipitation of one inch or more of rain these midges appear in tremendous number within 3-4 days after precipitation. This species referred to now as *L. torrens* (Townsend) is believed to be causing similar problems in Arizona (Navajo and Hopi reservations), NE Arizona, SE Arizona and New Mexico. This species probably goes into northern portions of Mexico. In all these areas the problem has become acute now because of the establishment of residential subdivisions into the infested areas.

I have noted heavy populations of *Leptoconops* midges in the Skeleton Canyon, Sulfur Draw and other canyons of the Chiracaua Mountains in SE Arizona. The biting activity of these midges was so high that it was impossible to take students and classes to the infested areas for entomological and botanical observations.

Current Status of Biosystematics. - - The systematic status of *Leptoconops* midges is in a state of flux now and no readily available characters are available for species differentiation in the *L. kerteszi* complex. In recent years, specialists have produced many new species descriptions in this complex and it is extremely difficult to precisely determine material to species in this complex. A brief chronology of the systematics of species of *Leptoconops* occurring in California is presented below.

Wirth (1952) in his monograph of the Heleidae of California, reported 3 species: *L. (Holoconops) kerteszi* Kieffer 1908, *L. (Leptoconops torrens* (Townsend) 1893, and *L. (styloconops) freeborni* Wirth 1952 as new species. All *Leptoconops* material collected from that time to about 1973 were placed in one or more of these 3 species. In 1973, Wirth and Atchley (1973) published a review of the North American species of *Leptoconops*, basing their subgeneric treatment on the work of Carter (1921) who established the 3 subgenera *Holoconops, Leptoconops, Acanthoconops = Styloconops*. In their Review, Wirth and Atchley (1973) established 5 subgenera with 13 known species of which 6 were described as new species. Among these, 8 species were reported from California of which 4 were described as new species. The California species reported were: *L. (Holoconops) belkini* n.s., *L. (Brachyconops) californicus* n.s., *L. (Leptoconops) carteri* Hoffman, *L. (Leptoconops) freeborni* Wirth, *L. (Leptoconops) kerteszi* Kieffer, *L. (Leptoconops) mohavensis* n.s., *L. (Leptoconops) torrens* (Townsend) and *L. (Leptoconops) werneri* n.s.

As a further follow-up of these studies, Clastrier and Wirth (1978) made a revision of the *L. (Holoconops) kerteszi* complex in North America. They concluded that *L. keteszi* Kieffer does not occur in North America and reported and described 11 species in the *kerteszi* complex from N. America. With the exception of *L. americanus* Carter, in this complex, the remaining 10 species were described as new species. Of the 10 new species recognized in this complex from N. America, 8 were described from material collected in California.

The California species placed in the *L. (Holoconops) kerteszi* (Clastrier and Wirth 1978) complex are: *L. americanus* Carter, *L. andersoni* n.s., *L. arnaudi* n.s., *L. asilomar* n.s., *L. atchleyi* n.s., *L. foulki* n.s., *L. knowltoni* n.s., *L. sublettei* n.s., *L. whitseli*.

At the present time, no practical keys are available for the separation of all the species in this complex. Biological and behavioral data are needed to support and confirm the specific status and the various populations that we have in California. Laboratory colonization methods and genetic studies will be needed to advance our knowledge regarding the species identity of the many biotypes of *Leptoconops* species in California.

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# ASSESSMENT OF BITING MIDGES (DIPTERA: CERATOPOGONIDAE) IN THE SALTON SEA BASIN


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The Coachella Valley of southern California (Riverside County) has a rapidly rising human population, and in the southern and eastern regions, an extensive population of domestic animals. Both are subject to various species of biting midges in the family Ceratopogonidae throughout most of the year. Following heavy rainfall in late spring, summer, and early autumn, large broods of *Leptoconops torrens* are produced near the densely populated cove cities. During the cooler months of the year, large numbers of *L. foulki* and *Culicoides variipennis occidentalis* emerge on the shores of the Salton Sea, along drainage ditches, and along seeps and springs of Riverside and Imperial Counties where the soil is permanently damp.

My objectives during the winter season have been to characterize larval development sites at the Salton Sea with the hope of developing a model for delineating larval habitats and predicting larval densities. Secondly, the host-seeking behavior and gonotrophic cycle of *L. foulki* were studied to gain an understanding of reproductive potential, flight range, and vector potential.

The area chosen for study was on the shores of the Salton Sea at North Shore where Foulk (1969) reported large numbers of adults in a bimodal frequency-distribution with populations peaking in mid-morning and again in mid-afternoon. The distribution of immatures was determined by extracting life stages from cores of soil (5.5 cm. dia x 5 cm. deep) using saturated sodium chloride solution (Horsfall 1956). This technique routinely recovers 85% of the immatures present in the core. Eleven samples were taken every 5.5 m along each of 3 parallel transects extending inland from the Salton Sea shoreline. The elevation of each sample was measured with a Craftsman transit to the nearest mm in relation to the water level of the Salton Sea and the level of free soil water; the latter being determined by digging numerous holes at strategic locations. Following a preliminary survey for larvae, replicated soil samples from each zone were collected for soil chemistry analysis, performed by the UCR Cooperative Extension laboratory.

This study identified 4 ecological zones. The first, a relatively narrow band at the ecotone of the Sea and shoreline, contained immatures of *C. variipennis occidentalis*. A second zone was characterized by saturated soils. In such areas, larvae of *Dasyhelea* spp., a non-hematophagous ceratopogonid were collected in large numbers. When such sites were adjacent to the shoreline, some overlap with *C. variipennis occidentalis* was noted. *L. foulki* larvae were found in soils above the level of free soil water. The inland margin of this zone was not easily delineated by visual inspections; however, the communities of vegetation generally described the distribution of *Leptoconops*. As Iodine bush (*Allenrolfea occidentalis*) became sparse and arrowweed (*Pluchea sericea*) began to dominate, the density of larvae dropped rapidly. The 4th zone was characterized by nearly pure stands of arrowweed, and no larval ceratopogonids were found in soil samples.

Soil chemistry analysis revealed some trends that vaguely

correlated with distribution of larval ceratopogonids. Readings of pH varied without an obvious pattern, however, values of free chlorides and electrical conductivity increased with increasing distance inland. Other parameters found not to be significantly different between zones were percent organic matter and the soil particle size.

Having delineated the macrohabitat of *L. foulki*, the microhabitat was next studied by taking 15 contiguous samples (each replicated three times) well within the *L. foulki* zone. This transect extended from a depression to the top of a small hummock. As previously described, the elevation of each sample was measured and additional samples were collected for soil chemistry analysis.

Once again, trends in soil chemistry were evident but unsatisfactory in describing larval density. However, larval numbers correlated with elevation above the free water line ($r=-.90$). This is an indirect measurement of soil moisture. It now seems likely that this measurement, combined with patterns of vegetation, will enable us to quickly identify and evaluate development sites of larval *L. foulki*.

The reproductive bionomics of adults was also studied at this location. The vertical and horizontal distribution of host-seeking midges was determined using paired dry ice baited CDC traps placed at ground and vegetation canopy levels (2 m). Pairs of traps were placed 5, 50, and 120 m inland and operated during morning hours and again during the afternoon. Midges were counted and 5-10 *L. foulki* were randomly selected from each of the 6 traps and were dissected to determine the physiological age of the population by time collected. This was done by examining the ovaries for follicular relics (Detinova 1962), the presence of which indicate that oviposition has occurred and that the female is therefore parous. The spermathecae were crushed to determine if mating had occurred and the stage of ovariole development was recorded (Christophers 1911). The remainder of the midge was crushed in cold anthrone reagent (Van Handel 1972). If nectar was present in the crop, the reagent changed color from yellow to deep blue within 20 minutes. These procedures were initiated in March 1981 and are being continued at monthly intervals.

Several strong trends have persisted to date. Only 2 species of biting midges have been present in large numbers during the day. Both *L. foulki* and *C. variipennis occidentalis* were more abundant in ground level traps regardless of weather conditions or distance from the Salton Sea. More females have also been captured 120 m inland than 5 m inland.

Because of Foulk's studies, and because the summer pests are also of this genus, only females of *L. foulki* were dissected. A distinct difference was seen in the "age" of the AM versus PM populations. In March and April parous flies were rare in the morning (<10%) but abundant in the afternoon (>35%). These differences were statistically significant on each of the 3 sampling dates. Furthermore, virtually all females were mated, and no nullipars exhibited ovarioles beyond the resting stage, indicating an obligate anautogenous reproductive strategy (Magnarelli and Cupp 1977). No significant difference was noted in the prevalence of nectar-positive females between AM and PM populations; at least 35% of the population had imbibed nector relatively recently.

In May, the age structure shifted. Parous midges constituted over 70% of both AM and PM populations, indicating that the number emerging had diminished greatly. In addition, the proportion of nectar-positive females had increased to greater than 60%.

What is the significance of these findings? First, a distinct pattern in the behavior of *L. foulki* is clear; a pattern that warns of vector potential. It is now apparent that this species seeks nectar and is fairly successful in obtaining it. With these nectars, *L. foulki* can disperse beyond the larval site in search of blood, and can survive beyond the first gonotrophic cycle. In fact, examination of the ovaries has shown that parous flies are not only common, but concentrated during the afternoon hours and late in the season. Consequently, if this species is a vector of pathogenic agents, suscepts are more likely to become hosts in the afternoon rather than morning. Information taken from the literature reveals that this species readily attacks man, as well as domestic and wild animals. Therefore, although no study has rigorously examined the role of *Leptoconops* in transmitting pathogens, based on these findings, one cannot discount the possibility that it may be involved in the cycle of animal or zoonotic diseases. With the human population of Coachella Valley increasing, and the presence of domestic livestock in large numbers, further rigorous studies are warranted.

Finally, it may be possible to quickly identify larval sites and to predict population levels by a combination of vegetation communities and elevation above free soil water. If so, larval control may be practical.

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# THE EFFECTS OF THE BITE OF *LEPTOCONOPS TORRENS*[1] ON HUMANS

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This lecture describes an episode of *Leptoconops torrens* bites during which many people were attacked. I shall emphasize the weather conditions which precipitated the emergence of *L. torrens*, the response of the human skin to the bite, the clinical course of the disease, the histopathology of the bite and the possibility of specific desensitization.

The epidemic of midge bites occurred in the vicinity of Palm Springs which lies in the Coachella Valley, one of the low inland desert valleys of Southern California. During the summer of 1979 tropical storms deposited heavy rains during one day in July (2.5″) and again on one day in August (1.69″). This was the only rainfall during those two months. The subsequent flooding of the soil precipitated a massive emergence of adult midges. This, apparently, is not in agreement with previous observations on the valley black gnat that drying of the soil favors the larvae to pupate (Smith and Lowe, Hilgardia 18:177, 1948.)

We could not tell how many people were bitten during the epidemic of July and August of 1979, However, inhabitants were forced to curtail outdoor recreational activities and many construction workers had to leave jobsites. The misery caused by the bites was so widespread the front page articles appeared in the local newspapers describing the midge and assuring the populace that the infestation would soon abate.

The bites of the midge, *L. torrens*, produced 3 types of reaction on the skin; urticarial weals, 2-3mm papules at times topped by vesicles, and hard papules as large as 1.0cm in diameter. The weals disappeared within hours. However, in some patients the indurated papules lasted for months itching intensely all the while. Some patients exhibited all 3 types of lesions, and each type might blend into the other.

Undoubtedly, in the majority of cases, gnat bites produced only small weals which disappeared quickly without treatment. Only patients with the most severe reactions came to the physician's office or the hospital emergency room. Still, persistent papular reactions were common.

Midge bites are supposed to cause immediate pain, while the bite of the blackfly does not, because the blackfly (*Simulium*) injects an anesthetic into its prey (Frazier CA, Cutis 19: 439, 1977). Some patients did say that the bite of the midge produced immediate pain. However, others denied this. They did not know when they were bitten. To my knowledge there were no patients who had systemic reactions following midge bites. Thus the bite of the midge may differ from that of the blackfly which can cause "blackfly fever".

Patients testified that insect repellents did not deter midges. Although I used oral and parenteral steroids as therapy, I cannot say that they shortened the the disease process.

I shall discuss 3 cases in detail.

The first case was a 63 year old man who noted itching of the trunk 2 days prior to being seen. He had been fishing on one of the local lakes and remembered being bitten. There were papulo-vesicles scattered over the chest. Histologically, there was a superficial perivascular infiltrate of lymphocytes and histiocytes, while deeper the infiltrate was composed of eosinophils.

The second case, a 48 year old woman, had 3-5mm papules over the trunk. Histologically, the epidermis was ulcerated at the bite site. Again the infiltrate was composed of lymphocytes and histiocytes superficially, and eosinophils in the deeper dermis and fat. I biopsied a 2 week old bite. Only lymphocytes and histiocytes surrounded the superficial vessels. There were no eosinophils and no deep infiltrate.

The third case, a 75 year old man, had rock hard papules over the arms legs and back. Histologically, the picture was the same as the first 2 patients, round cells superficially and eosinophils in the deep dermis and fat. I biopsied a 3 week old bite. There were fibroblasts and fibrosis present, signs of early scar formation. However, a year later he had no visible scars.

I have not been able to obtain any previous publication describing the clinical features and histopathology of midge bites. There have been a few studies on the dermatologic effects of the blackfly. The histopathology seems to be similar to *L. torrens* bites. Eosinophils are a common histologic finding in arthropod bites. However, the intense eosinophilia found at *L. torrens* sites was unique.

The antigen injected by a biting insect produces immediate type hypersensitivity. IgE is the antibody involved. After sensitization a subset of B cell lymphocytes manufactures specific IgE which attaches to mast cells. Antigen injected with a subsequent bite cross links IgE antibody on the mast cell membrane which triggers the mast cell granules to release histamine and other anaphylactic mediators.

Histamine causes vascular dilatation (the clinical erythema) and escape of serum into the tissue (the weal and papule). The mast cell granules also release an eosinophilic factor of anaphylaxis which attracts eosinophils. Enzymes released by eosinophils do damage helminths in the gut, but also degrade histamine and , by feed back, dampen the whole inflammatory reaction.

Unlike the mosquito and blackfly which pupate in water and can be controlled by spraying, midges have eluded mass control; nor is specific desensitization possible because so little antigen can be obtained.

[1]Taxonomic position is still under study.

# BITING MIDGES (*CULICOIDES OCCIDENTALIS*) AT BORAX LAKE, CALIFORNIA

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Borax Lake is located within the city limits of Clearlake (Lake County, California) at an elevation of 406 m. The lake is in an interior drainage system (has no outlet) with a watershed of 6.5 $km^2$ (Wetzel 1964). During the last 20 years the maximum depth has varied from 0.5 to 3.7 m and the area of the lake has varied from 34 to 55 ha. Borax Lake experiences considerable seasonal and yearly variation in some parameters. During normal years the lake has a conductivity range of 3,000 to 130,000 micromhos/cm, a pH range of 9.2 to 10.0, a total alkalinity range of 1000 to 5000 parts per million (ppm), a water temperature range of 5 to 34°C, and a boron concentration range of 200 to 900 ppm. Borax was mined from the lakebed during the 1860's.

The primary biting midge which breeds in Borax Lake is *Culicoides occidentalis* of Downes, 1978 (=*Culicoides variipennis* of Wirth & Jones 1957). Adult females deposit their eggs on moist algae or detritus in the eulittoral zone of Borax Lake and also on floating algae (primarily *Ctenocladus circinnatus*) in the lake.

Benthic samples from Borax Lake indicated the larvae were concentrated near the shoreline (Table 1). Additional studies near the shoreline were conducted by taking small core samples (as per Kelson et al. 1980) at 0-3 and 3-6 cm above, and 0.3 and 3-6 cm below the waterline at various Borax Lake locations. These daytime samples indicated larvae were most abundant just below the water line and pupae were most numerous just above the waterline during the spring (April-June). It is possible that diel or seasonal changes occur in this distribution pattern at Borax Lake.

The *C. occidentalis* apparently do not undergo a true diapause but experience a winter quiescence (as defined by Chapman 1971) in this area. Late-instar larvae collected during January and held in the laboratory at 25°C pupated and started emerging within 10 days. Adult *C. occidentalis* have been collected from Borax Lake in February. Typically, significant adult emergence begins in March and continues through November (Kelson et al. 1980).

There are few livestock within the flight range of the midges from Borax Lake, so vectoring bluetongue virus (Luedke et al. 1967) is not a major problem in the area. However, residents of nearby communities are bitten by the gnats which occur in high densities. A New Jersey light trap in a residential area 1.6 km from the lake sometimes collects more than 50,000 *C. occidentalis* per trap-week.

**Table 1.** Number of *C. occidentalis* larvae per $cm^2$ collected by Ekman dredge during a transect at Borax Lake on April 15, 1977.

| Water depth (cm) | Total larvae | Late instars* | Early instars |
|---|---|---|---|
| 0 (West shoreline) | 1.83 | 1.47 | 0.36 |
| 20 | 0.39 | 0.25 | 0.14 |
| 40 | 0.08 | 0.03 | 0.05 |
| 60 | 0.03 | 0.02 | 0.01 |
| 80 | 0.13 | 0.10 | 0.03 |
| 100 | 0.03 | 0.03 | 0.00 |
| 120 (Center) | 0.12 | 0.11 | 0.01 |
| 100 | 0.06 | 0.03 | 0.03 |
| 80 | 0.03 | 0.01 | 0.02 |
| 60 | 0.19 | 0.09 | 0.10 |
| 40 | 0.34 | 0.16 | 0.18 |
| 20 | 0.61 | 0.44 | 0.17 |
| 0 (East shoreline) | 5.28 | 4.84 | 0.44 |

*"Late instars" are larvae retained by a brass sieve having 19.7 meshes/cm.

Chemical control of the *C. occidentalis* is difficult at Borax Lake due to the effects of the alkaline waters on pesticides (Apperson 1975). The most promising new larvicides are pyrethroids. Permethrin and fenvalerate have $LC_{90}$ values of 13 and 28 ppb, respectively, in 24 hr laboratory tests. However, small scale field studies have indicated there are some formulation and/or stability problems which reduce their efficacy in the alkaline waters.

Insect growth regulators have shown poor efficacy against *C. occidentalis* (Apperson and Yows 1976). A recent small scale field trial in cylinders (as per Kelson et al. 1980) indicated that even with an initial water concentration of 10.0 ppm, MV-678 provided only a 7% reduction in emergence.

Any chemical which could improve water quality so that effective predators or competitors could survive in Borax Lake would be of interest. A flocculating agent or complexing agent which would tie up boron (primarily $Na_2B_4O_7 \cdot 10H_2O$) might be useful. However, in laboratory tests the survival time of mosquitofish in Borax Lake water was not significantly increased by the addition of any of several suggested agents, including 1,2-propanediol, 1,2-butanediol, 1,2-hexanediol, or $NH_4Al(SO_4)_2$ when tested at various concentrations (up to 25,000 ppm).

A physical control possibility at Borax Lake involves the pupae. At times the *C. occidentalis* pupae occur in high densities on the surface of the mud just above the waterline where it appears they could be destroyed by mechanical or thermal treatment. However, adults were found to emerge from a test area which had been flame-treated. This suggested that additional pupae might occur below the pupae visible on the surface. Sectional coring (method of Anderson et al. 1980) revealed that 79% of the pupae occurred in the top 2 cm of the mud (Table 2), but enough pupae occurred at greater depths to indicate a surface physical control method would not sufficiently reduce the *C. occidentalis* population.

**Table 2.** Abundance of immature *C. occidentalis* in mud 0-3 cm above shoreline at Borax Lake on May 1, 1980.

| Depth in mud (cm) | Pupae (number/dm$^3$) | Larvae (number/dm$^3$) |
|---|---|---|
| 0-1 | 487.6 | 82.8 |
| 1-2 | 152.0 | 29.9 |
| 2-3 | 39.7 | 7.8 |
| 3-4 | 16.7 | 5.4 |
| 4-5 | 20.6 | 3.4 |
| 5-6 | 30.9 | 5.9 |
| 6-7 | 10.8 | 4.4 |
| 7-8 | 11.8 | 3.4 |
| 8-10 | 9.3 | 1.2 |
| 10-12 | 11.3 | 0.2 |

**Table 3.** Invertebrates collected from Borax Lake.

| Organism | Month collected |
|---|---|
| *Culicoides occidentalis* | Jan-Dec |
| *Hydropyrus hians* | Jan-Dec |
| *Diaptomus sicilis* | Mar-Nov |
| *Moina hutchinsoni* | Mar-Nov |
| *Corisella inscripta* | Nar-Nov |
| *Hexarthra* sp. | Apr-Nov |
| *Tabanus punctifer* | June-Oct |
| *Bezzia glabra* | July-Aug |
| *Cyclops* sp. | Mar-June |
| *Enochrus conjunctus* | Apr-June |
| *Eulalia tumida* | Apr-June |
| *Lepadella* sp. | May-June |
| *Notonecta shooteri* | Nov |
| *Notonecta unifasciata* | Mar-June |
| *Saldula pallipes* | Mar-May |
| Chironomidae | Apr-May |
| *Culex tarsalis* | Apr-May |
| Heteroceridae | May |
| Ostracoda | May |
| *Bosmina* sp. | Mar |
| *Gerris incognitus* | Mar |
| *Tarnetrum corruptum* | Mar |
| *Culiseta incidens* | Mar |
| *Culiseta inornata* | Mar |
| *Agabus* sp. | Mar |
| Eristalinae | Mar |
| *Hygrotus* sp. | Mar |
| *Ochthebius rectus* | Feb-Mar |
| Hypogastruridae | Feb |

Natural biological agents have not provided adequate control of the *C. occidentalis* at Borax Lake. Several invertebrate competitors or predators of the *C. occidentalis* occur in Borax Lake during the spring when the fresh runoff waters have improved the water quality (Table 3). However, as higher temperatures and evaporation increase the salt concentrations and pH of Borax Lake during the summer, most of these populations are reduced or eliminated.

Laboratory studies yielded larval $LC_{90}$ values of >1.0 ppm for both *Bacillus sphaericus* (Strain 1593-4) and *B. thuringiensis* var. *israelensis* (Bactimos™ strain). Small scale field studies also demonstrated low efficacy, perhaps because the non-filter-feeding *C. occidentalis* has a low probability of ingesting a lethal dose of these materials.

Borax Lake is fishless, so a vertebrate which would prey on aquatic invertebrates should reduce the *C. occidentalis* density. However, most species of fish collected from a variety of saline and/or alkaline habitats are unable to acclimate to Borax Lake water (Kelson et al. 1980). In recent tests fish have been placed in aquaria containing 10% Borax Lake water and the concentration has been increased 10% every thirty minutes. This procedure permits comparison of fish collected at different locations and different times. The only fish species tested to date which have been able to survive these increasing concentration experiments until they are in 100% Borax Lake water are the Alvord chub (*Gila alvordensis*), the Borax Lake (Oregon) chub (*G. boraxobius*), the Amargosa pupfish (*Cyprinodon nevadensis*), the Salt Creek pupfish (*C. salinus*), and the Cottonball Marsh pupfish (*C. milleri*).

*Cyprinodon milleri* and *C. salinus* are of interest because these species were collected from waters (in Death Valley, California) with high (>45 ppm) boron concentrations and high (>45,000 micromhos/cm) conductivities (in March 1981). These euryhaline species are feeding generalists (Soltz and Naiman 1978) and readily feed on larvae and pupae of *C. occidentalis* in the laboratory. Further studies of the abilities of these fish to reproduce in water from Borax Lake are in progress.

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# *CULICOIDES VARIIPENNIS* AND BLUETONGUE DISEASE IN CALIFORNIA[1]

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Bluetongue (BT) is an arthropod-borne virus disease of domestic and wild ruminants. This disease was first described in South Africa in the 1930's, but it was not until 1944 that BT virus transmission was linked to *Culicoides* gnats (Du Toit 1944).

BT virus causes clinical illness more in sheep than in other domestic ruminants with symptoms of fever, edema of the ears and head ("big head") and oral and nasal tissues ("bluetongue"), as well as inflamation of the hooves with resulting lameness. In BT-infected cattle there usually are no clinical signs except in severely affected animals which may show abnormal nasal discharge and ulcerative stomatitis ("sore muzzle") and a stiff gait from inflammed hooves; more serious are reproductive abnormalities such as abortions, and deformed and unthrifty newborn calves. Recent studies have shown that BT virus may be transmitted in bull semen and that fetal exposure can result via placental transmission from BT-infected cows (Luedke et al. 1975, 1977).

Currently, there are 20 serotypes of BT virus worldwide but only types 10, 11, 13, and 17 are known to occur in the United States and all four serotypes occur in California. Bluetongue vaccines are available for sheep only and contain serotypes 10 and 17. There is little or no cross protection of these vaccines to serotypes 11 and 13. Since the vaccines contain attenuated live virus, the subsequent viremia following inoculation of sheep may be of sufficient titer to be acquired by the gnat vector with possible later transmission to non-vaccinated animals.

Following a statewide epizootic of BT in sheep in 1977 and completion of a national survey of BT in cattle in 1978 by APHIS, USDA, research funds became available to conduct a more thorough epidemiological study of this disease. Time does not permit a review of this total research effort, but results to date from investigations in California must be credited to cooperative staff in a number of agencies -- among these are the Arthropod-borne Animal Disease Research Laboratory at Denver, CO. (USDA), the Bureau of Animal Health, California Department of Food and Agriculture, the California Department of Fish and Game, the School of Veterinary Medicine, Univerity of California at Davis, private practitioners in the California Veterinary Medical Association, and several California Livestock Associations.

The primary objectives of our research are to: 1) develop simpler, more efficient and rapid procedures for BT diagnosis, 2) define the incidence of BT infection in sheep, goats, cattle, and wildlife on a statewide basis in order to better define reservior hosts of this disease, 3) develop means of controlling and eventual eradication of BT infection from sheep, goats, and cattle, and 4) develop control measures to minimize and eventually eliminate direct and indirect losses due to BT for the livestock industries in California. Objectives 3 and 4 include studies on the biology, surveillance and eventual control of *Culicoides* gnat vectors.

Results from blood samples tested for virus isolations, August 1978 through March 1981, are shown in Table 1. Bloods from all hosts show the predominance of serotypes 11 and 17, followed by types 13 and 10. Similar serotype results are evident from sheep and cattle bloods since these hosts reflect over 76% of the total animal bloods tested. Although few BT isolations were made from blood samples on wildlife, sufficient data show that antelope and elk are infected with more than one BT serotype and can serve, like cattle, as important reservoirs of this disease (Osburn et al. 1981).

The geographical distribution of positive BT isolations by hosts in California include sheep and cattle sampled from 24 counties; from Modoc and Siskiyou Counties in the north, through the length of the Central Valley and extending through San Bernardino, Riverside and Imperial Counties in the southern region. Coastal counties involved are Humboldt, Mendocino, Solano, San Luis Obispo, and Santa Barbara. BT isolations in goats represent herds sampled in Stanislaus, Mer-

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**Table 1.** BT Virus isolations from domestic livestock, wildlife, and *Culicoides variipennis*, CA. 1978-1981 1/

| Species | Total No. Blood Samples Tested | Total No. Virus Isolations | BT Virus Isolations By Serotypes (%) 10 | 11 | 13 | 17 |
|---|---|---|---|---|---|---|
| BLOOD | 18,878 | 299 | 28(9) | 145(48) | 52(17) | 74(25) |
| Bovine | 4,736 | 104 | 5(5) | 49(47) | 22(21) | 28(27) |
| Ovine | 9,713 | 173 | 21(2) | 92(53) | 21(12) | 39(22) |
| Caprine | 3.584 | 13 | 1 | 3 | 4 | 5 |
| Antelope | 107 | 2 | 1 | | | 1 |
| Bighorn Sheep | 50 | | | | | |
| Deer | 130 | 1 | | | | 1 |
| Elk | 155 | 6 | | 1 | 5 | |
| (Culicoides gnats) | (240) | 5 | | 3 | 2 | |

1/ Update to data, by Osburn *et al.* (1981).

ced and Madera Counties only, whereas positive isolations were made from antelope and deer in Modoc County and from elk in Inyo County. Seasonal distribution for all hosts show that the 4 serotypes are particularly prevelant in animal bloods taken from July through November with little or no isolations made during the first 6 months of each calendar year. These data support the occurrence of typical epizootics of BT in sheep in late summer and early fall when the vector gnat populations are reported to reach dense numbers.

*Culicoides* vector studies were initiated in 4 endemic BT areas of Butte, Imperial, Sonoma, and Stanislaus Counties. The objectives included in these studies are 1) to determine seasonal adult population data on *Culicoides* species, 2) to identify *Culicoides* breeding sources for evaluation of immature population densities, and 3) to collect live *Culicoides* for virus isolation and BT serotype infection rate tests at U. C. Davis and the USDA laboratory at Denver, respectively. To-date, serotypes 11 and 13 have been isolated from *C. variipennis* collected during September and October in Butte and Stanislaus study areas. Laboratory tests by the Denver research group have shown infection rates (IR) for all 4 virus serotypes in *C. variipennis* collected in Imperial and Stanislaus areas, IR for serotypes 10, 11, and 13 in *C. variipennis* from the Butte area, and IR for types 10 and 11 from this species taken in the Sonoma area.

The use of CDC light traps, and vehicle-mounted net traps in all 4 study areas has shown *C. variipennis* to be the predominant species. *C. crepuscularis, C. freeborni, C. (Selfia)* spp., and *C. stellifer* have been collected in Butte, Sonoma, and Stanislaus Counties but in too few numbers to warrant evaluation of population densities. In the Imperial Valley, *C. calexicanus, C. mohave, and C. saltonensis* were collected in light traps operated in desert regions adjacent to pastured livestock but *C. variipennis* was still the predominant species.

Seasonal adult population data for *C. variipennis* was established in Butte, Sonoma, and Stanislaus study areas from 1979 through 1980. In general, adults first appeared in April and gradually reached peak densities from August through September with a sharp decrease after October. In the Stanislaus area, however, adult densities continued through the winter months and declined during February. No absolute cause was determined for this unusual extension of winter adult activity in this area. *Culicoides* light trap studies in 1966-67 in Kern County showed the prevalence of adult *C. variipennis* (64%) of 6 species collected; in decreasing order of adult densities these were *C. crepuscularis, C. freeborni* and *C. posoensis, C. haematopotus*, and *C. (Selfia)* spp. (Nelson and Bellamy 1971).

Earlier studies by other investigators have shown a wide range of development sites for the immature stages of *C. variipennis*; from fresh-, salt-, and alkaline-water soil habitats. Polluted water sites, however, are reported to favor the occurence of *C. variipennis* (Wirth and Jones 1957, Jones 1961). The results from larval habitat surveys made from 1978 through 1979 in California have verified the versatility of sites which produce *Culicoides*. These can be classified into natural, agricultural, industrial, and domestic water sources. Sites in these habitats were identified by collections of immature stages in substrate samples taken from the soil-water interface of suspected habitats. Natural habitats included margins of salt water sources such as Borax Lake, or along the Salton Sea and in brackish water of wildlife refuge ponds, and in gently-sloped muddy banks of rivers and creeks. Agricultural habitats consisted mainly of man-made water impoundment facilities which included dairy waste water lagoons, dairy waste water discharged into pastures, orchards, ditches, and natural water channels, ponded water inside cattle feedlot pens, water trough overflow areas including animal hoofprints, low spots in animal-occupied irrigated pastures, and drainage canals receiving crop irrigation runoff water. Industrial habitats included collections of oil well runoff water, oxidation ponds connected to runoff water from sewage treatment plants, and other water impoundment areas subject to flooding from commercial washing of transportation equipment. Urban habitats consisted chiefly of waste water runoff from homes, ponded water from lawn and garden irrigation, and shallow water impoundment areas in back-yards holding a mixture of domestic livestock (horses, sheep, pigs) and poultry.

Organic-contaminated water impoundments such as dairy lagoons consistently produced the highest densities of *C. variipennis*. These findings, therefore, coincide with the repetitive large numbers of BT virus isolations from blood tests in indigenous dairy animals. As a result of these larval habitat investigations, a statewide dairy waste water survey was conducted in 1980. One hundred thirteen (113) dairies were sampled at the start and ending of summer within the Central Valley and Coastal and Southern regions. Fifty-five percent (55%) of the dairy lagoons were found to support *Culicoides* development in the early summer while only 40% of these sources were *Culicoides*-positive in the fall months (Table 2). Quantitative analysis showed some lagoons to continually produce dense immature populations while others converted from dense to light or remained totally free of immature gnat populations. The reasons for this variation involves a complex of waste water and solids management practices and lagoon construction which warrant further study. Also, records from local mosquito abatement districts showed routine application of oil and/or chlorpyrifos (Dursban) for mosquito control in

many dairy waste water lagoons. The relationships of these chemical applications for indirect control of *Culicoides* is not clear although a pilot study on one dairy demonstrated that chlorpyrifos granules or solutions at ½ lb a.i./acre of dairy lagoon margins controlled adult *C. variipennis* emergence.

**Table 2.** Culicoides gnats found in dairy lagoons, CA 1980.

| Region (No. Counties) | May-July No. Dairies: Insp. | Positive(%) | Sept.-October No. Dairies: Insp. | Positive(%) |
|---|---|---|---|---|
| Sacramento Valley (5) | 13 | 7 (54) | 16 | 6 (38) |
| San Joaquin Valley (5) | 75 | 41 (55) | 25 | 8 (32) |
| Coastal (3) | 14 | 4 (29) | 6 | 3 (50) |
| Southern (2) | 11 | 10 (91) | 6 | 4 (66) |
| 4 Region Total | 113 | 62 (55) | 53 | 21 (40) |

The implication of dense *Culicoides* breeding in organic contaminated water impoundment areas relates closely to the more extensive operational programs now conducted by many mosquito and vector control agencies. Cooperative studies are needed to determine the application of physical, cultural, and chemical control strategies to help minimize adult *Culicoides* production from such water sources with ultimate reduction in BT disease transmission.

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# LABORATORY OBSERVATIONS OF THE DEVELOPMENTAL BIOLOGY OF *ANOPHELES FREEBORNI* AITKEN

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## ABSTRACT

Observations were made on various developmental phenomena in a recently established laboratory colony of *Anopheles freeborni* Aitken. Indications are presented for overall developmental rates including: mean time of occurrence, duration, and survivorship of the aquatic stages, differential developmental rates for male and female; and length of the gonotrophic cycle and mean fecundity per cycle.

INTRODUCTION:—Since Freeborn's initial studies of California anophelines (Freeborn, 1926) only one major attempt to colonize and mass rear *Anopheles freeborni* Aitken in a laboratory situation (Hardman 1947) has appeared in the literature. To further research endeavors at the University of California at Davis on the ecology of *An. freeborni* a laboratory colony of this mosquito was established from the progeny of approximately 3500 gravid females collected from a variety of mosquito resting sites in Sacramento and Yolo Counties from May-July 1979. After the tenth generation of development in the laboratory, a series of observations was initiated on the developmental biology of *An. freeborni* in a laboratory situation.

MATERIALS AND METHODS:—Insectary: The following methods have proven to be quite satisfactory for mass rearing *An. freeborni* in a laboratory situation. The insectary is illuminated with four double banks of fluorescent lights timed for a constant photoperiod of 14½ hours. In addition a 40 watt incandescent lamp is timed to provide a dawn and dusk period of ½ hour prior and subsequent to the overhead light period. The room is maintained at 30° ± 2°C and humidified with a cold humidifier to a minimum of 40% R. H. Larvae are reared in rectangular enameled pans of 24cm x 40cm x 6cm in 3 liters of water (equal parts tap and distilled water for a conductance range of 150-200 micro-ohms/$cm^2$). Larvae are fed daily a finely ground mixture of equal parts by weight Tetra Min fish-food and guinea pig chow. Pupae are removed from the larvae pans with a large bore pipet and transferred into small plastic cups filled with 125 ml of water for emergence in adult cages. Adults are maintained in .03 $m^3$ screen sleeve cages wrapped with plastic lining. A 10% sucrose solution in 150 ml flasks dispensed through cotton wicks provides the carbohydrate source; a supplement of raisins is also made available in the cages. The pupal emergence cups provide oviposition sites for the females after blood-feeding. The blood source is closely-shaved guinea pigs held tightly restrained in cylindrical wire cages. Eggs are harvested daily and transferred into enameled pans.

Developmental Biology Studies: Several sets of observations were performed in this insectary situation. The first concerned overall development from egg to adult, and entailed counts at 24 hour intervals of the occurrence of the various life stages beginning with a known number of eggs. All counts were performed in situ to minimize any deleterious effects from handling. Three counts were made for each and the average recorded. One replicate of 4 pans was performed at a density of 50 eggs per pan, 3 replicates of 4 pans were performed at a density of 100 eggs per pan. Each pan was provided the following feeding regimen:

| day after oviposition | quantity of food |
|---|---|
| 1 | 40mg |
| 2 | - |
| 3 | - |
| 4 | 20mg |
| 5 | 20mg |
| 6 | 40mg |
| 7 | 40mg |
| 8 | 50mg |
| 9 | 100mg |
| 10 | 100mg |
| 11... | ... decreasing with increasing pupation |

Pupae from each replicate were pooled into a single emergence cage and counts of adults were performed at 24 hour intervals; these counts were continued for 2 weeks after final pupation.

The second set of observations concerned the rates of pupal eclosion and relative proportions of male and female emergence. Four replicates of 200 eggs were reared in enamel pans.

Pupae were removed and segregated into adult cages according to age at pupation. Counts by sex of adult emergence were recorded at 24 hr intervals.

The third set of observations involved the length of the gonotrophic cycle from blood-meal to oviposition. Eight replicates of 100 blooded females were set up in .03 $m^3$ cages and provided with oviposition sites. Counts of eggs deposited were performed at 24 hour intervals under a magnifying viewer, taking 3 counts for each and recording the average. Two days after oviposition had ceased, a random sample of 10 females from each replicate was dissected and spermathecae examined for insemination.

The fourth set of observations concerned fecundity. One hundred 3 day old, nulliparous females were blooded and isolated in individual large, foam-stoppered test tubes provisioned with raisins suspended on hooks, a thin strip of absorbent paper which was saturated with water daily using a wash bottle, and a section of applicator stick on which the mosquito could cling. Eggs were counted as before. All females were dissected after oviposition or death to determine insemination.

RESULTS.–Results of the observations of overall development are summarized in Fig. 1 and Tables 1-3. Of the initial 1400 eggs, 704 reached adulthood for an approximately 50% survivorship. These surviving mosquitoes had all emerged as adults within 17 days after oviposition (Fig. 1). Of the survivors 50% had reached adulthood by the 12th day after oviposition with a weighted average of 13 days.

Successful egg hatch was determined by cumulative larval counts which reached their highest numbers, (i.e. 1055) on the fifth day after oviposition (Table 1). Most of the eggs successfully developing to 1st instar larvae had done so between 24-48 hours after oviposition. The total egg hatch was 75% of the eggs deposited.

The four larval instars could be readily distinguished by their head capsule sizes. The early instar larvae were very dark in color and became progressively lighter until taking on the characteristic light brown color of *An. freeborni* in the fourth instar. At this time several other color traits also appeared; these included a green color trait, a trait for a white thoracic region, and a trait for a longitudinal white stripe on the abdomen. At least 50% of the surviving larvae had reached pupation by the 10th day after oviposition with a weighted average of about 11 days (Table 2). The duration of each instar ranged from 1.64-2.47 days as estimated by subtracting the mean occurrence of each from the succeeding stage. The fourth larval stage exhibited the most protracted duration. The survivorship through the larval stages was high, (i.e. 96% from 1st-4th instar). However, only 77% survived from 4th instar larvae to pupae (Table 3).

The results of the pupal eclosion observations are summarized in Table 4. Of the 800 eggs set up, 532 pupated. The average duration of the pupal stage was just over 2 days, with males developing to pupation more rapidly than females.

The results of observations of the length of the gonotrophic cycle and fecundity are summarized in Table 5. Of the eggs deposited by the 800 blood fed females, about half were deposited on the 3rd and half on the 4th days after blood feeding. Of the 80 mosquitoes dissected in the random samples, 79 were positive for insemination. Only 31 of 100 isolated females oviposited. The others perished apparently from drowning in the small amount of water at the bottom of the tubes. All were positive for insemination. Of the 31 ovipositing an average of 133 eggs each was produced in one gonotrophic cycle.

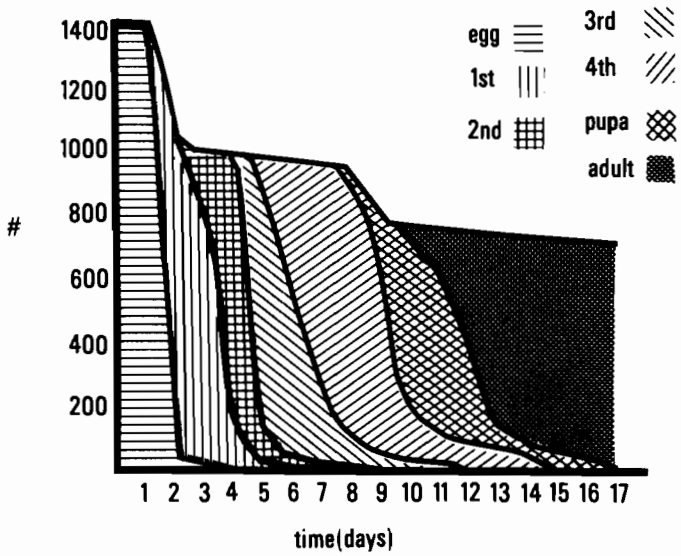


**Figure 1.** *Anopheles freeborni* Development. The upper line indicates the total number surviving through time. Shaded areas correspond to the proportional occurrence of the life stages through time. At T=0, n → 1400 eggs.

**Table 1.** *Anopheles freeborni* egg hatch.

| hours* | # | % | s |
|---|---|---|---|
| 24 | 0 | 0 | 0 |
| 48 | 1012 | 73 | 2 |
| 72 | 32 | 2 | 4 |
| 96 | 7 | – | 2 |
| 120 | 4 | – | 4 |
| total | 1055 | 75 | 2 |

*after oviposition

Table 2. *Anopheles freeborni* Larval-pupal development. Weighted averages for the time of occurrence of each instar and the pupal stage are indicated.

| | 1st | 2nd | 3rd | 4th | pupa |
|---|---|---|---|---|---|
| occurrence $\bar{x}$ time* | 2.73 | 4.37 | 6.04 | 8.27 | 10.74 |
| s | 1.04 | 1.33 | 1.47 | 1.82 | 2.15 |
| duration (days) | 1.64 | 1.67 | 2.23 | 2.47 | 2.15 |

*days after oviposition

Table 3. *Anopheles freeborni* Survivorship. Real and apparent survivorship to selected stages is indicated.

| stage | lx(%from egg) | s | lx(%from prior stage) | s |
|---|---|---|---|---|
| 1st | 75 | 2.0 | 75 | 2.0 |
| pupa | 52 | 5.5 | 77 | 5.5 |
| adult | 50 | 5.6 | 96 | 7.4 |

Table 4. *Anopheles freeborni* Pupal eclosion. Weighted averages for pupal duration are indicated. N= 532 pupae.

| day after oviposition | emergence $\bar{x}$ time(days) | s | dx(%) | male(%) | female(%) |
|---|---|---|---|---|---|
| 9 | 2.60 | 0.52 | 6 | 75 | 25 |
| 10 | 2.24 | 0.39 | 5 | 62 | 38 |
| 11 | 2.00 | 0.14 | – | 45 | 55 |
| 12 | 2.20 | 0.15 | – | 36 | 64 |
| 13 | 2.90 | 0.26 | 2 | 10 | 90 |
| 14 | 2.00 | 0 | 22 | 0 | 100 |
| total | 2.15 | 0.36 | 4 | 48 | 52 |

Table 5. *Anopheles freeborni* Gonotrophic cycle: oviposition and fecundity. Egg deposition through time is indicated for 800 blood fed females. Fecundity is calculated as the mean eggs deposited by 31 isolated females.

| hours* | # eggs | % total | fecundity ** | |
|---|---|---|---|---|
| 24 | 0 | 0 | | |
| 48 | 0 | 0 | $\bar{x}$ | 133 |
| 72 | 13684 | 47 | s | 64 |
| 96 | 14067 | 48 | range | 22–279 |
| 120 | 799 | 3 | | |
| 144 | 472 | 2 | | |
| 168 | 32 | – | | |
| | total 29054 | | | |

*after blood meal **eggs/ovipositing female

DISCUSSION.–A summary of the developmental biology of *Anopheles freeborni* in a laboratory situation is as follows:

On the average 133 eggs are deposited by each ovipositing female 3.52 days after blood feeding. Of eggs deposited an average of 75% hatch 2.03 days after blood feeding. Of the lst instar larvae 98% survive to the 2nd instar in a mean time of 1.64 days. Of 2nd instar larvae 99% survive to 3rd instar in a mean time of 1.67 days. Of 3rd instar larvae 98% survive to the 4th instar in a mean time of 2.23 days. Of the 4th instar larvae 77% survive to pupation in a mean time of 2.47 days. Of the pupae 96% survive to adulthood in a mean time of 2.15 days. Males develop to adulthood on the average more rapidly than females do, but the shorter duration of the larval instars does not correspond to a shorter duration of the pupal stage.

A brief comment about survivorship must be made at this point prior to more general discussion of these results. Two regions of substantially reduced survivorship may be viewed in Fig. 1. The first of these occurs between the egg and first larval stage and corresponds to a 25% failure in egg hatch. The survivorship curve of Fig. 1 depicts this as mortality; however, it is impossible to accurately state from these results how much of this 25% failure to hatch is actual mortality and how much is simply due to infertility. Although the observed insemination rates clearly indicate that all females had mated, it isn't clear whether all the eggs produced had been fertilized since embryonation rates were not determined. Further observations including rates of embryonation would clarify this question.

The second region of reduced survivorship occurs during the fourth larval stage (i.e., seen between days 10 and 11 on Fig. 1). This time period corresponds closely to the mean occurrence of the pupal stage, 10.74 days after oviposition. It would therefore be reasonable to assume that the mortality associated with the fourth larval stage actually occurs during the

process of moulting to pupa and is likely due to increased physiological stresses associated with pupation.

In general these results reflect a pattern of development in the laboratory for *An. freeborni* similar to that described by Hardman (1947). The differences in developmental rates between the results of this study and Hardman's can primarily be attributed to the differing diets and temperature regimes. Huffaker (1944) clearly delineates the differential rates of development for *Anopheles quadrimaculatus* in different constant temperature regimes. Bailey and Geike (1968) demonstrated the same phenomenon with *An. freeborni*. In light of the effects of temperature on developmental velocity it seems reasonable to conclude that the more rapid development observed in this study as compared to Hardman's (i.e., egg to pupa 10.74 days vs. 17.2 days) is due in part to the higher temperature (30°C vs. 27°C). In fact, data presented by Bailey and Geike closely conform with the calculated developmental rate of 30°C seen in this study.

However, temperature differences alone do not sufficiently explain the differences in observed egg hatch, mortality, and variability of developmental rates. One other factor which could play a significant role is the feeding regimen and diet. The problem with pellicle formation or "scumming" in the rearing pans is noted by all these authors. Hardman (1947) noted that measures to reduce pellicle resulted in a decrease of pupal mortality to 4%, the same pupal mortality observed in this study. Bailey and Geike (1968) refer to high mortality due to adverse effects from pellicle formation. The lower overall mortality in this study, 50% compared with 59% and 63% noted by the other authors, could well be due to the substitution of diet (i.e., ground guinea pig and fishfood instead of ground dog biscuits). The increased survivorship could either be due to enhanced access to air by reduction of surface film and/or an actual nutritional benefit from the substituted diet. This same reasoning may be used to explain the greater viability of eggs, (i.e., hatch rate 75% vs. 53%) and decreased variability of developmental rates, (i.e., egg-pupa: 9-15 days vs. 10-27 days). Better fed, less stressed larvae could be expected to develop more normally and exhibit greater reproductive potential as adults. However, these arguements fall in the realm of conjecture and more rigorous physiological determinations of these phenomena are certainly merited.

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# TERRESTRIAL BREEDING SITES OF RHAGIONDAE (DIPTERA) IN NORTHERN CALIFORNIA

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## ABSTRACT

The potential breeding sites of snipe flies in woodland-grass soils were investigated by a combination of emergence trapping and sampling for immatures in Mendocino County, California, 1978-81. Adults of 8 rhagionid species (*Chrysopilus*, 2; *Ptiolina*, 1; *Rhagio*, 1; *Symphoromyia*, 4) were taken inside emergence traps, and immatures of *Ptiolina* sp. near *zonata* Hardy & McGuire and *Rhagio costatus* (Loew) were reared to adults. Seventy-one hours spent hand-sorting woodland soils in 1980-81 yielded 29 rhagionid immatures or approximately 1 every 2.45 hr. Berlese funnels and hand-sorting appeared to be almost equally efficient for extracting larvae of *Ptiolina* sp., which occupied the upper 5 cm of soil. These findings corroborate previous reports that immature snipe flies are terrestrial.

INTRODUCTION.–Adult Rhagionidae, or snipe flies, are primarily non-hematophagous, though some species avidly suck the blood of animals such as man, deer, and domestic livestock. In western North American, certain species in the genus *Symphoromyia* are vicious man-biters, and recently a case of severe hypersensitivity response to *Symphoromyia* bites that resulted in apparent anaphylactic shock was reported from the State of Washington (Turner 1979). Hoy and Anderson (1966) gave accounts of several *Symphoromyia* species attacking man in California, and Turner (1979) recently reviewed published reports of *Symphoromyia* biting man in North America. Despite the potential medical/veterinary importance of *Symphoromyia*, however, breeding sites of less than 10% of the described North American species are known (Lane and Anderson, *in press*).

During biologic studies of Tabanidae in northern California, several rhagionid immatures were extracted from woodland-grass soils and reared to adults including *Rhagio costatus* (Loew) (reported as *Rhagio*) and *Symphoromyia inconspicua* Turner & Chillcott (Lane 1976). Further studies were initiated in 1978 to locate the breeding sources of other *Symphoromyia* as well as rhagionids in related genera by sampling terrestrial environments for immature stages and by trapping newly emerged adults in screen-houses set over woodland soils. Since results of the 1978-80 investigations will be presented in detail elsewhere (Lane and Anderson, *in press* ), the purpose of this communication is to briefly summarize those findings and to more fully document data gathered in 1981.

MATERIALS AND METHODS.–Investigations were carried out at the University of California, Hopland Field Station (UCHFS), a 2,168-ha agricultural sciences research facility in southeastern Mendocino County. A description of the study area has been presented by Hoy and Anderson (1978).

Adult rhagionids and other flies emerging from woodland-grass soils were captured inside 3 screen-houses 3 x 3 m wide by 2 m tall having woven netting screen walls and 1 or 2 doors. Traps were assembled at 3 sites in April 1980 and checked twice weekly for presence of flies until early July; also, a single trap was similarly operated between mid-November 1980 and early January 1981. All flies observed inside the traps were collected.

Woodland-grass soil samples up to 23 cm (usually 5-10 cm) deep were taken near emergence traps and in comparable biotopes with a trowel and examined for rhagionid immatures by hand-sorting on 35 dates from March to November 1978-81; also, 1.74-$m^2$ of sod were processed in Berlese funnels for 4-7 days in 1981. We attempted to rear most rhagionid larvae collected on a diet of mealworms and/or highly organic soils from the natural habitat.

RESULTS AND DISCUSSION.–Emergence trap studies--Sixty-one snipe flies representing 4 species of *Symphoromyia*, 2 species of *Chrysopilus*, and 1 species each of *Ptiolina* and *Rhagio* were collected. Except for *Ptiolina* sp., which emerged in December, all rhagionids were taken from April to June. Emergence densities ranged 0.04 to 1.30 flies/$m^2$ for different species. The breeding sites of 6 of these species had not been found heretofore. Although our 3 emergence traps covered only 28.3 $m^2$ of a vegetational type that occupies over 1,700 acres at UCHFS, we found 4 of the 10 *Symphoromyia* species that have been recorded there.

In addition to Rhagionidae, 197 flies distributed among 20 families and approximately 48 species also were taken, with Cyclorrhapha predominating with 147 individuals in 11 families and 29 species.

Collections of rhagionid immatures --The only rhagionid immatures found before 1981 belonged to the genus *Ptiolina*,

which had not been recorded previously from the state. Twenty-three larvae and a pupa were collected 1 to 4-cm deep in soils colonized by mosses and shaded by madrone or oaks. A mature larva and pupa taken in November 1980 each yielded an adult of a *Ptiolina* sp. near *zonata* Hardy & McGuire.

In 1981, hand-sorting woodland-grass soils produced a greater diversity and density of rhagionid immatures than were obtained in preceding years, perhaps because sampling was performed from mid-March to early May prior to emergence of most rhagionids. Five *Ptiolina*, 3 *Symphoromyia*, and 7 *Chrysopilus* and/or *Rhagio* immatures were extracted from 16.64-$m^2$ of soil for a mean density of 0.90 individuals/$m^2$ and 0.92 individuals/hr of sorting. An additional 8½ hr spent sampling in early May produced another *Symphoromyia* larva. At least 1 more *Ptiolina* and 2 *Chrysopilus* and/or *Rhagio* larvae were funnel extracted from soil samples 1.74-$m^2$ x 5 cm deep. Two females of *R. costatus* were reared from pupae collected in the shade of oaks *(Quercus)*.

All 4 *Symphoromyia* larvae shared several important features in common with those of the 4 undetermined Alaskan *Symphoromyia* species characterized by Sommerman (1962). Mature larvae are creamy white with a 12-segmented, cylindrical, elongate body tapering anteriorly to a partly retractile head. The 8th abdominal segment is deeply cleft horizontally, with upper and lower surfaces covered by semi-circular sclerotized plates each bearing 4 tubercles along their outer margins.

Table 1 compares the mean number of rhagionid immatures found/hr by hand-sorting woodland-grass soils in 1980-1981 with those taken by hand-sorting soils/mosses bordering vernal pools and intermittent creeks during a previous study at UCHFS (Anderson, J.R. and J.B. Hoy, unpublished data). On the average, it took ca 2.45 and 2.2 hr to collect each rhagionid immature from woodland-grass and the banks of intermittent creeks, respectively, whereas 17.5 hr were expended for each larva obtained from the margins of vernal pools. Although these data suggest that woodland soils and intermittent creek borders are of comparable productivity as breeding sources for rhagionid immatures, woodland soils actually yield a much higher biomass area-wide because they encompass a far more extensive habitat in the Hopland area.

**Table 1.** Comparison of the mean number of rhagionid immatures extracted/hour by hand-sorting soil samples from 3 habitat types, University of California, Hopland Field Station, 1964-65, 1980-81.

| HABITAT | APPROXIMATE NO. HOURS SPENT SORTING | MEAN NO. RHAGIONIDS FOUND/HOUR | ASSOCIATED TAXA* |
|---|---|---|---|
| WOODLAND-GRASS SOILS | 71 | 0.408 | *Ptiolina* sp.<br>*Rhagio costatus*<br>*Symphoromyia* |
| BANKS (MARGINS) OF: | | | |
| INTERMITTENT CREEKS | 150 | 0.453 | *Chrysopilus* spp. |
| VERNAL POOLS | 70 | 0.057 | *Symphoromyia pachyceras* |
| TOTAL | 291 | 0.347 | |

*Associations were based largely upon specimens reared to adults. However, most immatures were undetermined, particularly those taken from soil bordering intermittent creeks and vernal pools.

Finally, to determine the relative efficiencies of our extraction methods as well as the depth occupied by rhagionid larvae in spring-time, we compared hand-sorting and Berlese funnels for extracting snipe fly larvae from woodland soils at each of 2 depths (i.e., 0-5 cm, 5-10 cm). Ten 0.1-$m^2$ samples taken at both depths were hand-sorted individually in enamel pans for approximately 15 min., and the number of rhagionid larvae noted. All larvae were then returned to their respective samples, which were placed in sealed plastic bags for transport to the laboratory, and each was processed in a Berlese funnel for 6 days. As shown in Table 2, the number of rhagionid immatures extracted by each method was nearly identical and none of the larvae (=*Ptiolina*) occupied soil deeper than 5 cm. Since such low numbers of only a single rhagionid species were obtained, however, further studies are needed involving many more replicates, and other genera of larval rhagionids, before substantive conclusions can be reached about the relative efficiencies of these methods.

**Table 2.** Comparison of Berlese funnels and hand-sorting for extracting rhagionoid larvae from ten 0.1-$M^2$ woodland-grass soil samples taken at each of 2 depths, University of California, Hopland Field Station, 1981.

| DEPTH (CM) | NO. RHAGIONIDS EXTRACTED* BERLESE FUNNEL | HANDSORTING |
|---|---|---|
| 0 - 5 | 4 | 5 |
| 5 -10 | 0 | 0 |

**Ptiolina* sp. was the only rhagionid extracted.

ACKNOWLEDGMENT.–A. H. Murphy, Superintendent, and other personnel at the Hopland Field Station, as well as P. Rayces and S. Chew, are gratefully acknowledged for their support and technical assistance. Special thanks go to W. J Turner for confirming or determining the specific identities of most adult rhagionids.

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# SEASONAL VARIATION OF WING LENGTH AND EGG RAFT SIZE IN *CULEX TARSALIS*

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## ABSTRACT

A population of *Culex tarsalis* was sampled to determine seasonal variation in female wing length, raft size, hatch rate and relative abundance. Wing lengths of females ranged from 3.1 to 5.3 mm, and rafts from 30 to 438 eggs. Raft size and wing length were positively correlated, and both were correlated positively with hatch rate and negatively with air temperature at the time of female trapping. Relative abundance (females caught per trap night) was not significantly related to any of the variables studied.

Adult mosquito size may be influenced by several factors during immature life. Density dependent conditions include the accumulation of metabolites, food depletion and production of a growth retardant factor associated with overcrowding (White 1980; Moore and Fisher 1969; Jones 1960; Ikeshoji and Mulla 1970). Temperature is a density independent factor and influences the rate of larval development. Previous studies have shown that fecundity decreases as a function of decreasing adult body size in such species as *Anopheles stephensi* Reisen 1975) and *Aedes aegypti* (Jones 1960). White (1980) reported a negative correlation between air temperature and wing length of *Culex tarsalis* females in Kern County. He also observed that small females laid significantly fewer eggs than large females. Thus, an increased proportion of small females might explain the midsummer decline in *Cx. tarsalis* abunddance observed in earlier studies (Nelson et al. 1978; Asman et al. 1979; Milby et al. 1980).

The present study attempts to document this trend, using data from a sterile male release experiment at Breckenridge, Kern County, California in 1980 (Milby et al. 1981). The study relates adult female size to egg production, fertility, population abundance and air temperature.

METHODS.--The Breckenridge study site is located 12.5 km east of Bakersfield in Kern County, California, and consists of 3 more-or-less parallel canyons. Wastewater from oil fields goes into a series of holding ponds. During the summer, water is sprinkled onto the hillsides to produce grass for cattle-grazing. The ponds are stocked with *Gambusia*, but some mosquito breeding does occur in watercourses between the ponds, in cattle hoofprints, and in dense tule growth on the pond margins. In 1980, 93% of the mosquitoes collected at Breckenridge were *Cx. tarsalis*.

Female *Cx. tarsalis* were collected in $CO_2$ light traps, released into 30 x 30 x 30 cm cages and allowed to feed on a restrained chicken for 2 nights. The engorged females were held a minimum of 4 days for egg development, then transferred to individual 6 dram shell vials containing about 5 ml of tap water for oviposition. This procedure excluded autogenous females from the study. Egg rafts were removed after 3 days, placed on a strip of filter paper, disrupted, and counted under a dissecting microscope. Each egg was classified as hatched -- no apparent material left in egg case and micropilar cap opened; empty -- unhatched with no apparent embryo; or embryonated -- unhatched or partially hatched with an embryo visible.

Concomitant with raft counting, the length of one wing of the parent female was measured from the alular notch to the distal margin excluding the fringe scales, using an ocular micrometer (Figure 1). Females which died before completing oviposition, or which oviposited fewer than 30 eggs, were not included in further analyses.

Hatch rates were calculated for individual rafts, then averaged for each week from June 1 through September 28. Mean raft size and female wing length were also calculated for these 18 weeks.

Daily maximum and minimum temperatures, recorded at Kern County Airport by the National Oceanic and Atmospheric Administration (NOAA), were averaged, then combined to obtain mean temperature for each week.

The weekly female *Cx. tarsalis* light trap index for the Breckenridge area -- based on collections in CDC light traps supplemented with $CO_2$ -- was used as a measure of variations in population abundance.


**Figure 1.** Wing length measurement of *Cx. tarsalis* females.

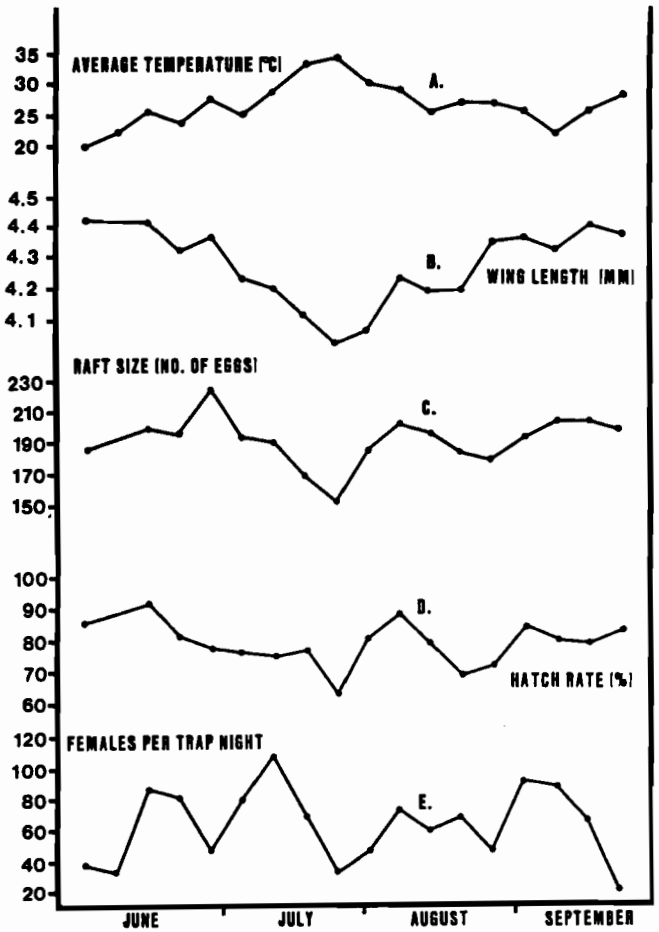


**Figure 2.** Weekly comparisons of average temperature (A) with mean wing length (B), raft size (C), hatch rate (D) and relative abundance (E) of *Cx. tarsalis* females collected at Breckenridge, Kern County, California, in 1980.

RESULTS.–Egg rafts with at least 30 eggs were oviposited by 2,422 female *Cx. tarsalis*. Raft size averaged 193 eggs (with a maximum of 438), and wing length ranged from 3.1 to 5.3 mm, with a mean value of 4.3 mm. These 2 variables showed a strong positive correlation ($p < 0.001$). The weekly means had a similar significant positive correlation (Figure 2B and 2C).

Weekly mean wing length was lowest (4.0 mm) for females collected in late July, when temperatures were highest (Figure 2A). Weekly mean raft size was also lowest (150 eggs/raft) at this time. There was a significant negative correlation of both variables with temperature.

In addition to temperature at the time of collection of females, we also examined the correlation with temperature one week before the females were collected. This is referred to as temperature lag 1 and was even more highly correlated with wing length and raft size than temperature during the week of collection ($r = -0.83$ vs. $-0.72$).

Weekly mean hatch rates were positively correlated with fecundity and wing length. There was a negative correlation with temperature (Figure 2D). Fertility followed the same general trend as raft size and wing length, starting with a high of 92% in June, declining to 63% during the week of peak temperature, and rising in late summer.

The light trap index showed a slight bimodal trend (Figure 2E), and reached a mid-summer low point when temperature was highest, but the population never exhibited the extreme fluctuations observed in other studies in Kern County (Asman et al. 1979).

DISCUSSION.–Temperature appears to be a major factor affecting the size of adult mosquitoes. It accelerates larval metamorphosis so that capacity to form tissue is inhibited. This could explain why wing length correlated better with temperature lag 1 than with temperature at the time of collection. Since temperature lag 1 measures the temperature during the late larval and pupal stages of the trapped females, it could indicate that the temperature at that time has a major influence on larval development and thus modifies the ultimate size of the adult.

Size of egg rafts correlated directly with the size of the female. This was shown previously for *Cx. tarsalis* in Kern County by White (1980), and is supported by Reisen (1975) who found that small *An. stephensi* females reared from crowded larvae imbibed less blood, produced fewer and smaller eggs, and had reduced survival compared to non-crowded groups. Also, Colless and Chellapah (1960) found the number of ovarioles was directly related to the size of the female in *Ae. aegypti*.

Both hatch rate and population abundance may have been modified by the experimental release of sterilized males from late June through the end of August. Although release efforts were hampered by low numbers of available individuals, there were 3 periods when releases exceeded 2,000 sterilized males per day. Two of these periods coincided with the 2 observed dips in mean hatch rate.

The trends in female size and fecundity at the Breckenridge

study site may be expressions of the population dynamics of *Cx. tarsalis*. Small females could have several effects on a local population. They probably have reduced flight range (Nayar 1969; Nayar and Sauerman 1970; Schiefer et al. 1973), imbibe less blood, produce fewer eggs and have reduced survival (Reisen 1975). These characteristics would contribute to a population decline, especially in marginal habitats during periods of unfavorable weather. If smaller females have reduced survival, they also would be less efficient disease vectors.

ACKNOWLEDGMENTS.–The authors thank the staff of the Arbovirus Field Station for their technical assistance and the Valley Waste Disposal Co., Bakersfield, for allowing access to the Breckenridge area. This research was supported in part by Research Grant AI-3028 from the National Institute of Allergy and Infectious Disease, General Research Support Grant I-SO1-FR-0441 from the National Institutes of Health and by special funds for mosquito control research appropriated annually by the California Legislature.

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## INFLUENCE OF AQUATIC SURFACE AREA ON THE PERCENT EMERGENCE OF *AEDES SIERRENSIS* ADULTS

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ABSTRACT

Synchronously hatched first-instar larvae of the western tree hole mosquito, *Aedes sierrensis* (Ludlow), were reared (50/100 ml) in four different types of containers that mimicked variously-shaped tree holes. The type A container (surface area = 143 $mm^2$) had a narrow neck and a large lower area; type B was deep and narrow (surface area = 1662 $mm^2$), type C was shallow (30 mm) with a surface area of 8825 $mm^2$; and type D was the shallowest (16 mm) and had the largest surface area (26,591 $mm^2$). In a fifth treatment the larval density of the type C container was doubled (100/100 ml).

After completing eight replicates of each treatment a stepwise multiple regression analysis (dependent variable being the percentage of adults emerging and the independent variables surface area, volume, depth and larval density) revealed that surface area was highly significant in predicting the percentage of adults emerging, whereas none of the other variables was significant ($p < .05$). The percentage of emerging adults ranged from 4.5 ± 9.9 (A container) to 77.4± 13.67 (D container).

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## CORRELATION OF MOSQUITO ABUNDANCE TO ASSOCIATED ENVIRONMENTAL VARIABLES FOR FORECASTING MOSQUITO POPULATION LEVELS

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ABSTRACT

Utilizing mosquito collection data from over 22 years of light trap operations in 3 areas in the Sacramento Valley of California, mosquito abundance was correlated to associated environmental variables. On the basis of stepwise regression with the best $R^2$ and adjusted $R^2$ values for *Anopheles freeborni, Culex tarsalis* and *Aedes melanimon,* predicted values for mosquito abundance were calculated and compared to observed values for the past three decades.

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# THE SELECTED AQUATIC FAUNA OF A RICE FIELD ECOSYSTEM WITH NOTES ON THEIR ABUNDANCES, SEASONAL DISTRIBUTIONS AND TROPHIC RELATIONSHIPS

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## ABSTRACT

The aquatic fauna of rice fields is remarkably rich; 62 species or species groups of organisms were regularly collected and sporadically another 23 different kinds of organisms were collected. The most abundant organisms in the rice fields were crustaceans, while insects dominated in the species composition.

The mosquitofish, *Gambusia affinis* (Baird and Girard), is a top predator but predacious insects, damselflies, notonectid bugs and beetle larvae probably play an important role in suppressing mosquito and chironomid larval populations in the Fresno Westside rice fields because they are so abundant in the ecosystem.

Insect and other animal pests of rice plants and their control have been reported in varying degrees of completeness mainly from the Sacramento Valley of California (Darby 1962, Lange 1970 and Willson 1979). However, relatively little information exists on aquatic organisms associated with rice fields located in the vicinity of South Dos Palos, California.

MATERIALS AND METHODS.–Two permanent rice fields measuring 20 and 27 acres of Koda Farms, Inc. near the Eagle Field Air Strip were used for this study. The fields were flooded with the Delta-Mendota canal water on April 20 and seeded on April 28. The water was continuously irrigated at a depth of 6 to 8 inches during the growing season and it was drained on September 1 for harvest.

Planktonic, nektonic and benthic organisms associated with rice fields were sampled by 2 methods: dipping and trapping. Dip samples were taken with standard white enamel dippers (450 ml capacity) and Husbands' concentrators (Husbands 1969) using fine nylon screen vials (200 mesh). Fifty dip samples were taken weekly from each field by transecting the field from north to south (Fig. 1). All samples were placed into vials containing 95% ethyl alcohol for later examination in the laboratory. One paddy approximately 1.1 acre size, on rice field no. 4, was arbitrarily divided into 100 sections in a grid pattern, a single dip sample was taken from each section and was kept separately in a screw-cap vial. In the laboratory all samples were examined under dissecting microscopes where they were counted and tabulated.

Gee® minnow traps were slightly modified by lining the inner sides of the traps with a piece of window screen. These were used to study the abundance of nektonic organisms and their seasonal fluctuations. Each week 5 unbaited traps were set in each field (Fig. 1) on a morning and retrieved the following morning. All organisms captured were counted and tabulated in the field and then released at the respective capture sites.

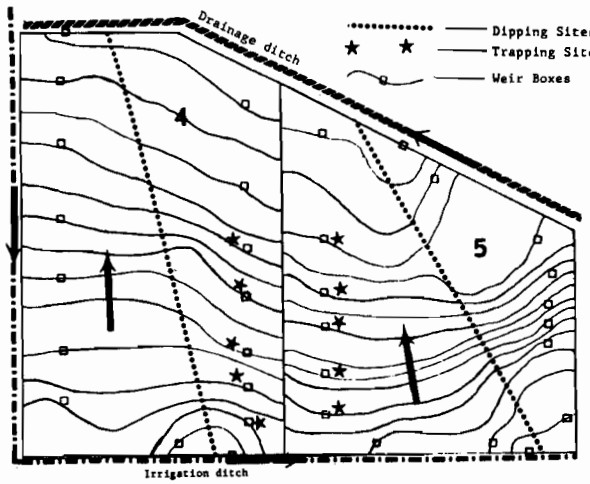


**Figure 1.** Study area showing sampling sites and irrigation water movement (arrows).

The samplings were started on June 18 and continued until August 31, 1980.

RESULTS AND DISCUSSION.–Table 1 shows the list of organisms collected in this study. The rice field ecosystem supports a tremendous number of organisms; 62 species or species groups of organisms were collected regularly while another 23 taxa were collected sporadically. Among collections, insects dominated the list of species; Coleoptera, Diptera and Hemiptera accounted for most species. Ephemeroptera and Odonata were present but the number of species were limited.

This is still a preliminary stage of study; however, this list is included here to serve as a base line to which further information may be added at a later date.

**Table 1.** A list of aquatic organisms found in rice fields in Fresno County (excluding mosquitoes).

- Rotifera
  - Ploima
    - *Asplanchana* spp.
- Oligochaeta
  - Plesiopora
    - *Aulophorus furcatus* (Muller)
- Hirudinae
  - Leeches
- Crustacea
  - Cladocera
    - *Ceriodaphnia* spp.
    - *Simocephalus* spp.
    - *Scapholeberis kingi* Sars
    - *Macrothrix rosea* (Jurine)
    - *Alona* spp.
  - Eucopepoda
    - *Cyclops vernalis* Fischer
    - *Diaptomus* spp.
  - Podocopa
    - *Chlamydotheca unispinosa* (Baird)
    - *Chlamydotheca acuata* (Sars)
    - *Cypris subglobosa* Sowerby
    - *Stenocypris* spp.
  - Decapoda
    - *Procambarus clarki* (Girard)
- Insecta
  - Ephemeroptera
    - *Callibaetis* sp.
  - Odonata
    - *Anax junius* (Drury)
    - *Enallagma civile* (Hagen)
    - *Ischnura salva* (Hagen)
  - Hemiptera
    - *Gerris* sp.
    - *Belostoma flumineum* Say
    - *Corisella decolor* (Uhler)
    - *Notonecta unifasciata* Guerin
    - *Buenoa scimitra* Bare
    - *Microvelia pulchella* Westwood
  - Coleoptera
    - *Hygrotus* spp.
    - *Laccophilus mexicanus mexicanus* Aube'
    - *Laccophilus mexicanus atristernalis* Crotch
    - *Laccophilus maculosus decipiens* LeConte
    - *Rhantus gutticollis* (Say)
    - *Colymbetes* spp.
    - *Liodessus affinis* (Say)
    - *Thermonectus basillaris* (Harris)
    - *Cybister explanatus* LeConte
    - *Hydrovatus brevipes* Sharp
    - *Berosus* spp.
    - *Hydrophilus triangularis* Say
    - *Tropisternus lateralis* (F.)
    - *Tropisternus ellipticus* (LeConte)
    - *Helophorus* spp.
    - *Heliplus* spp.
    - *Enochrus hamiltoni pacificus* Leech
    - *Bagous tingi* Tanner
  - Diptera
    - *Chironomus* spp.
    - *Goeldichironomus holoprasinus* (Goeldi)
    - *Pentaneura monilis* (L.)
    - *Polypedilum* spp.
    - *Procladius culiciforms* (L.)
    - *Corynoneura* spp.
    - *Cricotopus sylvestris* (Fabr.)
    - *Paralauterborniella* spp.
    - *Paratanytarsus* spp.
    - *Brachydeutera argentata* (Walker)
    - *Culicoides variipennis* (Coquillett)
    - *Chrysops* sp.
    - *Tabanus* spp.
    - *Limonia* spp.
- Arachnida
  - Acarina
    - *Arrenurus* sp.
    - *Hydrachna* spp.
- Osteichthyes
  - Microcyprini
    - *Gambusia affinis* (Baird and Girard)
- Amphibia
  - Salientia
    - *Hyla* spp.
    - *Rana* spp.
- Miscellaneous Collection
- Tardigrada
  - Water bears
- Coelenterata
  - Hydras
- Porifera
  - Sponges
- Insecta
  - Collembola
    - Smynthuridae
    - Isotomidae
  - Thysanoptera
    - Thrips
  - Homoptera
    - Aphids
    - Leaf hoppers
    - Tree hoppers
  - Trichoptera
    - Hydroptilidae
  - Diptera
    - Psychodidae
    - Ephydridae; *Hydrellia griseola* (Fallen)
- Arachnida
  - Acarina
    - Phytoseiidae
  - Araneida
    - *Lycosa* spp.
    - *Tetragnatha* spp.
    - *Pardosa* spp.
- Gastropoda *Physa* spp. *Helisoma* spp.
- Osteichthyes *Cyprinus carpio* L.
  - *Pomoxis nigromaculatus* (Lesueur)
  - *Percina macrolepida* Stevenson
  - *Lepomis cyanellus* Rafinesque
  - *Morone saxatilis* (Walbaum)
  - *Menidia audens* Hay

[a] Number of miscellaneous organisms (aquatic, semi-aquatic or terrestrial were not counted.

Figure 2 shows the relative abundance of organisms collected during the study. Crustaceans predominated among the number of individual collections with ostracods being the most

abundant followed by cladocerans and copepods. The most abundant insect was damselfly nymphs and were followed by dytiscid beetle adults, notonectid bugs and chironomid midges. Mosquito larvae and belostomatid collections were small but consistently collected during the study period.

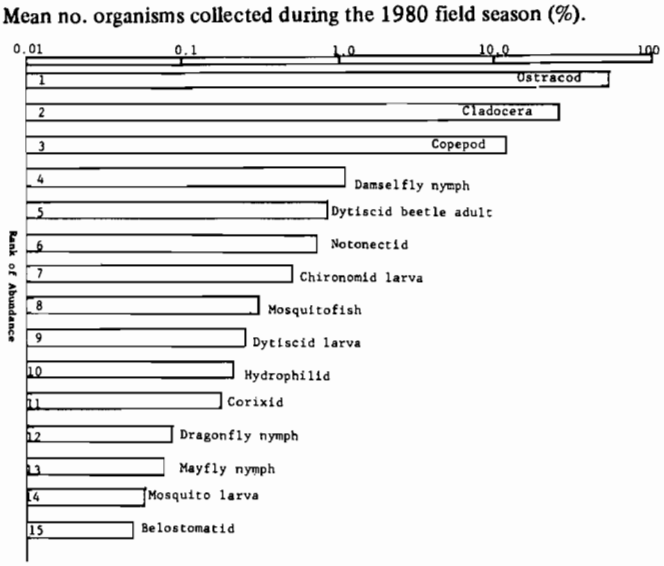


**Figure 2.** Relative abundance of major aquatic organisms collected during the 1980 study period from the Fresno Westside rice fields.

Figures 3 thru 7 show the visual display of spatial distribution of crustaceans, chironomid midge larvae and damselfly nymphs. All crustaceans examined exhibited a contagious distribution in the rice paddy i. e., the population is dense in some areas and sparse in others; the frequency distribution of individual per unit area is skewed to the left and the variance exceeds the mean. Chironomid larvae also exhibited a contagious distribution which agrees with the findings of Paterson and Fernando (1972), Shiozawa and Barnes (1977) and Clement and Christensen (1979). However, the variance and mean of damselfly nymphal population approximated to 1 (1.02 and 0.92) which implies a more random distribution than is described by a contgagious distribution.

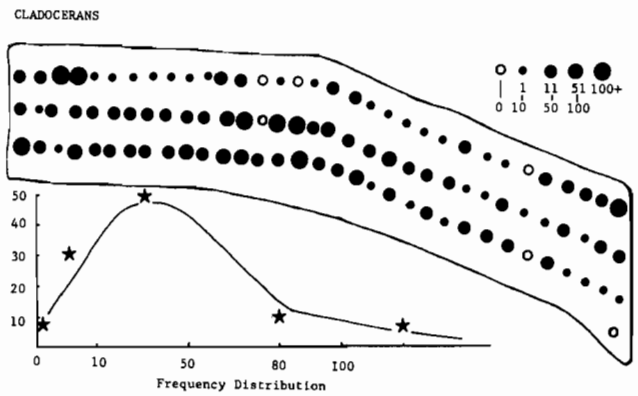


**Figure 3.** Spatial and frequency distributions of cladoceran population in a 1.1 acre paddy.

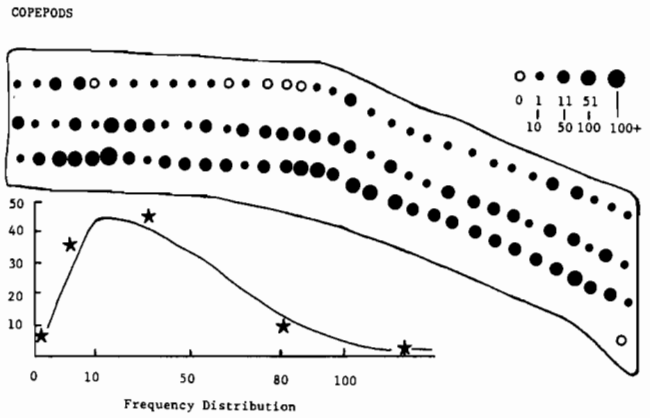


**Figure 4.** Spatial and frequency distributions of copepod population in a 1.1 acre paddy.

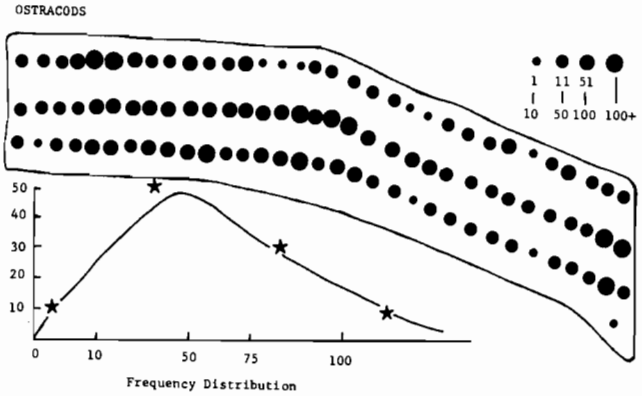


**Figure 5.** Spatial and frequency distributions of ostracod population in a 1.1 acre paddy.

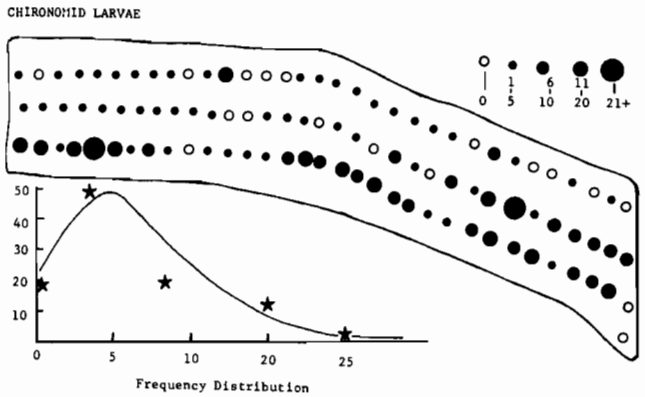


**Figure 6.** Spatial and frequency distributions of chironomid population in a 1.1 acre paddy.

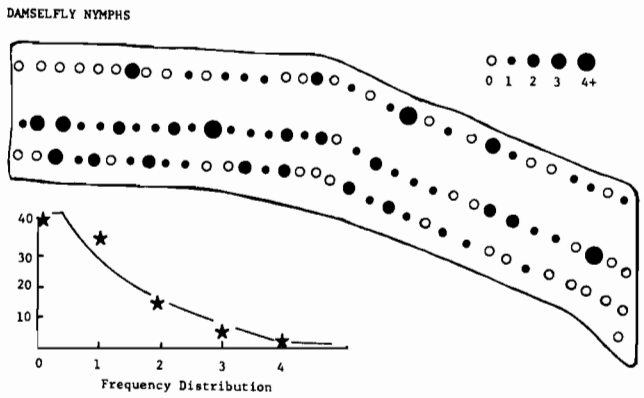


**Figure 7.** Spatial and frequency distribution of damselfly population in a 1.1 acre paddy.

Table 2 presents data on the seasonal abundance of the principal taxa collected in the rice fields. Rice fields create a unique aquatic environment; they are man-made shallow temporary bodies of water in which only animals such as winged insects or planktonic and nektonic organisms in the irrigation water can establish themselves and develop in enormous numbers in a short period of time. Crustacean populations reached equilibrium levels by the 2nd week of July and the levels were maintained throughout the season. Among insects, damselfly *Enallagma civil* nymphs dytiscid adult beetles *Hygrotus* spp., *Laccophilus* spp. and Chronomid larvae dominated population counts.

Hemipterans *Notonecta unifasciata, Corisella decolor* and ephemerids *Callibaetis* sp. were abundant in the early part of rice growing season but as the season progressed they disappeared from the fields. Hydrophilid beetle populations were few compared to dytiscid populations and they were poorly distributed.

Mosquitofish were not stocked in these study fields but were established in the fields through the irrigation water and is the only organism which increased in population steadily as the season progressed.

Figure 8 presents trophic relationship for those taxa more regularly collected during the study period. The numbers (%) of each major group of predators collected indicated each entry. There are many different predators in rice fields; among them, the mosquitofish is a top predator and is definitely more effective on a per-individual basis but insect predeators, such as damselfly nymphs, notonectid bugs and beetle larvae are

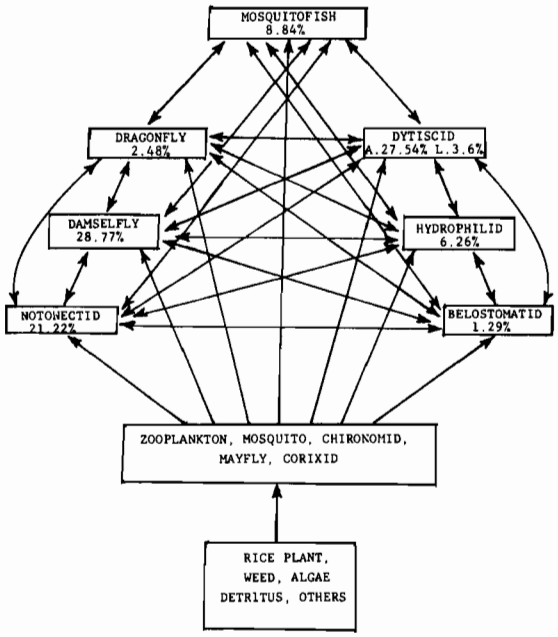


**Figure 8.** Trophic relationships between major organisms in rice fields based upon the 1980 study. Numbers indicate relative abundance (%) of predators. A = adults, L = larvae.

**Table 2.** Seasonal distribution of major taxa collected in South Dos Palos, California rice fields.

| organism | June | | July | | | | | August | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 18 | 25 | 2 | 9 | 16 | 23 | 30 | 6 | 13 | 20 | 27 |
| Cladocera D/ | 48 | 54 | 126 | 352 | 311 | 203 | 218 | 301 | 223 | 248 | 286 |
| Copepod D/ | 21 | 77 | 97 | 136 | 97 | 86 | 115 | 150 | 125 | 146 | 118 |
| Ostracod D/ | 9 | 80 | 89 | 446 | 660 | 434 | 726 | 787 | 487 | 580 | 643 |
| Mayfly D/ | 13 | 10 | 10 | 12 | 14 | 8 | 11 | 0 | 1 | 0 | 0 |
| Damselfly D/ | 28 | 25 | 120 | 84 | 96 | 58 | 64 | 103 | 123 | 84 | 70 |
| Dragonfly D/ | 10 | 4 | 8 | 8 | 10 | 7 | 6 | 5 | 12 | 4 | 4 |
| Belostomatid D/ | 1 | 1 | 1 | 2 | 5 | 5 | 5 | 5 | 6 | 6 | 3 |
| Notonectid T/ | 294 | 96 | 155 | 38 | 46 | 15 | 1 | 1 | 2 | 2 | 0 |
| Corixid T/ | 76 | 56 | 20 | 2 | 4 | 4 | 1 | 1 | 2 | 0 | 0 |
| Chironomid D/ | 54 | 53 | 33 | 34 | 32 | 38 | 29 | 36 | 32 | 20 | 24 |
| Dytiscid Adult T/ | 158 | 45 | 123 | 63 | 137 | 157 | 84 | 22 | 22 | 16 | 9 |
| Dytiscid Larva D/T/ | 13 | 12 | 12 | 9 | 8 | 11 | 8 | 4 | 17 | 12 | 5 |
| Hydrophilid Adult T/ | 9 | 8 | 6 | 6 | 8 | 16 | 37 | 20 | 16 | 14 | 5 |
| Hydrophilid Larva D/T/ | 18 | 4 | 6 | 4 | 4 | 3 | 0 | 4 | 2 | 2 | 2 |
| Mosquito D/ | 3 | 4 | 2 | 4 | 4 | 3 | 4 | 6 | 7 | 9 | 7 |
| Mosquitofish T/ | 6 | 1 | 8 | 4 | 10 | 14 | 27 | 52 | 49 | 44 | 54 |

D - 100 dip collection (Cladocerans, coepods and ostracods = 10 dip collection)

T - 10 trap collection

much more abundant than fish. Therefore, on a per population basis insect predators may play an important role in suppressing mosquito and chironomid midge larval populations in rice field ecosystems in the San Joaquin Valley of California.

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# ACTIVITY OF NEW LARVICIDES AGAINST MOSQUITOES

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INTRODUCTION.–Since the appearance of resistance in larvae to various organochlorine and organophosphorus larvicides, considerable advances have been made in the evaluation and development of substitute materials having various modes of action for the control of mosquito larvae. Such materials include pyrethroids (decamethrin and permethrin), insect growth regulators (methoprene and diflurobenzuron), microbial insecticides (*Bacillus thuringiensis* serotype H-14) and the parasitic nematodes (*Romanomermis culicivoras*).

In recent studies, a number of the new control agents displayed excellent biological activity against mosquitoes (Darwazeh et al. 1978, Goldberg and Margalit, 1977, Mulla and Darwazeh 1976). Some of these agents exhibited results equal to or superior than some of the active and widely used organophosphorus larvicides such as chlorpyrifos and temephos. In addition, these new materials were found to be nonresidual in water and they possess a high margin of safety against some freshwater fishes and other nontarget organisms (Mulla et al. 1978, 1980, Miura et al. 1980).

The following studies were initiated to evaluate newly developed synthetic materials against mosquito larvae in the laboratory and under field conditions, and to determine the optimum larvicidal rate for the control of stagnant and floodwater mosquitoes.

METHODS AND MATERIALS.–Seven new compounds were evaluated in the laboratory against 4th stage larvae of *Culex quinquefasciatus* Say. Suppliers and formulations of the materials tested are listed in Table 1. The compounds evaluated were: Bay FCR-1272 (Cyano [4-fluro-3-phenoxyphenyl] methyl 3-[2,2-dichloro-ethenyl]-2,2-dimethylcyclopropane-carboxylate), Sumithrin (d-[*cis*, *trans*] phenothrin), OMS-2000 (fenvalerate): Butyric acid, 2-(3-chlorophenyl-3-methyl α-cyano-3-phenoxybenzylester, ZR-3210, DPX-5444 (1,5-Bis [*P*-trifluoromethyl phenyl]-3-cyanoformazan), OMS-1356 (1,1-bis-[paraethoxyphenyl]-2-nitropropane, and Physan-20 (10%) of (n-alkyl [60% $C_{14}$, 30% $C_{16}$, 5% $C_{18}$] dimethylbenzyl ammonium chlorides) and (10%) of (n-alkyl [68% $C_{12}$, 32% $C_{14}$] dimethyl ethylbenzyl ammonium chlorides). Mosquito larvae utilized in these studies were obtained from laboratory colonies at the University of California, Riverside.

Procedures utilized in the laboratory evaluation are those described by Mulla et al. (1980). In brief, (1%) stock solution from the EC formulations (w/v) were prepared in acetone, and serial dilutions were prepared in acetone as needed. The required aliquot of the dilute solution was added to 4 oz disposable cups (Sweetheart Cup Division of Maryland, Baltimore, MD) containing 20 4th stage larvae in 100 ml of tap water. After 24 h of exposure of test organisms in a controlled temperature holding room (78°F±1), mortality readings were taken and $LC_{50}$-$LC_{90}$ in ppm were obtained from dosage response lines on probit log paper based on mean mortality values versus concentrations. Each material was tested at several concentrations in triplicate on 2-3 different occasions.

Field evaluations were conducted against *Culex tarsalis* Coquillett larvae in experimental ponds at the Aquatic and Vector Control Research Facility at the University of California, Riverside and in the Coachella Valley of southern California. Detailed descriptions of these facilities are reported by Mulla et al. (1974, 1975). The Coachella Valley ponds measure 18 x 18 ft, while the ponds at Riverside measure 12 x 24 ft. Water depth at both locations was maintained constant (12 in.) by use of float valves.

The required amount of toxicant as EC formulation was mixed with 125 ml of tap water and applied with an all purpose (1 liter) household sprayer, utilizing 3 replicates per application rate. Three ponds were left untreated as checks. Prior to and 2, 7 and 14 days after treatment, 5 dips per pond were taken and composited into one sample after removing excess water through 150-mesh stainless steel strainer cloth. The composite sample was preserved with 95% ethyl alcohol. All organisms collected by the dipper were counted and identified in the laboratory under a dissecting microscope.

Studies on the floodwater mosquito *Psorophora columbiae* (Dyer and Knab) were conducted in 1/32 or 1/16 acre plots in irrigated pastures of El Rancho Del Rio in Imperial County, located 5 miles south of the town of Palo Verde on State highway #78. The required amount of the EC formulation of the toxicants were mixed with 500 or 1000 ml of water (depending on plot size) and applied with a 1-gal. stainless steel pressurized hand sprayer. Each material was tested at 2 or 3 rates, utilizing 3 replicates per rate. Each rate was tested in a separate irrigation check to avoid mixing the high rates with the low rates. Along with each test, 3 separate plots were left untreated as checks. Prior to and 24 hr after treatment, 10 dips per plot were taken and percent reduction was calculated

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on the basis of the mean number of all larval stages and pupae in the posttreatment count versus pretreatment.

**Table 1.** Evaluation of various mosquito larvicides against 4th stage larvae of *Cx. quinquefasciatus* in the lab.

| Material | Supplier | ppm $LC_{50}$-$LC_{90}$ |
|---|---|---|
| | Pyrethroids | |
| Bay FCR-1272 | Mobay Chem. Corp. | 0.0001-0.0002 |
| Sumithrin | McLaughlin Gormley King Co. | 0.0025-0.0046 |
| OMS-2000 | World Health Organization | 0.020 -0.080 |
| ZR-3210 | Zoecon Corp. | 0.050 -0.090 |
| | General Larvicides | |
| DPX-5444 | DuPont | 0.005 -0.010 |
| OMS-1356 | World Health Organization | 0.018 -0.035 |
| Physan 20 | Consan Pacific Inc. | 17.0 -42.0 |

RESULTS AND DISCUSSION.- -Laboratory: The pyrethroid Bay FCR-1272 displayed high level of activity against 4th stage larvae of *Cx. quinquefasciatus* in the laboratory, causing 50 and 90 percent mortality at the concentrations of 0.1 and 0.2 ppb. Sumithrin was 5 times less active but caused 50 and 90 percent mortality at 2.5 and 4.6 ppb. The other two pyrethroids (OMS-2000 and ZR-3210) were less active than the former two. Their $LC_{90}$ level against 4th stage larvae was in the range of 80-90 ppb (Table 1).

Bay FCR-1272 was the most active pyrethroid tested during these studies but was 10 times less active than some of the previously tested pyrethroids, namely decamethrin (FMC-45498). Sumithrin produced results similar to those obtained with permethrin in earlier studies (Mulla et al. 1980).

DPX-5444, an orange dye, displayed good larvicidal activity, similar to the activity of the pyrethroid sumithrin, causing 50 and 90 percent mortality at 5 and 10 ppb. OMS 1356 (related to DDT) and ZR-3210 were equal in activity, causing 90 percent mortality at 80 ppb. Physan 20, containing quarternary ammonium salts, was the least effective material tested with an $LC_{90}$ in excess of 42 ppm (Table 1).

Field Evaluation. - -Against a field population of *Cx. tarsalis* larvae in experimental ponds, Bay FCR-1272 eliminated the late larval stages at the rate of 0.01 lb/A (11.2 g/ha). At the lower rates of 0.001, 0.0025 and 0.005 lb/A, 82, 97 and 99 percent reduction of the late larval stages was obtained 2 days after treatment. No residual activity in water was apparent, and at all rates applied, the larval population began to recover within 7 days after treatment. ZR-3210 also was highly active at the rates of 0.01, 0.025 and 0.05 lb/A, causing complete control of the late larval stages within two days after treatment. Poor results, however, were obtained at the lower rates of 0.0025 and 0.005 lb/A. Physan 20, in one test, produced 93, 86 and 97 percent reduction in the larval population at the rates of 6, 12, 18 lbs/A (4, 8 and 12 gal formulation/A), while the lower rates, in another test, produced negligible results. At the rates of 1.5, 3 and 6 lb/A, only 42, 23 and 17 percent reduction was obtained 2 days after treatment (Table 2). Physan 20 is an algicide, and at algicidal rates (2-4

**Table 2.** Evaluation of various mosquito larvicides against *Cx. tarsalis* in experimental ponds.

| Material and formulations | Rate lb/A | Rate g/ha | Avg. no. of larvae/5 dips pre and posttreat.(days) Pretreat 1-2 | Pretreat 3-4 | 2 1-2 | 2 3-4 | 2 (%R) | 7 1-2 | 7 3-4 | 7 (%R) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | UC Riverside, 6/26/1980 (72°-84°F)[a/] | | | | | | | |
| Bay FCR-1272 EC(18.5%) | 0.0010 | 1.1 | 191 | 343 | 14 | 62 | 82 | 18 | 41 | 88 |
| | 0.0025 | 2.8 | 153 | 152 | 4 | 5 | 97 | 79 | 350 | 0 |
| | 0.005 | 5.6 | 321 | 257 | 27 | 3 | 99 | 63 | 120 | 53 |
| | 0.010 | 11.2 | 253 | 239 | 3 | 0 | 100 | 59 | 282 | 0 |
| Check | - | - | 338 | 613 | 367 | 830 | 0 | 94 | 733 | 0 |
| | | | Coachella Valley, 5/18/1981 (65°-88°) | | | | | | | |
| ZR-3210 EC(10%) | 0.0025 | 2.8 | 11 | 7 | 5 | 5 | 29 | 12 | 3 | 57 |
| | 0.005 | 5.6 | 13 | 20 | 4 | 13 | 35 | 26 | 3 | 85 |
| | 0.010 | 11.2 | 13 | 5 | 2 | 3 | 40 | 9 | 0 | 100 |
| Check | - | - | 19 | 11 | 31 | 16 | 0 | 47 | 7 | 36 |
| | | | Coachella Valley, 4/13/1981 (66°-84°) | | | | | | | |
| ZR-3210 EC(10%) | 0.010 | 11.2 | 13 | 11 | 0 | 0 | 100 | 8 | 0 | 100 |
| | 0.025 | 28.0 | 72 | 13 | 4 | 0 | 100 | 40 | 0 | 100 |
| | 0.050 | 56.0 | 12 | 8 | 1 | 0 | 100 | 1 | 0 | 100 |
| Physan 20 (20%) | 1.5 | - | 80 | 24 | 54 | 14 | 42 | 41 | 22 | 8 |
| | 3.0 | - | 77 | 43 | 71 | 33 | 23 | 33 | 32 | 26 |
| | 6.0 | - | 92 | 35 | 47 | 29 | 17 | 105 | 65 | 0 |
| Check | - | - | 47 | 19 | 36 | 16 | 16 | 27 | 33 | 0 |
| | | | Coachella Valley, 4/21/1980 (72°-84°F) | | | | | | | |
| Physan 20 (20%) | 6.0 | - | 26 | 29 | 0 | 2 | 93 | 7 | 4 | 86 |
| | 12.0 | - | 66 | 21 | 0 | 3 | 86 | 7 | 3 | 86 |
| | 18.0 | - | 72 | 35 | 2 | 1 | 97 | 3 | 2 | 94 |
| Check | - | - | 19 | 25 | 2 | 29 | 0 | 2 | 16 | 36 |

Mean min. and mean max. water temp.

gal/A) no significant reduction of the mosquito larval population was apparent. Higher rates are required (8-12 lb/A) to obtain satisfactory results. At these high rates, Physan 20 will be impractical to use as mosquito larvicide in mosquito control operation due to high costs.

DPX-5444 EC 2 produced complete control of all larval stages at the rate of 0.05 lb/A (56 g/ha) in the Coachella Valley ponds. At the same rate the late larval stages were eliminated in the Riverside ponds, but younger larvae, however, persisted during the entire test duration. Similar results at both locations were obtained at the rate of 0.025 lb/A (28 g/ha), and 97 and 86 percent control of late larval stages was achieved. At the lower rates of 0.005 and 0.01 lb/A, poor results (20 and 64% reduction) were obtained in the Riverside ponds, while good control (82 and 90% reduction) was possible in the coachella Valley ponds (Table 3).

Effectiveness of DPX-5444 in the Coachella Valley ponds at the lower rates could be attributed to several factors. The Riverside ponds harbor heavier density of larval populations, free of vegetative growth, water percolation in the ponds is in excess of 2 gal/min and water pH is in the range of 8-8.2. Coachella Valley ponds are vegetated, percolation rate is less than 1 liter/min and pH is in the range of 9.1-9.4. Water percolation rate in Riverside ponds is extremely high, therefore dilution of toxicants in the ponds is rapid, resulting in inferior results.

Against 3rd and early 4th stage larvae of the floodwater

**Table 3.** Evaluation of DPX-5444 EC2 against mosquito larvae of *Culex tarsalis* in experimental ponds.

| Rate | | Avg. no. of larvae and pupae/5 dips pre and posttreat.(days) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pretreat. | | | 2 | | | | 7[a] | | | |
| lb/A | g/ha | 1-2 | 3-4 | P | 1-2 | 3-4 | P | (%R) | 1-2 | 3-4 | P | (%R)[b] |
| **UCR Aquatic & Vector Cont. Facility** | | | | | | | | | | | | |
| **Test A, June 18, 1980** | | | | | | | | | | | | |
| 0.005 | 5.6 | 514 | 384 | 29 | 410 | 308 | 52 | 20 | 288 | 251 | 42 | 35 |
| 0.010 | 11.2 | 314 | 131 | 14 | 141 | 47 | 21 | 64 | 145 | 183 | 4 | 0 |
| 0.025 | 28.0 | 294 | 235 | 18 | 107 | 7 | 6 | 97 | 143 | 236 | 0 | 0 |
| Check | - | 96 | 233 | 32 | 677 | 233 | 48 | 0 | 264 | 409 | 16 | 0 |
| **Test B, June 2, 1980** | | | | | | | | | | | | |
| 0.025 | 28.0 | 29 | 108 | 12 | 5 | 0 | 5 | 100 | 114 | 47 | 0 | 56 |
| 0.050 | 56.0 | 51 | 175 | 17 | 2 | 0 | 11 | 100 | 177 | 31 | 0 | 82 |
| 0.100 | 112.0 | 67 | 228 | 66 | 12 | 0 | 28 | 100 | 440 | 541 | 0 | 0 |
| Check | - | 22 | 148 | 29 | 30 | 206 | 17 | 0 | 122 | 106 | 39 | 28 |
| **Coachella Valley Aquatic & Vector Cont. Facility** | | | | | | | | | | | | |
| **Test A, June 25, 1980** | | | | | | | | | | | | |
| 0.005 | 5.6 | 13 | 17 | 17 | 12 | 3 | 2 | 82 | 18 | 9 | 0 | 47 |
| 0.010 | 11.2 | 12 | 55 | 5 | 10 | 5 | 2 | 90 | 19 | 5 | 2 | 90 |
| 0.025 | 28.0 | 14 | 14 | 1 | 7 | 2 | 1 | 86 | 30 | 6 | 0 | 57 |
| Check | - | 6 | 9 | 3 | 3 | 11 | 2 | 0 | 13 | 5 | 1 | 44 |
| **Test B, April 21, 1980** | | | | | | | | | | | | |
| 0.050 | 56.0 | 71 | 50 | 2 | 0 | 0 | 0 | 100 | 13 | 8 | 0 | 84 |
| 0.100 | 112.0 | 38 | 45 | 4 | 0 | 0 | 0 | 100 | 17 | 4 | 0 | 91 |
| Check | - | 19 | 25 | 2 | 2 | 29 | 0 | 0 | 2 | 16 | 2 | 36 |

[a] Population recovered and pupae were present 14 days posttreat. in all plots.

[b] (%R) Percent reduction calculation is based on no. of 3rd and 4th stage larvae in posttreat. v. pretreat.

**Table 4.** Evaluation of various mosquito larvicides against *Psorophora columbiae* in irrigated pastures. (Palo Verde Valley, Calif.)

| Material & formulation | Rate | | Avg. no. larvae & pupae/dip | | | | |
|---|---|---|---|---|---|---|---|
| | | | Pretreat. | | Posttreat.(24h) | | |
| | lb/A | g/ha | L | P | L | P | (%) Reduction[c] |
| | | | **July 29, 1980[a]** | | | | |
| ZR-3210 | 0.005 | 5.6 | 4.3 | 0.6 | 3.5 | 1.1 | 6 |
| EC(10%) | 0.010 | 11.2 | 5.6 | 1.1 | 1.8 | 0.9 | 60 |
| | 0.025 | 28.0 | 8.2 | 2.7 | 1.7 | 1.5 | 71 |
| Bay FCR-1272 | 0.01 | 11.2 | 1.9 | 3.1 | 0.4 | 1.5 | 62 |
| EC(18.5%) | 0.025 | 28.0 | 4.5 | 4.8 | 0.5 | 0.4 | 90 |
| | 0.05 | 56.0 | 2.9 | 2.9 | 0.2 | 0.2 | 93 |
| Check | - | - | 2.8 | 2.3 | 4.9 | 6.5 | 0 |
| | | | **Aug. 3, 1980[b]** | | | | |
| DPX-5444 | 0.005 | 5.6 | 121 | 0 | 43 | 0 | 64 |
| EC2 | 0.010 | 11.2 | 36 | 0 | 8 | 0 | 78 |
| | 0.025 | 28.0 | 43 | 0 | 3 | 0 | 93 |
| Bay FCR-1272 | 0.0025 | 2.8 | 131 | 0 | 114 | 0 | 13 |
| EC(18.5%) | 0.005 | 5.6 | 68 | 0 | 28 | 0 | 59 |
| | 0.010 | 11.2 | 36 | 0 | 7 | 0 | 81 |
| Check | - | - | 125 | 0 | 158 | 0 | 0 |

[a] Conducted in 1/16 acre plots. Population consisted of late 4th stage larvae and pupae.

[b] Conducted in 1/32 acre plots. Population consisted of 3rd and 4th stage larvae.

[c] (%R) percent reduction is based on no. of larvae and pupae in posttreat. vs. pretreat.

mosquito *Ps. columbiae*, Bay FCR-1272 produced 81% control at the rate of 0.01 lb/A (11.2 g/ha). At the same rate, however, only 62% reduction occurred in a mixed population of late 4th instars and pupae, and higher rates of 0.025 and 0.05 lb/A yielded better results (90 and 93% reduction). Against the younger stages (3rd and 4th), DPX-5444 EC2 produced excellent control (93% at the rate of 0.025 lb/A.) Lower rates (0.005 and 0.01 lb/A) also caused substantial reduction in the larval population, causing 64 and 78% reduction. ZR-3210 was less effective than Bay FCR-1272 and DPX-5444, yielding only 71% reduction of the late 4th instars and pupae at the rate of 0.25 lb/A (Table 4).

From the above studies it can be concluded that the pyrethroid Bay FCR-1272 is the most effective material tested during these studies, exhibiting excellent activity against both stagnant and floodwater mosquitoes. This material, however, was less effective than previously tested pyrethroids such as decamethrin or FMC-45498 (Mulla et al. 1980). Bay FCR-1272 and DPX-5444 seem to be promising as mosquito larvicides and could be used effectively for the control of floodwater mosquito larvae at the rate of 0.025 lb/A. At this rate DPX-5444 was equally effective against the stagnant water mosquito *Cx. tarsalis*, but Bay FCR-1272 was more effective (10 fold) and yielded excellent control of this species at the rate of 0.0025 lb/A (2.8 g/ha)--about 3 times the required rate of decamethrin.

The pyrethroids (MS-2000 (fenvalerate) and Sumithrin were evaluated earlier (Mulla et al. 1980). In these earlier studies, Sumithrin produced excellent control of *Ae. nigromaculis* and *Cx. tarsalis* at the rate of 0.025 lb/A. The effective rate against these two species with OMS-2000 (fenvalerate) was in the range of 0.025-0.05 lb/A. Fenvalerate, due to its high fish toxicity (Mulla et al., 1978), will probably not be a material of choice for mosquito control in many aquatic habitats.

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# COMPARATIVE EFFECTS OF AEROSOLS OF BENDIOCARB, CHLORPYRIFOS, MALATHION, AND PYRETHRINS UPON CAGED HONEY BEES AND MOSQUITOES

E. L. Atkins[1], D. J. Womeldorf[2], and L.A. Lewis[3]

INTRODUCTION.–Nonthermal aerosol or ultra-low volume (ULV) generators have been widely incorporated into mosquito control programs in California over the past several years. These machines apply highly-concentrated insecticides to control adult insects. The droplets are produced in a relatively narrow spectrum with volume median diameters in the neighborhood of 10 microns. Observations have been made in California upon the effects against various non-target organisms of chlorpyrifos aerosols (Lusk et al. 1976) and of pyrethrins and pyrethroid aerosols (Washino et al. 1977). None of the California studies evaluated the effects of nonthermal aerosols upon honey bees (*Apis mellifera*). However, Atkins (1972) and Womeldorf et al. (1974) have reported on the effects upon honey bees of aerial applications of several mosquito control pesticides, including highly-concentrated chlorpyrifos, propoxur, fenthion, and dichlorvos. The present paper reports on tests which evaluated the effects on nonthermal aerosols of commercially available formulations of malathion, chlorpyrifos and pyrethrins, and of an experimental formation of bendiocarb, upon caged honey bees and caged mosquitoes.

METHODS AND MATERIALS.–Four assessment lines were established in rural Colusa County. Each was approximately one mile long. All extended north from the same east-west road. Distances between lines varied from 1 to 1.5 miles. Four-foot lath grade stakes were spaced at intervals from the point of aerosol release to the end of each assessment line.

Honey bees obtained from a local field hive were confined in wire mesh cages (Atkins 1972). *Culex pipiens* subsp. from a known-susceptible colony maintained by the California Department of Health Services' Vector Biology & Control Section laboratory at Sacramento were confined in disposable paper and nylon mesh assay ("paper") cages as described by Townzen and Natvig (1973). A paper cage of honey bees were attached near the top of each stake for all tests.

For the bendiocarb tests, *Cx. pipiens* subsp. from a known-susceptible colony maintained by the Disease Vector Ecology and Control Center, Naval Air Station, Alameda, were confined in window screen ("wire") cages as described by Stains et al. (1969). One wire cage was attached near the top of the stake at each station; during one test, a second wire cage was placed at ground level.

Following exposure, the bees and the mosquitoes in the wire cages were transferred to clean holding cages. Mortality was determined at 24 hours and corrected with Abbott's formula for mortality in untreated check cages.

Basic meteorological measurements were taken at intervals of about 10-15 minutes before, during and after each aerosol application. Relative humidity was determined with a sling psychrometer. An anemometer with a directional vane was hand-held at 6 ft above the ground to determine wind speed and direction. Temperatures were measured at 10 m (32 ft) and 2 m (6 ft) above the ground with an electronic thermistor thermometer. The tests were begun only when an inversion was detected (warmer air at 10 m than 2 m).

Tests 1-4 were run more or less simultaneously, using 4 ULV units, on 2 September, 1980. After allowing time for the aerosols to travel the length of the test lines, all cages were collected and new ones put into place. Tests 5-7 were then run. On 3 September, tests 8 and 9 were run by exposing two lines to a single pass of the aerosol generator; at the same time test 10 was run at another line. In every instance the truck-mounted generators were driven from east to west, beginning about 0.5 mile east of the line and continuing to about 0.5 mile west.

Table 1 lists the pertinent data about the tests. The aerosol generators noted as "MAD" were built by the designated mosquito abatement district. They were generally similar to the machine described by Whitesell (1973).

RESULTS.–Two separate findings were developed. The major one was the comparative effect of the aerosols upon mosquitoes and honey bees. The second one was a limited evvaluation of the experimental formulation of bendiocarb, a material never before tested as an aerosol against mosquitoes in California.

Comparative Effects Upon Mosquitoes and Honey Bees.- - The graphs representing tests 1 through 10 illustrate the mortality observed at 24 hours and corrected for check mortality. Only mosquitoes exposed in the paper cages are included in the graphs.

The toxicity to caged honey bees was compared by inspection with the toxicity to caged mosquitoes. The four insecticides showed this decreasing order of toxicity to honey bees: bendiocarb, chlorpyrifos, malathion, and pyrethrins. Bendiocarb was approximately equally toxic to the honey bees and the mosquitoes. Chlorpyrifos killed the bees which received the highest dosages. Malathion produced some mortality in

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**Table 1.** Materials, equipment and operating conditions for 10 tests of mosquito control aerosols applied against honey bees.

| | TEST | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Date | 9-2 | 9-2 | 9-2 | 9-2 | 9-2 | 9-2 | 9-2 | 9-3 | 9-3 | 9-3 |
| Time begun | 2016 | 2030 | 2016 | 2016 | 2112 | 2112 | 2112 | 1900 | 1900 | 1902 |
| Insecticide | pyrethrins | bendiocarb | malathion | chlorpyrifos | pyrethrins | malathion | chlorpyrifos | bendiocarb | bendiocarb | chlorpyrifos |
| Formulation | 12% | 25% | 8 lbs/gal | 6 lbs/gal | 12% | 8 lbs/gal | 6 lbs/gal | 25% | 25% | 6 lbs/gal |
| Diluent | Stoddard solvent; mineral oil | Klearol | None | Motor Oil | Stoddard solvent; mineral oil | None | Motor oil | Klearol | Klearol | Motor oil |
| Final concentration | 2.5% | 12.5% | 8 lbs/gal | 0.5 lbs/gal | 2.5% | 8 lbs/gal | 0.5 lb/gal | 12.5% | 12.5% | 0.5 lb/gal |
| Equipment | Butte County MAD | Microgen ED2-20A | Sutter-Yuba MAD | Colusa MAD | Butte County MAD | Sutter-Yuba MAD | Colusa MAD | Microgen ED2-20A | Microgen ED2-20A | Colusa MAD |
| Discharge (fl oz/min) | 2 | 4 | 4.09 | 12.5 | 2 | 4.09 | 12.5 | 2.5 | 2.5 | 2.5 |
| Vehicle speed (mph) | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Distance driven (miles) | 1± | 0.9 | 1.1 | 1± | 1± | 1.0 | 1± | 1.0 | 1.0 | 1± |
| Temperature @ 10m(°F) | 76.5 | 76.0 | 76.5 | 76.5 | 73.0 | 73.0 | 73.0 | 80.8 | 80.8 | 80.8 |
| Temperature @ 2m(°F) | 73.0 | 72.3 | 73.00 | 73.0 | 71.5 | 71.5 | 71.5 | 77.9 | 77.9 | 77.9 |
| Windspeed (mph) | 4.6 | 4.6 | 4.6 | 4.6 | 5.2 | 5.2 | 5.2 | 4.6 | 4.6 | 4.6 |
| Wind direction | SE | SE | SE | SE | ESE | ESE | ESE | ESE | ESE | ESE |
| Relative humidity (%) | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 72 | 72 | 72 |

the caged bees. Pyrethrins were essentially nontoxic at the dosages applied.

Evaluation of Bendiocarb Against Mosquitoes. - - As noted in Table 1, the experimental formulation was 25% weight/volume (250 gm/liter). It was diluted 1:1 with Klearol® and applied with a Microgen ED2-20A. The experimental formulation utilized was found to be reasonably easy to mix and disperse. It also had no odor.

Table 2 lists the results of the observed mortalities of caged mosquitoes for three tests. Some apparent differences between mortalities of mosquitoes in the paper and wire cages are noted, but in general the results are comparable.

On the night of 2 September, the application was made using a flow rate of 4 fl. oz./minute of a 12.5% mixture (Test 2). An effective swath width of about 600 feet was achieved, even though continuing meteorological readings indicated a rapidly deteriorating inversion condition from the time the test was begun. Ground level placement (low) of wire-cage mosquitoes did not produce sginificant kill. These low level placement findings are consistent with results of earlier studies.

On the evening of 3 September, Test 8 produced a very significant kill to 1200 feet from the point of aerosol release, although the flow rate had been reduced to 2.5 fl. oz./minute of the 12.5% mixture. The improved swath width with this lesser dosage may have been due to the strong inversion condition which persisted for the duration of this test.

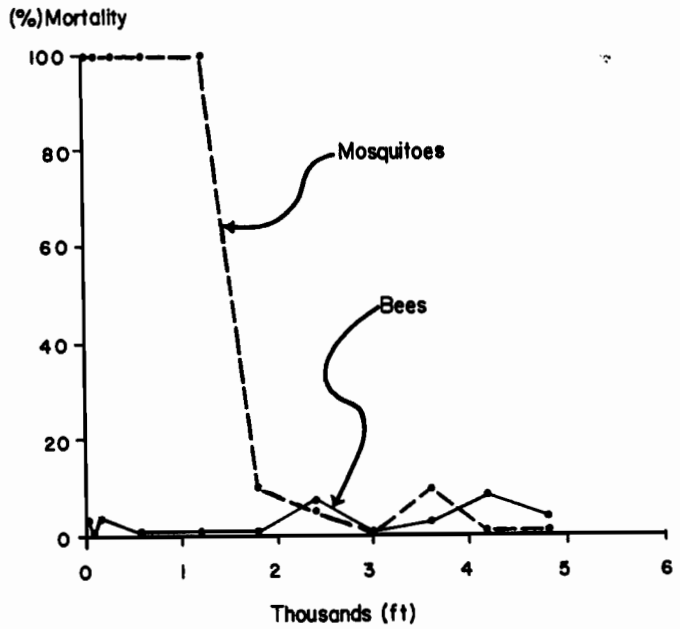
**Table 2.** Percent mortalities (corrected) of caged mosquitoes resulting from applications of an experimental formulation of bendiocarb.

| | Test 2 | | | Test 8 | | Test 9 | |
|---|---|---|---|---|---|---|---|
| | | Wire cage | | | | | |
| No. feet from discharge point | Paper cage | High | Low | Paper cage | Wire cage | Paper cage | Wire cage |
| 0 | 100 | 100 | 6 | 100 | 100 | 100 | 100 |
| 150 | 100 | 100 | 15 | 100 | 67 | 100 | 100 |
| 300 | 100 | 100 | 0 | 100 | 62 | 2/ | 49 |
| 600 | 100 | 57 | 32 | 100 | 65 | 100 | 0 |
| 900 | 1/ | 1/ | 1/ | 100 | 87 | 87 | 0 |
| 1200 | 1 | 28 | 5 | 100 | 76 | 64 | 0 |
| 1800 | 0 | 28 | 14 | 51 | 42 | 40 | 0 |
| 2400 | 0 | 8 | 0 | 0 | 18 | 64 | 3 |
| 3000 | 0 | 17 | 23 | 0 | 26 | 5 | 16 |
| 3600 | 0 | 5 | 7 | 0 | 50 | 22 | 0 |
| 4200 | 9 | 10 | 22 | 0 | 13 | 0 | 0 |
| 4500 | 1/ | 1/ | 1/ | 0 | 10 | 1/ | 1/ |
| 4620 | 1/ | 1/ | 1/ | 1/ | 1/ | 19 | 0 |
| 4770 | 1/ | 1/ | 1/ | 1/ | 1/ | 0 | 9 |
| 4800 | 7 | 18 | 0 | 0 | 27 | 1/ | 1/ |
| 4950 | 1/ | 1/ | 1/ | 4 | 2/ | 1/ | 1/ |
| 5400 | 0 | 23 | 0 | 1/ | 1/ | 1/ | 1/ |

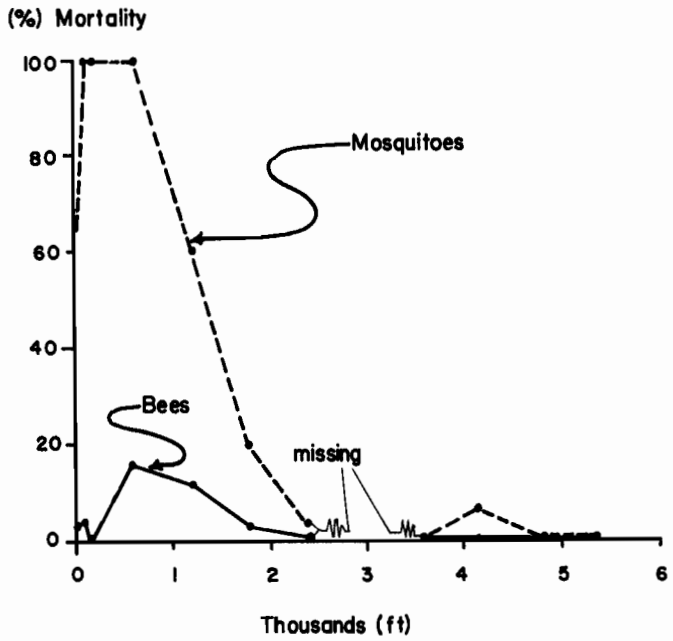
1/ No station at this location this test.

2/ Missing

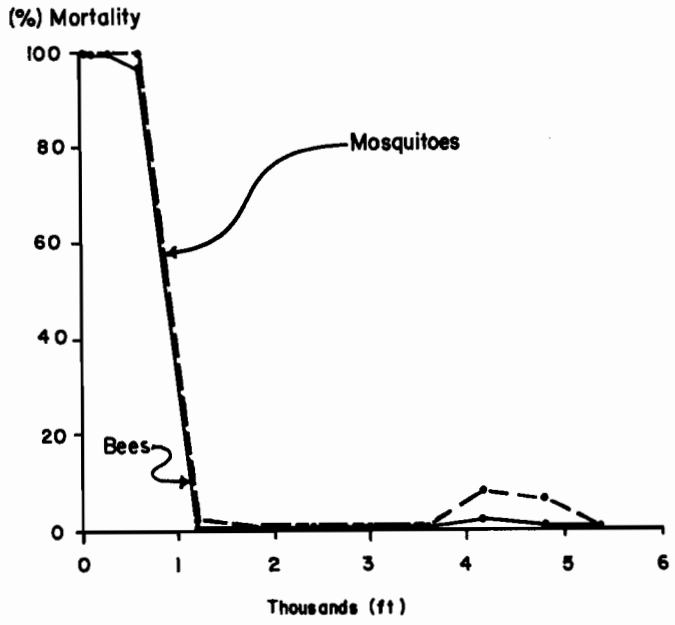
PYRETHRINS, TEST 1



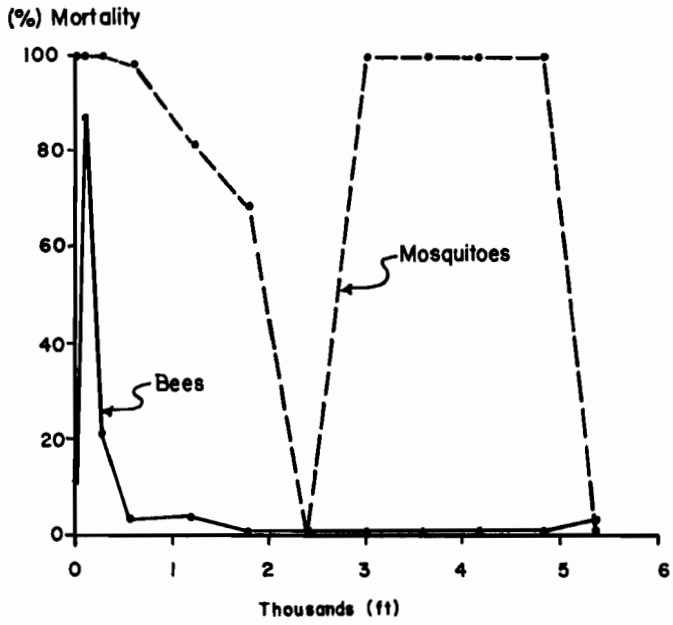

MALATHION, TEST 3



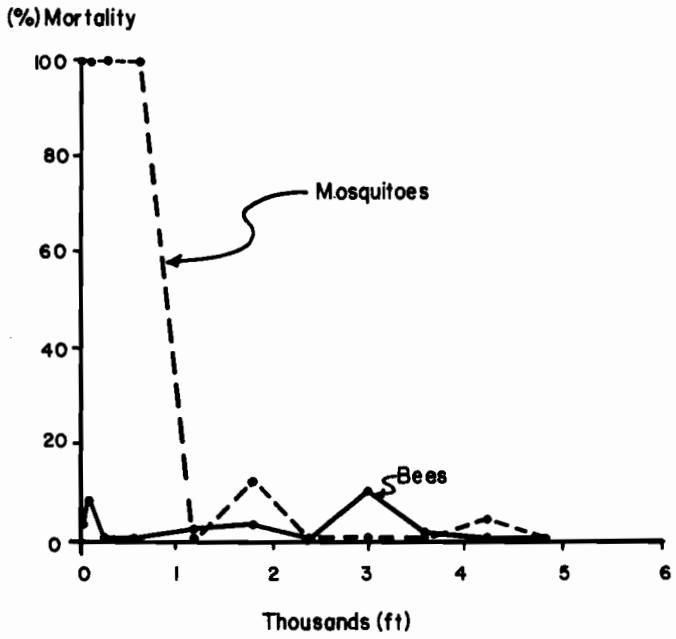

BENDIOCARB, TEST 2



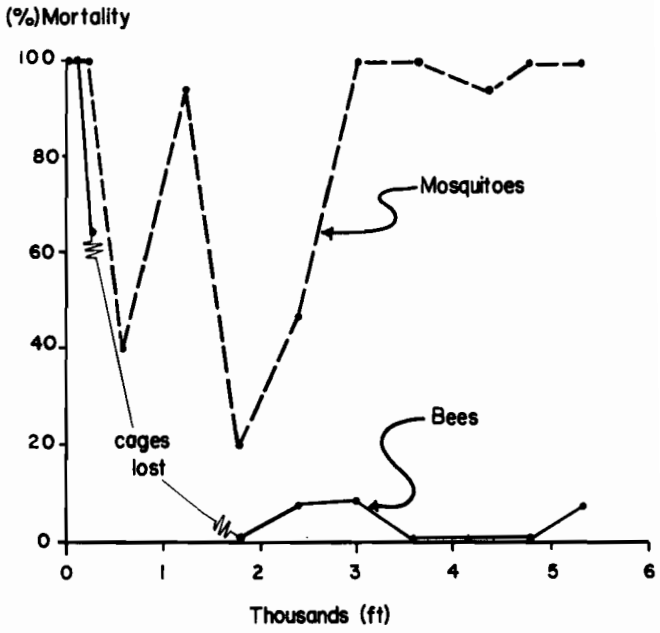

CHLORPYRIFOS, TEST 4



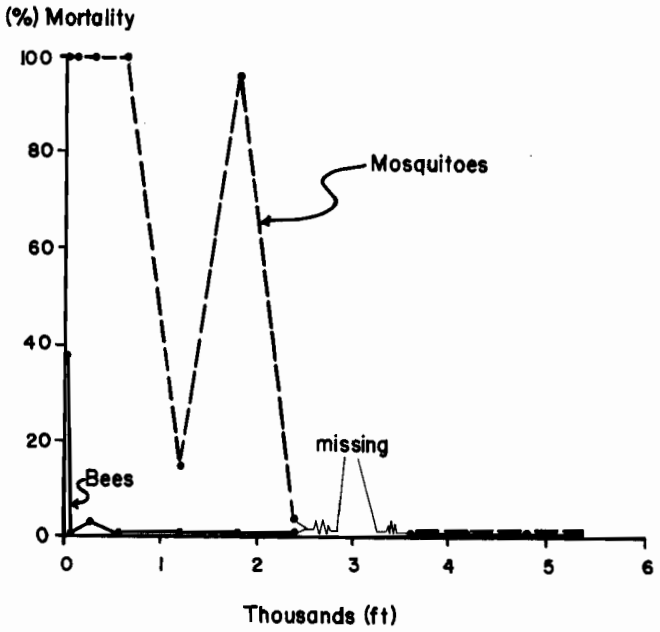
PYRETHRINS, TEST 5



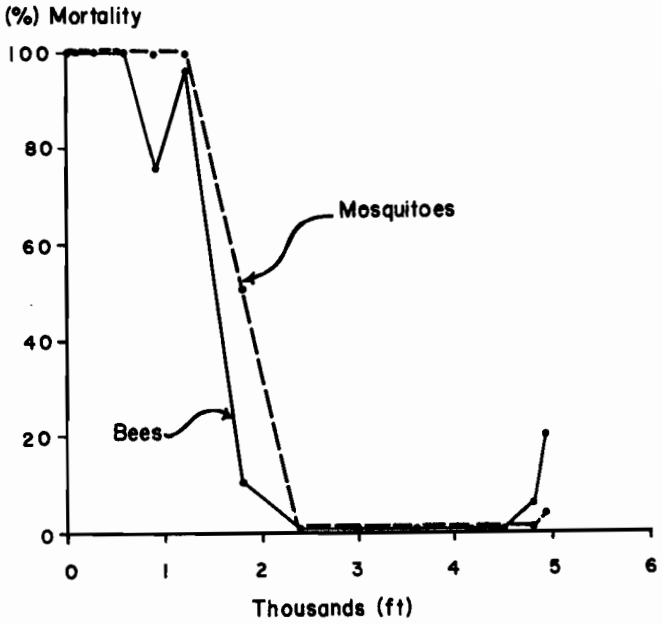

CHLORPYRIFOS, TEST 7



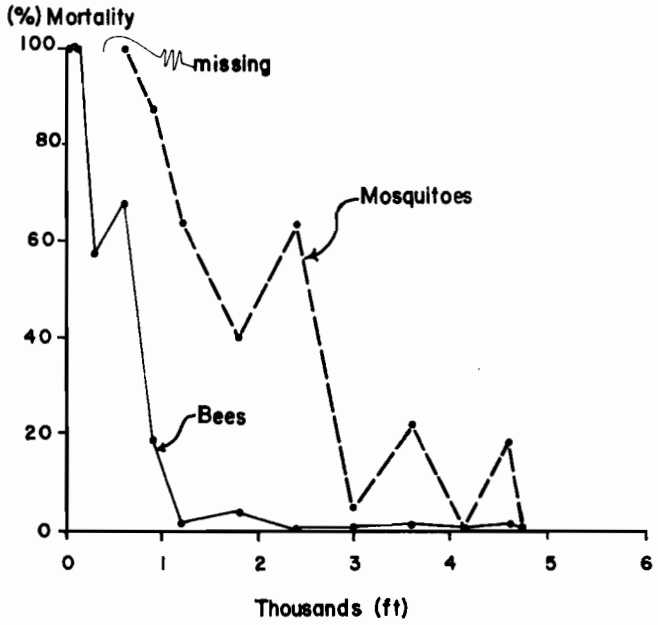

MALATHION, TEST 6



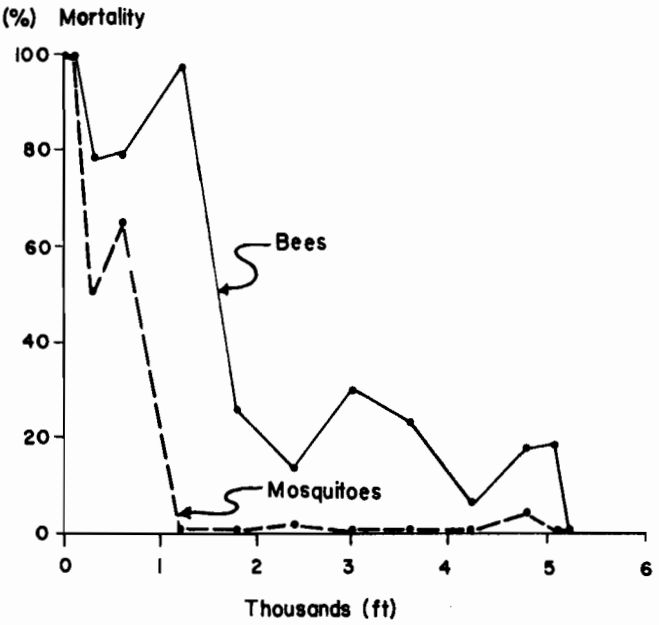

BENDIOCARB, TEST 8



BENDIOCARB, TEST 9




CHLORPYRIFOS, TEST 10




Test 9 was run on a line parallel to Test 8 but separated by a distance of nearly 1 mile. Both lines were treated by the same pass of the aerosol generator. Significant kill of mosquitoes in the paper cages was obtained out to 900 feet.

DISCUSSION AND CONCLUSIONS.–Effects Upon Honey Bees. - - Bendiocarb, malathion and chlorpyrifos were highly hazardous to caged worker honey bees which were directly contacted at the closer test site stations within the path of the nonthermal aerosol applications while pyrethrins had no substantive effect on caged honey bees.

We did not have colonies of honey bees placed in the assessment lines; therefore, we have no direct data demonstrating that nonthermal aerosols entered bee hives and affected or did not affect the bees within. Shaw and Armstrong (1966) showed that a pesticide which is highly toxic to bees upon direct contact, naled, was nonhazardous to bees in their hives. In addition, the senior author has done hundreds of field tests in agricultural crops at the higher agricultural crop dosages and has applied the sprays directly over unprotected colonies in the plots. This does not cause measurable bee mortality as long as the bees are in the hive and are not clustered on the outside of the hive, as might occur in warm weather. Clustering continues until the temperature has dropped to 70°F and lower and/or until a brisk wind prevails.

Because of these data, our opinion is that nonthermal aerosols containing these pesticides, at the dosages utilized and applied under the conditions which prevailed, would not be hazardous to worker honey bee adults in their hives during the time of the actual pesticide application. However, precautions should be taken to be certain that bees are not clustered on the hives at the time of application of the more toxic materials. In agricultural fields the bee colonies are inspected for clustering prior to application at night. If bees are clustering, the application is delayed until the bees enter their hive, or the bees are smoked into their hive and the hive entrance is closed with wet burlap. If these precautions are taken, we do not expect any hazard from the nonthermal aerosols to bees in their hives in areas within the swath width of the application. Bees outside of the expected swath width are not considered to be at risk.

Evaluation of Bendiocarb Against Mosquitoes. - - The beniocarb results were very encouraging, comparing well with the data of Tapley et al. (1980). An experimental use permit is pending which will allow testing the material against mosquitoes in the field.

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# THE ACCUMULATION AND DEGRADATION OF SIR-8514 BY FISH

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ABSTRACT

Methods for the chemical analyses of a promising chemical control agent, SIR-8514 and two metabolites, were found that allow for the quantitative determination of these compounds in water, soil and fish tissues. When SIR-8514 is tested under standardized static conditions, it degrades in soil and channel catfish do not accumulate it. However, under constant flow-through conditions bluegill sunfish accumulate SIR-8514 in their tissues up to 100X the concentration of the treated water. SIR-8514 is metabolized in fish tissues and trifluoromethoxyphenylurea and trifluoromethoxyaniline are produced.

SIR-8514 (2-chloro-N-[[[4-trifluoromethoxy)phenyl] -amino] carbonyl] benzamide) has been shown to be a highly effective inhibitor of mosquito development (Schaefer et al. 1978). Also when adult female mosquitoes are fed this compound, the eggs that they produce are not viable (Miura and Takahashi 1979). SIR-8514 acts through the inhibition of chitin synthesis (Hajjar and Casida 1978).

The stability of SIR-8514 in water is reduced as temperature and pH simultaneously increase but is not greatly affected by sunlight (Schaefer and Dupras 1979). When a pond was treated with 5 ppb SIR-8514, larvae of the Clear Lake gnat, *Chaoborus astictopus* Dyar and Shannon, were reduced by over 99%, planktonic arthropods decreased by $>90\%$, while planktonic rotifers and dinoflagellates and benthic organisms were less severely affected (Colwell and Schaefer 1981). During this study, bluegill sunfish, *Lepomis macrochirus* Rafinesque and mosquitofish, *Gambusia affinis* Baird and Girard, accumulated SIR-8514 from the water to ca. 400 ppb in their tissues within 48 hrs but no tissue residues remained by 28 days and no fish mortality was observed. Because SIR-8514 is regarded as having high potential as a mosquito control agent, laboratory studies were initiated to determine the accumulation of the parent compound into fish tissues and the subsequent formation of metabolites.

MATERIALS AND METHODS.--Analytical Methods. - - Procedures used for the analysis of diflubenzuron and 2 metabolites (Schaefer et al. 1980) were also employed in this study. A probably pathway of degradation (Figure 1) for SIR-8514 was devised; it appears likely, based on previous experence with diflubenzuron, that 4-trifluoromethoxyphenylurea and 4-trifluoromethoxyaniline would be degradation products. Methods previously developed for the analyses of diflubenzuron and its metabolites (DiPrima 1976, Rabenort et al. 1978) were evaluated for use with SIR-8514 and its suspected metabolites. Generally this involves separation of SIR-8514, 4-trifluoromethoxyphenylurea and 4-trifluoromethoxyaniline by pH adjustments and partitioning into organic solvents and the SIR-8514 and 4-trifluoromethoxyphenylurea fractions are

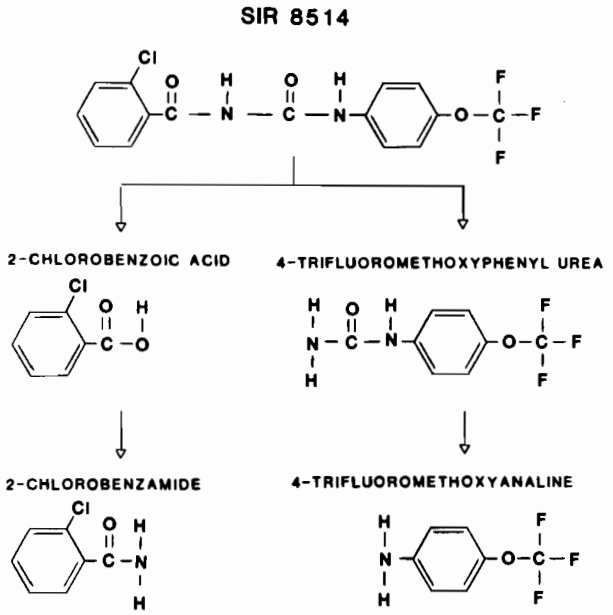


**Figure 1.** Probable degradation pattern for SIR-8514

hydrolyzed by refluxing in acid to form 4-trifluoromethoxyaniline; the latter is reacted with heptafluorobutyric anhydride to form the heptafluorobutyric-4-trifluoromethoxyanilide, which is then analyzed by gas-liquid chromatography using an electron capture detector.

Samples of soil, water and fish tissues were fortified with known amounts of SIR-8514, trifluoromethoxyphenylurea and trifluoromethoxyaniline. These were then subjected to extraction and analysis, as described above, and the recoveries of each were determined. In addition, it was of importance to determine the extent as to which SIR-8514 would hydrolize during the course of laboratory exposure studies. Therefore water samples were fortified at 0.02 ppm SIR-8514 (the maximum water solubility) and then extracted immediately (0 hrs) or held for 24, 48 or 72 hrs at temperatures of 24° and 38°C and under pH's of 7.7 and 10.0 after which each was analyzed for SIR-8514 and the 2 suspect metabolites.

Static Exposures. - - These studies were designed to comply with EPA data requirements for registration of pesticides. Soil is treated with the highest use rate projected for a given chemical; the soil is aged aerobically for thirty days, flooded with water and aged an additional thirty days, and then, channel catfish, *Ictalurus punctatus* Rafinesque are exposed for intervals up to thirty days and residues of the chemical in their tissues are then analyzed. In this case Atwater sandy loam soil was collected and sieved to remove debris (smallest mesh size 1.4 mm). Technical SIR-8514 (98.8% active ingredient) was dissolved in 10% acetone - - 90% methanol and batches of the soil were sprayed in a rotating cement mixer to give a treatment rate of 0.04 lb AI/acre (the highest projected use rate). Ten gallon aquaria received 10 pounds and larger tanks 60 pounds each of treated soil. The ten gallon aquaria were held indoors, while the larger tanks were held on a porch. All of the tanks were allowed to stand open for 30 days before being flooded. Ten gallon aquaria were flooded with 32 L tap water each and the larger tanks 192 L each and all tanks were held an additional 30 days before fish were added. Fish were acclimatized for 15 days before initiation of exposure under the same holding conditions as for the tanks. Fingerling channel catfish (5.0 to 7.0 cm in length and 1.5 to 5.0 gm each) were added to the tanks at loading rates up to 25.6 gm per 10 gallon tank and 153.6 gm per 60 gallon tank (10 gal. tanks averaged 7 fish each and 60 gal. tanks 43 fish each). Both tank sizes were sampled for fish after 0, 1, 3, 6, 10, 14, 22, and 30 days of fish exposure. The concentration of residues of SIR-8514 and 2 suspect metabolites was determined for the water and soil after each 30 day aging period and for the whole bodies, edible tissues and viscera of the fish after each exposure interval.

Dynamic Exposures. - - Under this condition, bluegill sunfish *Lepomis macrochirus* Rafinesque, are exposed to a continuous flow-through treatment of the test insecticide. SIR-8514 was dissolved in water at 20 ppb, the maximum water solubility, and this solution was continuously metered through the 10 gal aquaria at 150 ml per minute during the exposures; the water level in each tank (25 cm depth) was kept constant by a siphon devise. Initially 48 fish (average 7.8 cm length and 8.8 gm each) were placed in the treatment tank. After each given exposure regime, analyses were made for SIR-8514 and 2 suspected metabolites in whole bodies, edible tissues and viscera. Three different exposure regimes were followed: 1. the fish were exposed for 24, 48 or 72 hrs and then analyzed, 2. the fish were exposed for 24, 48 or 72 hrs and then placed in a rinse tank with flowing, untreated water passing through, for 24 hrs and were then analyzed and 3. the fish were exposed for 24 hrs and the tissues were analyzed immediately as well as after rinse periods with untreated water of 24,48 and of 72 hrs.

RESULTS AND DISCUSSION.–Analytical Methods. - - Recoveries of SIR-8514 and its two suspected metabolites from water, soil and fish tissues are shown in Table 1. It is clear that the recoveries are adequate to allow quantitative analyses of these residues. The hydrolysis of SIR-8514 in water at its maximum solubility (20 ppb) is shown in Table 2; under pH 7.7 it is quite stable at either 24 or 38°C. However, at pH 10.0 it hydrolyses to trifluoromethoxyphenylurea at increasing rates as temperature increases but there is no evidence of further degradation to the corresponding analine. Thus, at pH levels not higher than 7.7 i. e. in the fish tanks, no significant hydrolysis would be expected within a 72 hr exposure period.

Static Exposures. - - Analysis of soil samples taken from the 10 gal. and from the 60 gal. aquaria immediately after treatment showed an average level of 95.8% of the theoretical treatment dose (0.12 ppm); however, after the 30 day period of aerobic exposure and aging, the average soil concentration was only 2.6% of the treatment dose and after aging an additional 30 days under flooded conditions, no SIR-8514 was detected. Analysis of fish tissues after each of the exposure periods revealed no detectable amounts of SIR-8514, trifluoro-

**Table 1.** Recovery of SIR-8514, trifluoromethoxyphenylurea[a/] and trifluoromethoxyaniline[b/] from water, fish and soil following fortification of samples of 5 ppb.

| | Average % Recovery | Range | Lowest Detectible Limit (ppb) |
|---|---|---|---|
| Water | | | |
| SIR-8514 | 97.1 | 95.1 - 99.2 | 0.4 |
| "urea"[a/] | 95.1 | 92.7 - 96.5 | 0.3 |
| "aniline"[b/] | 95.0 | 91.9 - 97.9 | 0.2 |
| Soil | | | |
| SIR-8514 | 98.8 | 97.8 -100.0 | 0.3 |
| "urea"[a/] | 91.8 | 88.2 - 96.4 | 0.2 |
| "aniline"[b/] | 94.3 | 92.2 - 96.2 | 0.2 |
| Fish | | | |
| SIR-8514 | 97.5 | 96.0 - 99.1 | 0.4 |
| "urea"[a/] | 97.4 | 95.4 - 99.2 | 0.3 |
| "aniline"[b/] | 96.4 | 93.9 - 98.5 | 0.2 |

**Table 2.** Hydrolysis of SIR-8514 after treated water (0.02 ppm) was held at 24 and 38°C at pH's of 7.7 and 10.0.

| Compound: temp (°C): | SIR-8514 | "urea"[a/] 24° | "aniline"[b/] | SIR-8514 | "urea" 38° | "aniline" |
|---|---|---|---|---|---|---|
| pH 7.7 | | | | | | |
| holding time (hrs) | | | | | | |
| 0 | 19.4 | N.D.[c/] | N.D. | | | |
| 24 | 15.3 | N.D. | N.D. | 12.7 | N.D. | N.D. |
| 48 | 11.9 | N.D. | N.D. | 10.6 | N.D. | N.D. |
| 72 | 11.1 | N.D. | N.D. | 10.2 | 0.4 | N.D. |
| pH 10.0 | | | | | | |
| holding time (hrs) | | | | | | |
| 0 | 19.9 | N.D. | N.D. | | | |
| 24 | 11.5 | 1.7 | N.D. | 4.9 | 4.4 | N.D. |
| 48 | 8.5 | 2.0 | N.D. | 3.0 | 6.1 | N.D. |
| 72 | 8.3 | 2.9 | N.D. | 1.1 | 6.9 | N.D. |

[a/] trifluoromethoxyphenylurea

[b/] trifluoromethoxyaniline

[c/] not detectable

methoxyphenylurea or trifluoromethoxyaniline. Thus, SIR-8514 has limited persistence in soil and no fish toxicity or bioaccumulation resulted from these static exposures at the maximum projected use rate of 0.04 lb/acre.

Dynamic Exposures. - - Analysis of the water used for treatments showed an average of 19.3 ppb or 96% of the 20.0 ppb level sought; the lowest concentration measured was 18.4 ppb or 92% of theoretical.

Residues of SIR-8514, trifluoromethoxyphenylurea and trifluoromethoxyaniline in bluegill tissues following three different treatment and rinse regimes are shown in Table 3. Bioaccumulation of SIR-8514 occurs to ca. 100X (tissue concentration divided by water concentration). It is apparent that the viscera accumulates the highest concentration of the parent compound; however, the viscera only composes ca. 7% of the body mass. The edible tissues compose ca. 74% of the body mass and thus the major portion of SIR-8514 and the 2 metabolites was found there. No apparent toxicity to bluegills resulted from any of these exposures.

Both trifluoromethoxyphenylurea and trifluoromethoxyaniline are significant metabolites in fish tissues.

**Table 3.** Residues of SIR-8514, trifluoromethoxyaniline[a/] and trifluoromethoxyphenylurea[b/] in fish tissue following exposure to 20 ppb SIR-8514 (in ppb).

| | | | | |
|---|---|---|---|---|
| Test No. 1: Exposure but no rinse | | | | |
| Exposure period: | | 24 h | 48 h | 72 h |
| whole body | 8514 | 380.68 | 591.75 | 104.18 |
| " " | "aniline"[a/] | 65.66 | 30.26 | 32.79 |
| " " | "urea"[b/] | 65.07 | 68.69 | 129.88 |
| edible tissue | 8514 | 358.09 | 286.75 | 324.91 |
| " " | "aniline" | 17.46 | 13.43 | 31.81 |
| " " | "urea" | 75.85 | 63.60 | 18.15 |
| viscera | 8514 | 1794.38 | 1768.31 | 985.50 |
| " | "aniline" | 37.81 | 41.53 | 53.80 |
| " | "urea" | 139.02 | 60.01 | 68.53 |
| Test No. 2: Exposure followed by 24 h rinse period | | | | |
| Exposure period: | | 24 h | 48 h | 72 h |
| whole body | 8514 | 204.69 | 210.00 | 81.12 |
| " " | "aniline" | 6.48 | 16.16 | 12.58 |
| " " | "urea" | 23.40 | 69.14 | 45.29 |
| edible tissue | 8514 | 253.70 | 130.90 | 310.14 |
| " " | "aniline" | 10.74 | 89.01 | 25.31 |
| " " | "urea" | 166.23 | 58.10 | 30.86 |
| viscera | 8541 | 1224.79 | 733.49 | 530.14 |
| " | "aniline" | 54.23 | 149.63 | 77.16 |
| " | "urea" | 1075.57 | 184.85 | 413.87 |
| Test No. 3: Exposure for 24 h followed by rinse period | | | | |
| Rinse period: | | 0 h | 24 h | 48 h | 72 h |
| whole body | 8514 | 1962.34 | 807.77 | 483.89 | 321.07 |
| " " | "aniline" | 33.20 | 33.48 | 25.71 | 22.56 |
| " " | "urea" | 135.01 | 287.71 | 129.48 | 58.50 |
| edible tissue | 8514 | 665.55 | 603.61 | 280.52 | 156.80 |
| " " | "aniline" | 39.38 | 37.88 | 24.85 | 22.27 |
| | "urea" | 251.07 | 93.51 | 91.12 | 86.58 |
| viscera | 8514 | 2758.26 | 2143.50 | 1634.02 | 679.47 |
| " | "aniline" | 51.73 | 78.28 | 53.98 | 27.42 |
| " | "urea" | 383.71 | 254.15 | 330.89 | 166.14 |

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# EFFICACY OF PETROLEUM LARVICIDAL OILS AND THEIR IMPACT ON SOME AQUATIC NONTARGET ORGANISMS

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ABSTRACT

Complete control of stagnant water mosquito larvae of *Culex tarsalis* Coquillet, *Culiseta inornata* Williston and *Anopheles franciscanus* McCracken was achieved at the rate of 2 and 4 gals/A in experimental ponds with Golden Bear petroleum larvicidal oils. GB-1111 and GB-1313 produced excellent control for one week, while BG-1356 was effective for over 2 weeks. All these formulations were found to be harmless, at both rates applied, against bottom dwelling macroinvertebrates present which include mayflies, dragonflies and damselflies. Surface breathing organisms such as diving beetle larvae and adults, notonectids and corixids, however, were markedly affected, and mortality occurred soon after treatment upon contact with petroleum oil film on the water surface.

INTRODUCTION.—Mosquito control agencies in California utilized 270,000 gallons of petroleum hydrocarbon larvicidal oils for the control of mosquito larvae in 1954, at a time when chlorinated hydrocarbons were highly effective and widely used in mosquito abatement operations. In 1960, when resistance in the larval stages to chlorinated hydrocarbons became a critical problem, the use of petroleum oils as larvicide more than doubled, and over 550,000 gallons were utilized. Later on the use of petroleum oils decreased somewhat as the newer organophosphorus insecticides such as fenthion and chlorpyrifos became available. However, due to the development of resistance in the larval populations, these and other organophosphorus larvicides became relatively ineffective in certain areas of California. With the emergence of widespread resistance, the use of petroleum oil as mosquito larvicide increased markedly and exceeded 577,000 gallons in 1977 (Mulla (1977).

To meet the increased demand for more effective petroleum larvicidal oils, several petroleum hydrocarbon derivatives were evaluated, and reported to possess a high level of activity against larvae of the multiresistant strains of stagnant and floodwater mosquitoes at the rate of 2 gal/A. (Darwazeh 1973, Darwazeh and Ramke 1972, Micks et al. 1968, Mulla et al. 1971). Some of these materials include Flit MLO, Golden Bear GB-1111, GB-1313 and GB-1356 and others. Efficacy and longevity of Flit MLO and other petroleum hydrocarbons such as Toxisol FLC and Toxisol TB (Atlantic Richfield Co.) against mosquito larvae and other nontarget aquatic organisms were determined and reported by Mulla et al. (1971). However, no studies were conducted to determine the longevity and impact of the Golden Bear petroleum oils on nontarget aquatic organisms, and these formulations are widely employed for mosquito control in California and elsewhere.

The current studies, therefore, were carried out to investigate the efficacy and lonevity of Golden Bear petroleum larvicidal oils on mosquito larvae and to determine their impact on selected aquatic nontarget organisms in mosquito breeding habitat.

METHODS AND MATERIALS.—Investigations were conducted at the Aquatic Research Facility of the University of California, Riverside, located near the Salton Sea in the Coachella Valley of Southern California as described elsewhere (Mulla and Darwazeh 1971). The pond system is composed of 64 identical ponds of 8 rows, with 8 ponds in each row, and each pond is 36 $m^2$ (18′ x 18′) in size. Water for the ponds are provided from an artesian well, and water depth is kept constant (12″-15″) by float valves. The experimental materials were provided by Witco Chemical, Golden Bear Division, Bakersfield, California. The composition and properties of these oils are shown in (Table 1).

Each material was applied at 2 and 4 gals/A. The required amount was applied with 1 qt. all purpose household sprayer from the sides and the middle of each pond, covering the entire surface area. Two ponds were used per rate of application, and 2 left untreated as check. Mosquito populations consisted mostly of all the aquatic immature stages of *Culex tarsalis* Coquillet, but *Culiseta inornata* Williston and *Anopheles franciscanus* McCracken also were present in small numbers (5-10%).

Prior to treatment and 2, 7 and 14 days after treatment, 5 dips per pond were taken and were concentrated into one composite sample (Mulla et al. 1975) and organisms present were identified and counted under a dissecting microscope in the laboratory. These organisms include mayfly, dragonfly and damselfly naiads, mosquito larvae and pupae, diving beetle larvae, tadpoles and ostracods. Percent mosquito larval con-

**Table 1.** Properties and composition of Golden Bear petroleum larvicidal oils.

| Property | Pet. oils tested | | |
|---|---|---|---|
| | GB-1111 | GB-1313 | GB-1356 |
| Appearance | Colorless liquid | Colorless liquid | Colorless liquid |
| Specific Gravity @ 60°F | 0.892 | 0.875 | 0.891 |
| Pounds/gal. | 7.4 | 7.3 | 7.4 |
| Viscosity, SSU @ 100°F | 49.0 | 57.0 | 47.0 |
| Flash Point, COC, °F | 265.0 | 305.0 | 280.0 |
| Unsulfonated Residue (%) | - | - | - |
| Odor | slight | slight | slight |
| Additives (%): | | | |
| Surfactant | a | a | 1.3 |
| Aliphatic amines | 0 | 0 | 0.5 |

a/ Contain undisclosed surface active agents.

**Table 2.** Effectiveness of Golden Bear petroleum oils against mosquito larvae in experimental mosquito breeding ponds[a/] (Oasis, California, April 1978).

| Pet Oil | Rate | Avg. no. of larvae and pupae/5 dips composit sample pre and post-treat- (days) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-treat- | | | 2 | | | | 7 | | | | 14 | | | |
| | Gal/A | 1-2 | 3-4 | P | 1-2 | 3-4 | P | (% C) | 1-2 | 3-4 | P | (% C) | 1-3 | 3-4 | P | (% C) |
| GB-1111 | 2 | 12 | 8 | 1 | 0 | 0 | 0 | 100 | 7 | 0 | 0 | 67 | 10 | 3 | 0 | 38 |
| | 4 | 19 | 4 | 4 | 1 | 0 | 0 | 96 | 2 | 0 | 0 | 93 | 17 | 7 | 0 | 14 |
| GB-1313 | 2 | 13 | 4 | 1 | 1 | 0 | 0 | 94 | 3 | 0 | 0 | 83 | 6 | 2 | 0 | 56 |
| | 4 | 17 | 6 | 0 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 96 | 24 | 1 | 0 | 0 |
| GB-1356 | 2 | 10 | 4 | 0 | 0 | 0 | 0 | 100 | 3 | 0 | 0 | 79 | 3 | 0 | 0 | 79 |
| | 4 | 17 | 13 | 2 | 0 | 0 | 0 | 100 | 1 | 0 | 0 | 97 | 4 | 2 | 0 | 81 |
| Check | - | 4 | 5 | 1 | 8 | 11 | 0 | - | 18 | 11 | 1 | - | 9 | 3 | 1 | - |

a/ Mosquito population consisted mostly of *Cx. tarsalis*, but *An. franciscanus* and *Cu. inornata* also were present in small numbers.

trol was calculated by the formula: % Control = 100 - $(\frac{x}{y})$ 100, where x = number of larvae and pupae post-treatment, and y = number of larvae and pupae pre-treatment.

RESULTS AND DISCUSSION.–All three larvicidal oil formulations were equally effective for the control of larvae of stagnant mosquitoes *Cx. tarsalis*, *Cu. inornata*, and *An. franciscanus* at 2 and 4 gals/A. Effective control was achieved for one week with GB-1111 and GB-1313. GB-1356, which contains an aliphatic amine (0.5%), was superior to GB-1111 and GB-1313, and it yielded good control for over two weeks at both rates (Table 2). It is important to note that pupae disappeared completely from the treated ponds for more than 2 weeks even at the lower rate of 2 gal/A; therefore, further treatments, if necessary, should be spaced at 2-3 week intervals for adequate results.

On nontarget organisms, Golden Bear petroleum oils produced results similar to those obtained with Flit MLO, Toxisol FLC and Toxisol TB which was reported earlier by Mulla et al. (1969, 1971). At the rates of 2 and 4 gals/A, no effect was observed on the naiads of mayflies, dragonflies and damselflies. Even though the numbers of dragonflies and damselflies were low, they remained stable and prevailed at constant level during the duration of the experiment. Diving beetle larvae, however, were affected, but were not completely eliminated (Table 3).

None of the Golden Bear larvicidal oils had any apparent effect on the tadpole population at either rate of 2 and 4 gals/A. GB-1313 displayed high biological activity against ostracods at both rates tested, while GB-1111 caused drastic reduction in the population at the higher rate only. No drastic effects were observed at either rate with GB-1356 (Table 4).

From the data presented, Golden Bear petroleum larvicidal oils seem to have a good margin of safety at larvicidal rate (2 gals/A) against bottom dwelling macroinvertebrates such as mayflies, dragonflies and damselflies. Diving beetle larvae and adults as well as corixids and notonectids, which come to the surface for breathing, are markedly affected; and bulk of mortality occurred soon after treatment as observed during the course of these studies. In conclusion, these petroleum hydrocarbon larvicides produce effects similar to those reported earlier by these and other authors.

**Table 3.** Effects of Golden Bear petroleum oils against nontarget organisms in experimental ponds. (Oasis, California, April 1978).

| Pet. oil | Rate | Avg. no. of organisms/5 dips composit sample pre & Post-treat (days) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MFN | | | | DFN | | | | DBL | | | |
| | Gal/A. | Pre | 2 | 7 | 14 | Pre | 2 | 7 | 14 | Pre | 2 | 7 | 14 |
| GB-1111 | 2 | 1 | 2 | 6 | 6 | 0 | 0 | 0 | 1 | 4 | 0 | 3 | 3 |
| | 4 | 6 | 5 | 3 | 3 | 1 | 0 | 1 | 1 | 6 | 2 | 0 | 1 |
| GB-1313 | 2 | 9 | 19 | 15 | 19 | 1 | 2 | 2 | 1 | 7 | 3 | 5 | 7 |
| | 4 | 2 | 3 | 5 | 6 | 2 | 1 | 1 | 4 | 7 | 3 | 3 | 2 |
| GB-1356 | 2 | 13 | 43 | 18 | 14 | 2 | 1 | 0 | 1 | 15 | 6 | 6 | 3 |
| | 4 | 17 | 29 | 27 | 25 | 5 | 4 | 6 | 3 | 5 | 3 | 5 | 2 |
| Check | - | 13 | 25 | 8 | 14 | 1 | 1 | 0 | 0 | 6 | 10 | 4 | 2 |

MGN = Mayfly naiad (*Baetis* sp.), DFN = Dragonfly naiad (Aeshnidae and Libellulidae),

DBL = Diving beetle larvae (Dytiscidae and Hydrophilidae).

**Table 4.** Effects of Golden Bear petroleum oils against nontarget organisms in experimental ponds. (Oasis, California, April 1978).

| Pet. oil | Rate | Avg. No. of organisms/5 dips composit sample pre and post-treat (days) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ostracods | | | | Tadpoles | | | | Damselflies | | | |
| | Gal/A. | Pre | 2 | 7 | 14 | Pre | 2 | 7 | 14 | Pre | 2 | 7 | 14 |
| GB-1111 | 2 | 75 | 190 | 186 | 269 | 3 | 1 | 3 | 2 | 0 | 0 | 1 | 1 |
| | 4 | 22 | 8 | 0 | 3 | 4 | 4 | 13 | 9 | 0 | 0 | 0 | 0 |
| GB-1313 | 2 | 20 | 56 | 8 | 151 | 0 | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| | 4 | 79 | 252 | 12 | 30 | 0 | 1 | 4 | 2 | 4 | 3 | 4 | 0 |
| GB-1356 | 2 | 92 | 156 | 392 | 268 | 3 | 0 | 4 | 2 | 1 | 1 | 2 | 2 |
| | 4 | 43 | 96 | 230 | 263 | 1 | 1 | 2 | 2 | 1 | 2 | 6 | 2 |
| Check | - | 148 | 176 | 242 | 149 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |

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# *N,N*-DIALKYLALKANAMIDES AND ALKYL *N*-METHYLCARBAMATES AS MOSQUITO LARVICIDES

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During our investigations on larvicidal activity of unsubstituted and nitrogen-substituted aliphatic amides, we found that unsusbstituted amides and *N*-methylamides displayed no larvicidal activity whereas *N, N*-dimethylamides showed a high degree of activity (Hwang and Mulla 1980). *N, N*-Dimethylalkanamides from $C_{13}$ through $C_{18}$ demonstrated an excellent larvicidal activity against the first-instar larvae of *Culex quinquefasciatus* Say with both $LC_{50}$ and $LC_{90}$ below 1 ppm. The regression lines of the *N,N*-dimethylalkanamides had steep slopes on log-probit paper indicating that their $LC_{50}$ and $LC_{90}$ values were close together.

| | |
|---|---|
| $RCONH_2$ | not active |
| $RCONHCH_3$ | not active |
| $RCON(CH_3)_2$ | active |

R= $-C_{12}H_{25}$, $-C_{13}H_{27}$, $-C_{14}H_{29}$, $-C_{15}H_{31}$, $-C_{16}H_{33}$, or $-C_{17}H_{35}$

To study the effect of alkyl groups attached to the amide nitrogen on the larvicidal activity, we synthesized *N,N*-diethyl, *N,N*-dipropyl-, *N,N*-diisopropyl-, and *N,N*-diisobutylalkanamides from $C_8$ through $C_{22}$ and evaluated their activity against the first-instar larvae of *Cx. quinquefasciatus*.

The *N,N*-dialkylakanamides were prepared as follows: An alkanoic acid was allowed to react with an excess of boiling thionyl chloride. The resulting alkanoyl chloride was added into a solution of diethylamine, dipropylamine, diisopropylamine, or diisobutylamine in benzene. The crude product was distilled in vacuo or recrystallized from acetone or ethanol to give a pure *N,N*-dialkylalkanamide.

The *N,N*-dialkylalkanamides were bioassayed for toxicity against the first-instar larvae of *Cx. quinquefasciatus* according to the procedure reported elsewhere (Hwang et al. 1974). The bioassay data, obtained as percent mortalities at various concentrations, were analyzed for the log-probit regression analysis with a Compucorp model 145E computer. The larvicidal activity of the test compounds was expressed as lethal concentrations in parts per million affecting 50 and 90% of the population ($LC_{50}$ and $LC_{90}$). In this report, the level of toxicity of the amides is described as high ($LC_{50}$<1 ppm), moderate (1 ppm<$LC_{50}$<5 ppm), low (5 ppm<$LC_{50}$<10 ppm), or none ($LC_{50}$>10 ppm).

Of the *N,N*-diethylalkanamides [$RCON(C_2H_5)_2$] synthesized and evaluated, the lowest $C_8$ amide showed no larvicidal activity, but the activity increased as the carbon chain increassed. $C_9$ amide displayed a low degree of activity, and $C_{10}$ through $C_{13}$ amides showed moderate activity which gradually increased from the lower to the higher amides. $C_{14}$ amide was the most active with an $LC_{50}$ of 0.5 and an $LC_{90}$ of 0.7 ppm. When the carbon chain increased further, the activity gradually diminished. Thus amides from $C_{15}$ through $C_{19}$ showed low degrees of activity, and $C_{20}$ and higher amides showed no activity.

In the series of *N,N*-dipropylalkanamides [$RCON(C_3H_7)_2$], the larvicidal activity fluctuated at a moderate level from $C_8$ through $C_{14}$ amides with $C_{11}$ and $C_{14}$ amides showing an increased activity over others. Thereafter the activity diminished gradually from $C_{15}$ through $C_{18}$ and disappeared completely in $C_{19}$ and higher amides.

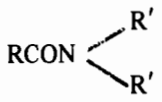
The larvicidal activity of the *N,N*-diisopropylalkanamides, $RCON[CH(CH_3)_2]_2$, followed a similar trend as those of the diethyl and dipropyl series of amides. With the increase in carbon chain, this series of alkanamides displayed an increasing activity which reached the highest level in $C_{11}$ amide with an $LC_{50}$ of 0.5 and an $LC_{90}$ of 1.0 ppm. The activity trend gradually decreased from $C_{12}$ through $C_{18}$ amides, and the activity completely disappeared in $C_{19}$ and higher amides.

$C_9$ and $C_{12}$ amides showed the highest degree of larvicidal activity in the series of *N,N*-diisobutylalkanamides, $RCON[CH_2CH(CH_3)_2]$. The activity increased from $C_8$ amide and reached the highest level from $C_9$ through $C_{12}$. Thereafter, the activity decreased from $C_{13}$ through $C_{16}$ and diminished completely in $C_{17}$ and higher amides.

It has been known that, in a homologous series of organic compounds, each member in the series is usually found to be more biologically active than its lower homologue until, suddenly, the addition of just one more $CH_2$ group severely diminishes, or even abolishes, the biological activity (Albert 1979). We also have found this tendency to be true with the larvicidal activity of branched-chain fatty acids and esters (Hwang et al. 1974, 1976, 1978), 2-haloalkanoic acids and esters (Hwang and Mulla 1976), and *N,N*-dimethylalkanamides (Hwang and Mulla 1980). The present studies again confirm the previous findings made in this and other laboratories.

To study the effect of the alkyl groups attached to the amide nitrogen on the larvicidal activity, we compared the larvicidal activities of the *N,N*-dialkylalkanamides with the RCO

group set at a constant length and with the nitrogen atom attached to various alkyl groups in the order of methyl, ethyl, propyl, isopropyl, and isobutyl.



R′=$CH_3$, $C_2H_5$, $C_3H_7$, $CH(CH_3)_2$, and $CH_2CH(CH_3)_2$

In the case of $C_9$ amides (R=$C_8H_{17}$), the activity declined as the R′ group varied from methyl through isobutyl groups. The activity of $C_{14}$ amides (R=$C_{13}H_{27}$), on the contrary, increased as the size of the R′ group increased. In $C_{17}$ amides (R=$C_{16}H_{33}$), the activity fluctuated irregularly. In conclusion, the size of the alkyl groups attached to the nitrogen atom did not bear any definite and clear-cut influence on the activity of a series of amides with a constant length of carbon chain.

Among the various *N,N*-dialkylalkanamides studied, *N,N*-dimethylalkanamides from $C_{13}$ through $C_{18}$ were the most active, demonstrating a high degree of toxicity against the first-instar larvae of *Cx quinquefasciatus*.

$RCON(CH_3)_2$
*N,N*-dimethylalkanamides

$ROCON(CH_3)_2$
Alkyl *N,N*-dimethylcarbamates

$ROCONHCH_3$
Alkyl *N*-methylcarbamates

In comparing the chemical structures given above, we noticed that alkyl *N,N*-dimethylcarbamates and alkyl *N*-methylcarbamates were structurally analogous to the *N,N*-dimethylalkanamides. To ascertain whether this structural similarity was associated with biological activity, we synthesized various aliphatic carbamates and evaluated their larvicidal activity against the first-instar larvae of *Cx. quinquefasciatus*.

The synthesis of the carbamates was conducted in the following manners. The alkyl *N,N*-dimethylcarbamates were prepared by treating a sodium alkoxide with dimethylcarbamyl chloride in refluxing benzene. The alkyl *N*-methylcarbamates were prepared by reacting an alkanol with methyl isocyanate in ether in the presence of triethylamine.

Of numerous aliphatic carbamates synthesized and evaluated, only a few showed some activity (Table 1). 1-Decyl *N,N*-dimethylcarbamate (1) was the only dimethylcarbamate which showed considerable larvicidal activity; however, the level of activity was by no means impressive. Although 1-decyl *N*-methylcarbamate (2) displayed moderate degree of activity, the other alkyl *N*-methylcarbamates, such as 1-undecy *N*-methylcarbamate (3), 1-dodecyl *N*-methylcarbamate (4), and 1-octadecyl *N*-methylcarbamate (5), were merely slightly active. We can therefore conslusively state that despite the structural similarity, the aliphatic carbamates studied here did not demonstrate a high degree of toxicity as shown by the *N,N*-dimethylalkanamides.

**Table 1.** Larvicidal activity of various aliphatic carbamates against first-instar larvae of *Cx. quinquefasciatus*.

| No. | Carbamates | ppm $LC_{50}$ | ppm $LC_{90}$ |
|---|---|---|---|
| 1 | $C_{10}H_{21}OCON(CH_3)_2$ | 2.6 | 10 |
| 2 | $C_{10}H_{21}OCONHCH_3$ | 1.2 | 2.3 |
| 3 | $C_{11}H_{23}OCONHCH_3$ | 2.7 | 40.7 |
| 4 | $C_{12}H_{25}OCONHCH_3$ | 3.1 | 9.0 |
| 5 | $C_{18}H_{37}OCONHCH_3$ | 3.0 | 6.6 |

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# ENCAPSULATED FORMULATIONS OF PRIMIPHOS-METHYL AGAINST MOSQUITO LARVAE AND SOME NONTARGET ORGANISMS

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ABSTRACT

The thinwall encapsultated formulations of primiphos-methyl rendered more superior results in the laboratory than the thickwall formulations. The $LC_{90}$ level against *Cx. quinquefasciatus* Say of the thinwall formulation was in the range of 0.004 - 0.017 ppm, while the thickwall formulations were 8 - 10 folds less effective, and their $LC_{90}$ was in the range of 0.058 - 0.08 ppm.

All formulations tested at 0.1 lb/A were equally effective, and caused 88 - 95% reduction in the larval population of *Cx. tarsalis* Coquillett in experimental ponds. At the same rate, the mayfly naiads *Callibaetis pacificus* (Seeman) and the Ostracoda population were completely eliminated, but began to recover one week after treatment. At all rates applied, dragonfly naiads *Erythemis simplicicollis* Say, diving beetles *Tropisternus lateralis* (Hydrophilidae), *Rhantus gutticollis* Say (Dytiscidae), and the damselfly naiad *Ischnura cervula* Selys were not affected.

All formulations showed no residual activity in water, and all organisms observed prior to treatment, which were eliminated due to chemical application, were recovered within one week after treatment.

During the course of studies on the development of new substitute materials for mosquito control, a large number of larvicidal chemicals were evaluated against various species of mosquitoes in the laboratory and under field conditions (Mulla et al. 1966, 1970, Gas and Rioux 1969). Some of these materials showed excellent biological activity against both stagnant and flood water mosquitoes. Intensive and frequent use of some of these mosquito control agents resulted in the emergence of resistant strains of the malaria mosquito *An. culicifacies*, and resurgence of malaria incidence in India, with an incidence of 5 million cases in 1975 as compared to 100,000 cases in 1965 (Samnotra and Kumar 1980).

In earlier studies reported by Mulla et al. 1973, primiphos-methyl, an organophosphorus compound whith a low mammalian toxicity comparable to that of temephos, was reported to be active against larvae of *Cx. tarsalis* Coquillett and *Ae. nigromaculus* Ludlow in the central San Joaquin Valley of California. Excellent control of these species was obtained at the rate of 0.05 and 0.1 lb/A (55 and 100 g/ha). In recent studies, primiphos-methyl was reported to be highly active as an adulticide as well as a larvicide against the multi-resistant strains of the malaria mosquitoes in India and Indonesia. At the adulticiding rate of 1 $g/m^2$, excellent control of *An. aconitus* was obtained for 8 weeks when applied on bamboo surface, while 18 weeks control was possible on wood surface (Pradhan et al. 1979). Weekly larvicidal applications at the rate of 12.5 g/ha also produced excellent control, and resulted in a reduction of transmission of malaria, lowering the incidence rate in children, and decreasing the *(Plasmodium falciparum* infection in the urban community of Bhiwani, India (Samnotra and Kumar 1980).

Therefore, in order to enhance and prolong the larvicidal activity of primiphos-methyl, several encapsulated formulations were prepared, each with various physiochemical properties. The activity of these formulations was studied against mosquito larvae in the laboratory and field, along with their impact on some nontarget organisms present in field test plots.

METHODS AND MATERIALS.--Thirteen formulations of primiphos-methyl (OMS-1424), *O*-[2-(diethylamino)-6-methyl-4-pyrimidinyl] *0,0*-dimethyl phosphorothioate, were prepared, 2 of which were solidified and disregarded. The remaining 11 formulations listed in Table 1 were evaluated in the laboratory and under field conditions as follows:

Laboratory. - - One percent (1%) stock suspension of each formulation (w/v) was prepared in water, and serial dilutions were prepared in water as needed. The required amount (0.2 - 1.0 ml) of a given strength suspension was added to 4 oz dixie cups containing 20 4th stage larvae of *Culex quinquefasciatus* (Say) in 100 ml of tap water. Larvae used in these studies were obtained from a laboratory colony at the University of California, Riverside. Each material was tested 3 - 4 times, utilizing 2 replicates per concentration each time, and along with each test, 2 cups were left untreated as checks. Prior to each test, new stock suspension and serial dilutions were made to

prevent any loss of activity which might occur in storage as aqueous suspension. After 24 hr of exposure of test organisms in a controlled temperature holding room (75°F±1), mortality readings were taken, and $LC_{50}$ and $LC_{90}$ values in ppm were obtained from a dosage response line on probit log paper, based on mean mortality values vs concentrations.

Field Evaluation. - - Field trials were conducted in ponds at the Aquatic and Vector Control Research Facility in the Coachella Valley of southern California (Mulla and Darwazeh 1976). These facilities consist of 64 ponds, measuring 18 x 18 ft each. Water to each pond is supplied from an artesian well through underground pipeline. Water depth in the ponds is kept constant at 12 in. by float valves. Mosquito populations during studies (May - June) consisted mostly of all the aquatic stages of *Culex tarsalis* Coquillett.

The required amount of material was mixed with 120 ml of water and applied with an all purpose household (1 qt) sprayer, utilizing 2 ponds per application rate and 2 as checks. Procedure employed in the evaluation against mosquito larvae and nontarget organisms were those described by Mulla et al. 1975. Five dips per pond were taken prior to treatment and 2 and 7 days after treatment. The five dips then were composited into one sample, preserved in 95% alcohol, and organisms present in the sample were identified and counted under a dissecting microscope in the laboratory. Percent larval reduction was based on the number of 3rd and 4th stage larvae in posttreatment vs pretreatment.

RESULTS AND DISCUSSION.- In laboratory tests, thinwall encapsulated formulations of primiphos-methyl showed higher initial activity than thickwall formulations. Size of particles in the range tested, however, did not have any appreciable effect on the activity of the formulations against 4th stage larvae of *Cx. quinquefasciatus*. The larger size particles (10 $\mu$ dia) of the thickwall formulations tended to coalesce and settle out of suspension rapidly, while the finer particles (4 $\mu$ dia or less) remained in suspension for considerable periods. The $LC_{90}$ level of the thinwall formulations was in the range of 0.004 - 0.017 ppm, while the thickwall formulations were 8 - 10 times less effective, and their $LC_{90}$ was in the range of 0.058 - 0.08 ppm (Table 1). Similarly, cross-linkage decreased initial activity, probably due to slow release characteristics of the formulations.

The three most effective formulations listed in Table 1 were then tested in the field at the rates of 0.025 and 0.05 lb/A (28 and 55 g/ha). At the lower rate, JF-2964B was the most effective, causing 95% reduction in the late stages of the larval populations 2 days after treatment. At the same rate, JF-6912A and JF-6915 were less effective, and produced 84 and 57% reduction respectively. At the higher rate (0.05 lb/A, all 3 formulations displayed good activity, causing 80 - 93% reduction in the late larval stages 2 days after treatment (Table 2).

**Table 1.** Initial activity of various formulations of primiphos-methyl against 4th stage larvae of *Culex pipiens quinquefasciatus* in the laboratory.

| Code No. | Formulation | (ppm) $LC_{50}$ - $LC_{90}$ |
|---|---|---|
| JF-2964/B | "Actellic" 20% Microencap, thinwall, 2 µ dia. | 0.0015 - 0.0040 |
| JF-6999 | As above with waxline blue | 0.0050 - 0.0088 |
| JF-6912/A | 20% encap, thinwalled, 1µ dia. | 0.0060 - 0.0090 |
| JF-6915 | 20% encap, floating capsule, 4µ dia. | 0.0070 - 0.0120 |
| JF-5742 | "Actellic" 25% WP | 0.0080 - 0.0160 |
| JF-6918 | 10% encap, thinwalled, 10µ dia. | 0.0090 - 0.0170 |
| YE-6704 | "Primotec", 20% microencap. | 0.0250 - 0.056 |
| JF-6914 | 20% encap, thickwalled, crosslinked, 4µ dia. | 0.0280 - 0.058 |
| JF-6914/A | 20% encap, thickwalled, crosslinked, 1µ dia. | 0.0330 - 0.080 |
| JF-6913/A | 20% encap, thickwalled, 1µ dia. | 0.0350 - 0.0750 |
| JF-6913 | 20% encap, thickwalled, 4µ dia. | 0.0480 - 0.080 |
| JF-6919 | 10% encap, thickwalled, crosslinked, 10µ dia. | solidified |
| JF-6920 | 10% encap, thickwalled, 10µ dia. | solidified |

**Table 2.** Short-term activity of various microencapsulated formulations of primiphos-methyl against larvae of *Culex tarsalis* in experimental ponds. (Coachella Valley Aq. Res. and Vector Cont. Facility)

| Material and Formulation | Rate lb/A | Rate g/ha | Avg. no of larvae/5 dips pre and posttreat (days) Pretreat. Larvae | 2 Larvae | 2 (%R) | 7 Larvae | 7 (%R) |
|---|---|---|---|---|---|---|---|
| | | | Test A, June 1979[a/] | | | | |
| JF-2964B | 0.025 | 28 | 19 | 1 | 95 | 4 | 79 |
| (20%) | 0.050 | 55 | 14 | 1 | 93 | 18 | 0 |
| JF-6912A | 0.025 | 28 | 19 | 3 | 84 | 9 | 53 |
| (20%) | 0.050 | 55 | 6 | 1 | 83 | 15 | 0 |
| JF-6915 | 0.025 | 28 | 7 | 3 | 57 | 13 | 0 |
| (20%) | 0.050 | 55 | 5 | 1 | 80 | 7 | 0 |
| Check | - | - | 3 | 7 | 0 | 5 | 0 |
| | | | Test B, May 1980[b/] | | | | |
| JF-2964B | 0.050 | 55 | 10 | 2 | 80 | 6 | 40 |
| (20%) | 0.100 | 110 | 14 | 0 | 100 | 21 | 0 |
| JF-6912A | 0.050 | 55 | 13 | 1 | 92 | 5 | 62 |
| (20%) | 0.100 | 110 | 14 | 1 | 93 | 3 | 79 |
| JF-6915 | 0.050 | 55 | 14 | 7 | 50 | 8 | 43 |
| (20%) | 0.100 | 110 | 44 | 8 | 82 | 8 | 82 |
| Check | - | - | 27 | 18 | 33 | 10 | 62 |

[a/] Water temp. mean min. 23°C and mean max. 33.8°C.

[b/] Water temp. mean min. 22.2°C and mean max. 28.8°C.
(%R) is based on no. of 3-4 stage larvae in posttreat. v. pretreat.

Due to a lack of complete control of the larval stages, the same 3 materials were retested at the higher rates of 0.05 and 0.1 lb/A (55 and 110 g/ha) along with remaining formulations. Again, JF-2964B was the most effective, eliminating the late larval stages at the rate of 0.1 lb/A, and causing 80% reduction at the lower rate of 0.05 lb/A. At both rates applied (0.05 and 0.1 lb/A, 92 and 93% reduction was obtained with JF-6912A, while 50 and 82% reduction occurred with JF-6915. However, somewhat higher control was obtained with the latter material seven days posttreatment (Table 2).

At the high rate of 0.1 lb/A, all formulations, with the exception of the 25 WP, were equally effective causing 88 - 95% control (Table 3). The 25 WP, was less effective, and caused only 75% reduction 2 days after treatment (Table 3). All formulations showed no residual activity in water, and the mosquito larval population was reestablished one week after treatment. Presence of younger larval stages (1st and 2nd) 2 days after chemical application indicates the lethal levels of the material persisted in water for a period of 24 - 48 hrs only.

Studies on the impact of microencapsulated formulations on non-target biota showed interesting trends. The mayfly naiads *Callibaetis pacificus* (Seeman) and Ostracoda populations were markedly affected at the low rate of 0.05 lb/A, and were almost completely eliminated at the higher rate of 0.1 lb/A. However, these two organisms began to recover one week after treatment. Dragonfly naiads, *Erythemis simplicicollis* Say, and the damselfly naiads, *Ischnura cervula* Selys, were not affected at all rates applied (Tables 4 & 5). Diving beetles, *Tropisternus lateralis* (Hydrophilidae), and *Rhantus gutticollis* Say (Dytiscidae) also were not affected (data on beetles not included).

In conclusion, no prolonged or increased activity against mosquito larvae was apparent with these microencapsulated formulations of primiphos-methyl. Emulsifiable concentrate formulations tested earlier (Mulla et al. 1973) displayed similar or better results, controlling the stagnant water mosquitoes *Cs. inornata* Williston, *Cx. tarsalis* Coquillett, and *Cx. peus* Speiser at the rate of 0.05 lb/A. At the rate of 0.1 lb/A, complete control of floodwater mosquito *Ae. nigromaculis* Ludlow also was reported.

From the data presented above, microencapsulated formulations of primiphos-methyl could be used effectively for mosquito larval control at the rate of 0.1 lb/A. However, weekly treatments will be necessary against asynchronous populations in semipermanent and permanent bodies of water.

**Table 3.** Short-term activity of various formulations of primiphos-methyl against larvae of *Culex tarsalis* in experimental ponds. (Coachella Valley Aq. Res. and Vector Cont. facility, June 1980[a/].)

| Material and Formulation | Rate lb/A | Rate g/ha | Avg. no. of larvae/5 dips pre-and posttreat (days) Pretreat. Larvae | 2 Larvae | 2 (%R) | 7 Larvae | 7 (%R) |
|---|---|---|---|---|---|---|---|
| JF-6913 | 0.05 | 55 | 21 | 8 | 62 | 16 | 24 |
| (20%) | 0.10 | 110 | 19 | 1 | 95 | 15 | 21 |
| JF-6914A | 0.05 | 55 | 7 | 4 | 43 | 17 | 0 |
| (20%) | 0.10 | 110 | 16 | 1 | 94 | 10 | 38 |
| JF-6913A | 0.05 | 55 | 22 | 8 | 64 | 11 | 50 |
| (20%) | 0.10 | 110 | 14 | 1 | 93 | 3 | 79 |
| JF-6999 | 0.05 | 55 | 28 | 5 | 82 | 5 | 82 |
| (20%) | 0.10 | 110 | 24 | 2 | 92 | 20 | 17 |
| YE-6704 | 0.05 | 55 | 23 | 10 | 57 | 24 | 0 |
| (20%) | 0.10 | 110 | 13 | 1 | 92 | 12 | 8 |
| JF-6914 | 0.05 | 55 | 8 | 1 | 88 | 6 | 25 |
| (20%) | 0.10 | 110 | 17 | 2 | 88 | 9 | 47 |
| JF-5742 | 0.05 | 55 | 24 | 15 | 38 | 26 | 0 |
| (25 WP) | 0.10 | 110 | 32 | 8 | 75 | 48 | 0 |
| Check | - | - | 27 | 18 | 33 | 10 | 63 |

[a/] Water temp. mean min. 23°C and mean max. 33.8°C, and (%R) is based on the no. of 3-4 stage larvae in posttreat vs. pretreat.

**Table 4.** Impact of various formulations of primiphos-methyl against nontarget organisms in experimental ponds. (Coachella Valley Aq. Res. and Vector Cont. Facility.)

| Material and Formulation | Rate lb/A | Rate g/ha | Avg. no. of organisms/5 dips sample pre and posttreat. (days) Mayflies Pre | Mayflies 2 | Mayflies 7 | Dragonflies Pre | Dragonflies 2 | Dragonflies 7 | Damselflies Pre | Damselflies 2 | Damselflies 7 | Ostracoda Pre | Ostracoda 2 | Ostracoda 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Test A, June 1979 | | | | | | | | |
| JF-2964B | 0.025 | 28 | 10 | 2 | 2 | 5 | 7 | 8 | 2 | 3 | 4 | + | 0 | + |
| (20%) | 0.050 | 55 | 10 | 0 | 3 | 9 | 7 | 4 | 2 | 3 | 3 | + | 0 | + |
| JF-6912A | 0.025 | 28 | 7 | 3 | 6 | 5 | 4 | 4 | 1 | 0 | 2 | + | 0 | + |
| (20%) | 0.050 | 55 | 6 | 2 | 3 | 1 | 3 | 4 | 2 | 1 | 2 | + | 0 | + |
| JF-6915 | 0.025 | 28 | 11 | 1 | 9 | 3 | 5 | 2 | 2 | 2 | 3 | + | 0 | + |
| (20%) | 0.050 | 55 | 27 | 1 | 2 | 7 | 2 | 2 | 4 | 3 | 2 | + | 0 | + |
| Check | - | - | 4 | 9 | 11 | 6 | 8 | 6 | 2 | 3 | 5 | + | + | + |
| | | | | | | Test B, May 1980 | | | | | | | | |
| JF-2964B | 0.05 | 55 | 2 | 1 | 1 | 0 | 1 | 0 | 2 | 4 | 0 | 45 | 4 | 0 |
| (20%) | 0.10 | 110 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 68 | 0 | 0 |
| JF-6912A | 0.05 | 55 | 5 | 0 | 3 | 0 | 0 | 1 | 1 | 2 | 1 | 70 | 0 | 2 |
| (20%) | 0.10 | 110 | 4 | 0 | 0 | 1 | 2 | 1 | 3 | 3 | 0 | 300 | 5 | 6 |
| JF-6915 | 0.05 | 55 | 6 | 2 | 2 | 1 | 0 | 0 | 0 | 4 | 3 | 0 | 0 | 0 |
| (20%) | 0.10 | 110 | 2 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 3 | 0 | 0 |
| Check | - | - | 2 | 3 | 2 | 2 | 2 | 1 | 8 | 10 | 10 | 38 | 93 | 91 |

Mayfly naiads = *C. pacificus*, Dragonfly naiads = *E. simplicicollis*, Damselfly naiads = *I. cervula.*

**Table 5.** Impact of various microencapsulated formulations of primiphos-methyl against nontarget organisms in experimental ponds. (Coachella Valley Aquatic and Vector Control Research Facility, May 1980.)

| Material and Formulation | Rate lb/A | Rate g/ha | Avg. no. of organisms/5 dips sample pre and posttreat. (days) Mayflies Pre | Mayflies 2 | Mayflies 7 | Dragonflies Pre | Dragonflies 2 | Dragonflies 7 | Damselflies Pre | Damselflies 2 | Damselflies 7 | Ostracoda Pre | Ostracoda 2 | Ostracoda 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JF-6913 | 0.05 | 55 | 20 | 0 | 0 | 1 | 1 | 0 | 2 | 2 | 4 | 0 | 0 | 1 |
| (20%) | 0.10 | 110 | 10 | 0 | 0 | 3 | 1 | 0 | 1 | 2 | 2 | 0 | 0 | 1 |
| JF-6914A | 0.05 | 55 | 4 | 0 | 1 | 0 | 0 | 1 | 1 | 3 | 1 | 30 | 5 | 8 |
| (20%) | 0.10 | 110 | 2 | 0 | 0 | 1 | 1 | 2 | 3 | 6 | 4 | 212 | 0 | 0 |
| JF-6913A | 0.05 | 55 | 5 | 0 | 0 | 2 | 0 | 0 | 2 | 3 | 0 | 190 | 0 | 0 |
| (20%) | 0.10 | 110 | 7 | 0 | 0 | 1 | 0 | 0 | 0 | 5 | 2 | 0 | 0 | 8 |
| JF-6999 | 0.05 | 55 | 3 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 4 | 9 | 0 | 0 |
| (20%) | 0.10 | 110 | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| YE-6704 | 0.05 | 55 | 2 | 0 | 0 | 2 | 1 | 0 | 0 | 9 | 2 | 0 | 0 | 0 |
| (20%) | 0.10 | 110 | 8 | 0 | 0 | 4 | 0 | 0 | 2 | 0 | 2 | 1 | 0 | 3 |
| JF-6914 | 0.05 | 55 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 15 | 0 | 0 |
| (20%) | 0.10 | 110 | 2 | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 20 | 0 | 0 |
| JF-5742 | 0.05 | 55 | 7 | 8 | 5 | 1 | 0 | 1 | 0 | 7 | 2 | 2 | 0 | 94 |
| (25 WP) | 0.10 | 110 | 8 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 3 | 0 | 0 | 0 |
| Check | - | - | 2 | 3 | 2 | 2 | 2 | 1 | 8 | 10 | 10 | 38 | 93 | 91 |

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# FIELD EVALUATION OF THE EFFECTS OF SLOW-RELEASE WETTABLE POWDER FORMULATION OF ALTOSID® ON NONTARGET ORGANISMS[1]

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ABSTRACT

Three identical shallow ponds were treated with one, two and four times recommended rates of 10% Altosid® wettable powder. Two sampling techniques were used to monitor pre- and post-treatment populations of three families of Coleoptera, two of Hemiptera and one of Diptera, and snails to compare population changes with a control pond. Adult emergence was used to gauge the length of effectiveness. Midge emergence was virtually eliminated for approximately three weeks. There were no significant deleterious effects on non-target animals.

INTRODUCTION.–With increased interest being focused on the use of slow-release formulations of the growth regulator, Methoprene (marketed as Altosid® by Zoecon Corp., Palo Alto, CA) for mosquito and midge control, it became imperative to assess the effects of truly chronic exposure of nontarget animals, particularly predatory insects, to the compound as it would be used in a rain pond or a shallow settling basin. The newest of these formulations, a 10% wettable powder was selected for this study.

METHOD AND MATERIALS.–A study site of four ponds (10m x 10m x 0.6m) was selected in the Rio Hondo Coastal Basin Spreading Ground in Montebello, CA. Sampling was carried out every two to four days for two weeks prior to the treatments and two weeks following. Two sampling devices were used, a 50cm plexiglass cylinder described by Legner et al. (1976) and a standard mosquito dip net (40cm diameter). Both devices were used to sample each pond four times in two ramdomly selected quadrants on each sampling date.

In addition to snails and Chironomid midges, the following families of Coleoptera (larvae and adults) and Hemiptera were sampled: Dytiscidae, Hydrophilidae, Dryopidae, Notonectidae, and Corixidae. The data presented represent the combined totals of specimens recovered by both sampling methods.

All animals were returned to the ponds after identification and counting over nylon screens.

A Chapin No. 121 compressed air sprayer was used to apply the wettable powder at dosage rates equivalent to 210, 420, and 840gm/hectare, leaving one pond untreated. Treatment was made on 20 July, 1980. To estimate the effective length of midge control, aquatic cone traps for adults as described by Mulla et al. (1974) were submerged from 25 to 40 cm in each pond. Adult midges were counted on the same days that the non-target forms were sampled and the traps were moved to new locations in each pond. All data was analyzed using two-way analysis of variance (ANOV).

RESULTS AND DISCUSSION.–The effects of Methoprene on midge emergence is shown in Figure 1 and Table 1. There were no significant differences in adult midge emergence from the four ponds prior to treatment but there were sharp significant drops by the time of the first sampling two days after application. Significantly ($P < 0.1$) fewer midges were recovered from all treated ponds for approximately three weeks following treatment. There were no differences noted between the treated ponds: midge control being almost absolute in all of them, while the check pond population remained relatively stable. In contrast, there was only one instance in which the nontarget insects or snails varied significantly after the wettable powder was applied. The number of notonectids rose significantly during the posttreatment period. All other changes that occurred in the treated ponds were paralleled by changes in the check pond. The data obtained from the two sampling methods were analyzed separately and combined with no changes in results. The F-values for significance at the 5% level were 9.28 for pond interaction and 10.13 for Altosid® effects. The largest values recorded in this trial were 1.49 for pond interaction 10.45 for Altosid® effects on notonectids. All others were lower than 8.45 for Altosid® effects.

Noteworthy is that there were approximately twice as many specimens recovered using the dip net method, but almost half the variation in counts between ponds using the Leg-

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Table 1. Collections using Legner and dip net samplers prior to and after methoprene application

| Order and Family | Pond | Collection Date | Pre-Treatment | | | | | | | Post-Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7/5 | 7/8 | 7/11 | 7/14 | 7/17 | 7/20 | Total | $\bar{x}$ | 7/22 | 7/23 | 7/27 | 7/29 | 8/4 | 8/7 | Total | $\bar{x}$ |
| Coleoptera | | | | | | | | | | | | | | | | | | |
| Dytiscidae (adult) | | | | | | | | | | | | | | | | | | |
| | Control | 1 | 6 | 19 | 29 | 35 | 70 | 49 | 208 | 35 | 28 | 29 | 34 | 28 | 30 | 21 | 170 | 28 |
| | | 2 | 7 | 31 | 12 | 12 | 26 | 28 | 116 | 19 | 28 | 34 | 14 | 16 | 20 | 24 | 136 | 23 |
| | | 3 | 2 | 25 | 3 | 7 | 10 | 17 | 64 | 11 | 23 | 10 | 24 | 25 | 19 | 24 | 125 | 21 |
| | | 4 | 7 | 21 | 3 | 2 | 21 | 24 | 78 | 13 | 31 | 6 | 11 | 15 | 20 | 12 | 95 | 16 |
| (larvae) | | | | | | | | | | | | | | | | | | |
| | Control | 1 | 1 | 2 | 5 | 13 | 27 | 58 | 106 | 18 | 30 | 15 | 25 | 25 | 31 | 12 | 138 | 23 |
| | | 2 | 5 | 5 | 5 | 3 | 10 | 7 | 35 | 6 | 17 | 13 | 11 | 17 | 22 | 6 | 86 | 14 |
| | | 3 | 4 | 7 | 1 | 4 | 8 | 13 | 37 | 6 | 6 | 5 | 12 | 18 | 5 | 11 | 57 | 10 |
| | | 4 | 1 | 17 | 1 | 1 | 9 | 5 | 34 | 6 | 2 | 3 | 14 | 22 | 4 | 4 | 49 | 8 |
| Hydrophilidae (adult) | | | | | | | | | | | | | | | | | | |
| | Control | 1 | 1 | 0 | 1 | 1 | 6 | 2 | 11 | 2 | 3 | 5 | 6 | 3 | 0 | 0 | 17 | 3 |
| | | 2 | 3 | 2 | 2 | 1 | 1 | 0 | 9 | 2 | 1 | 11 | 5 | 3 | 4 | 0 | 24 | 4 |
| | | 3 | 0 | 1 | 0 | 2 | 5 | 1 | 1 | 8 | 1 | 0 | 4 | 0 | 0 | 1 | 5 | 1 |
| | | 4 | 1 | 5 | 2 | 1 | 5 | 1 | 15 | 3 | 3 | 7 | 1 | 1 | 4 | 2 | 18 | 3 |
| (larvae) | | | | | | | | | | | | | | | | | | |
| | | 1 | 7 | 15 | 3 | 3 | 1 | 0 | 29 | 5 | 19 | 16 | 20 | 24 | 18 | 10 | 107 | 18 |
| | | 2 | 10 | 15 | 10 | 0 | 1 | 0 | 36 | 6 | 17 | 26 | 3 | 3 | 11 | 7 | 67 | 11 |
| | | 3 | 8 | 5 | 0 | 0 | 1 | 1 | 15 | 3 | 7 | 16 | 1 | 2 | 3 | 2 | 31 | 5 |
| | | 4 | 7 | 7 | 3 | 0 | 3 | 1 | 21 | 4 | 8 | 12 | 6 | 2 | 1 | 9 | 28 | 5 |
| Dryopidae | | | | | | | | | | | | | | | | | | |
| | Control | 1 | 1 | 6 | 15 | 35 | 173 | 30 | 260 | 43 | 13 | 65 | 11 | 11 | 0 | 3 | 103 | 17 |
| | | 2 | 10 | 10 | 58 | 81 | 157 | 22 | 338 | 56 | 34 | 84 | 17 | 29 | 3 | 6 | 173 | 29 |
| | | 3 | 11 | 13 | 95 | 87 | 20 | 25 | 251 | 42 | 28 | 64 | 60 | 37 | 14 | 6 | 209 | 35 |
| Hemiptera | | 4 | 19 | 45 | 114 | 272 | 31 | 103 | 584 | 97 | 32 | 96 | 64 | 88 | 41 | 25 | 346 | 58 |
| Notonectidae | | | | | | | | | | | | | | | | | | |
| | Control | 1 | 3 | 0 | 0 | 9 | 0 | 3 | 15 | 3 | 3 | 2 | 2 | 2 | 6 | 19 | 34 | 6 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 11 | 2 | 2 | 17 | 3 |
| | | 3 | 1 | 0 | 0 | 2 | 0 | 0 | 3 | 1 | 2 | 0 | 1 | 4 | 8 | 16 | 31 | 5 |
| | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 3 | 3 | 10 | 18 | 3 |
| Corixidae | | | | | | | | | | | | | | | | | | |
| | Control | 1 | 23 | 57 | 76 | 10 | 68 | 18 | 252 | 42 | 20 | 7 | 25 | 18 | 41 | 17 | 128 | 21 |
| | | 2 | 11 | 17 | 16 | 6 | 37 | 38 | 125 | 21 | 73 | 16 | 42 | 56 | 37 | 41 | 265 | 44 |
| | | 3 | 1 | 4 | 3 | 3 | 12 | 13 | 36 | 6 | 25 | 12 | 19 | 36 | 32 | 13 | 137 | 23 |
| | | 4 | 6 | 8 | 0 | 5 | 10 | 9 | 38 | 6 | 11 | 4 | 13 | 26 | 18 | 12 | 84 | 14 |
| Diptera | | | | | | | | | | | | | | | | | | |
| Chironomidae (Adults) | | | | | | | | | | | | | | | | | | |
| | Control | 1 | . | . | . | . | 12 | 5 | 17 | 9 | 4 | 8 | 8 | 12 | 24 | 7 | 63 | 10 |
| | | 2 | . | . | . | . | 6 | 19 | 25 | 13 | 2 | 0 | 1 | 0 | 2 | 1 | 6 | 1 |
| | | 3 | . | . | . | . | 6 | 5 | 11 | 6 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | .3 |
| | | 4 | . | . | . | . | 12 | 8 | 20 | 10 | 1 | 0 | 3 | 0 | 0 | 0 | 4 | .7 |
| (larvae) | | | | | | | | | | | | | | | | | | |
| | Control | 1 | 72 | 39 | 28 | 39 | 28 | 18 | 224 | 37 | 26 | 19 | 27 | 17 | 11 | 10 | 110 | 18 |
| | | 2 | 42 | 24 | 11 | 34 | 34 | 25 | 170 | 28 | 15 | 19 | 17 | 17 | 8 | 17 | 93 | 16 |
| | | 3 | 40 | 22 | 15 | 34 | 37 | 28 | 176 | 29 | 20 | 10 | 5 | 10 | 7 | 10 | 62 | 10 |
| | | 4 | 11 | 30 | 4 | 31 | 22 | 15 | 113 | 19 | 10 | 11 | 14 | 11 | 9 | 8 | 63 | 11 |
| Gastropoda | Control | 1 | 0 | 1 | 6 | 2 | 5 | 2 | 16 | 3 | 4 | 6 | 6 | 3 | 5 | 5 | 29 | 5 |
| | | 2 | 0 | 1 | 4 | 0 | 0 | 0 | 5 | 8 | 1 | 5 | 0 | 2 | 1 | 4 | 13 | 2 |
| | | 3 | 0 | 3 | 7 | 0 | 0 | 9 | 19 | 3 | 3 | 6 | 3 | 3 | 1 | 9 | 25 | 4 |
| | | 4 | 9 | 4 | 10 | 0 | 1 | 1 | 25 | 4 | 11 | 4 | 7 | 0 | 4 | 4 | 30 | 5 |

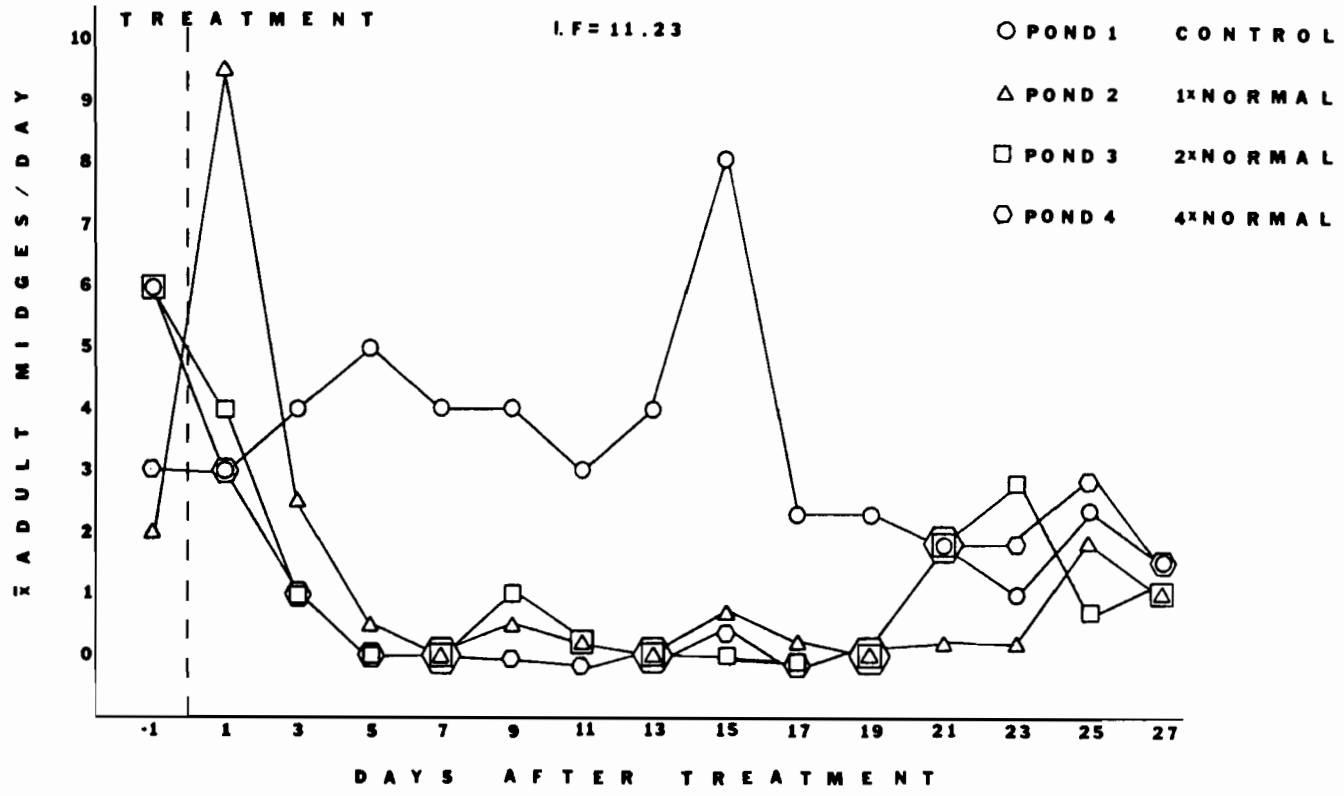


**Figure 1.** Effects of W. P. Altosid$^{T}$ on chironomidae

ner sampler. Indicating the far greater consistence obtainable with the latter method.

This study indicates that 10% Altosid® wettable powder used at the recommended rate gave excellent control of midges in these shallow ponds for approximately three weeks and that there were no statistically significant deleterious effects on any of the nontarget animals sampled when that rate was doubled or quadrupled.

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# FIELD EVALUATION OF MOSQUITO OVIPOSITIONAL REPELLENTS

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Volatile organic chemicals in breeding water are known to influence ovipositing behavior in mosquitoes. These volatile chemicals can be either attractive or repellent to ovipositing mosquitoes. Attractive compounds have been shown to be present in various organic infusions such as chicken manure infusions (Hayashi 1962) and grass infusions (Gjullin et al. 1965).

Compounds repellent to ovipositing mosquitoes were produced in a 1% lab chow infusion (Kramer and Mulla 1979). Through a series of experiments, this repellency was studied to chemically identify these compounds and to determine if they had a future in any mosquito control program. Hwang et al. (1980) isolated and identified the active chemicals from the lab chow infusion and identified the following lower aliphatic carboxylic acids showing ovipositional repellency: acetic, propionic, isobutyric, butyric, isovaleric, and caprioic acids. With laboratory olfactometers, each acid was tested for ovipositional repellency; they were repellent at $6x10^{-1}$%, and all but acetic acid were repellent at $6x10^{-2}$% against *Culex quinquefasciatus* Say (Kramer et al. 1980). This research was continued by testing additional aliphatic carboxylic acids from $C_5$ to $C_{13}$ for ovipositional activity (Hwang et al. 1981). Results showed that carboxylic acids in the $C_8$ to $C_{10}$ range were the most effective in reducing oviposition for *Cx quinquefasciatus*, *Cx. tarsalis* Coquillett, and *Aedes aegypti* L.

The next step in this study was the evaluation of repellents under semi-field and later field conditions. Semi-field tests consisted of testing portions of ponds with octanoic acid ($C_8$) as the repellent. Data from these semi-field tests gave us a better understanding of the problems involved in applying ovipositional repellents to water. The next step was testing first octanoic acid and later nonanoic acid ($C_9$) under field conditions for effectiveness in reducing oviposition.

Experiments were conducted at the Aquatic Research Facility, University of California, Riverside. Semi-field tests consisted of placing 4 sheet metal cylinders, about 45-cm diameter, in each of 4 ponds (3.6 x 7.2 m). The ponds and cylinders were filled with water. The top edges of the cylinders were a few centimeters higher than the water level in the ponds so that water in the cylinders would be isolated from the rest of the pond. Into the 4 cylinders were applied (1) chicken manure (0.2%) and 15 ppm octanoic acid, (2) chicken manure (0.2%) and 30 ppm octanoic acid, (3) only chicken manure (0.2%), and (4) nothing (as untreated checks). Chicken manure was added to increase the oviposition rate within the small surface area of the cylinders. The chicken manure was applied 1 day after flooding, and octanoic acid was applied 5 days after flooding. Egg rafts were collected post-flooding from the cylinders and counted every day for 20 days.

Results of this semi-field test showed that octanoic acid at both the 30 and 15 ppm concentration was effective in reducing oviposition. For example, after 3 days posttreatment, both the 30 and 15 ppm octanoic acid cylinders with manure had an average of 1 egg raft per cylinder. The check cylinder had an average of 3 egg rafts per cylinder, and those with manure only had an average of 8 egg rafts. Throughout the entire 15 days posttreatment, the number of egg rafts remained lower than either the check or manure only cylinders.

Several preliminary field tests with octanoic acid as repellent were conducted at low concentrations between 10 and 50 ppm. Although some reduction in egg-laying occurred, it did not give significant results. This is probably due to some of the repellent being absorbed by the pond's edges which could not occur in the metal cylinders. Each of the subsequent field tests were conducted with nonanoic acid as repellent.

Two field experiments were conducted. In each experiment, nonanoic acid was formulated with a solvent and a surfactant. The surface active agent was used for obtaining a relatively even distribution of the repellent on the water surface. In the first experiment Poly-Tergent G-300 was used; in the second experiment Triton X-100 was used. The solvent in both tests was xylene. The repellent was formulated in the ratio of 80-volume-parts nonanoic acid, 17-volume-parts solvent, and 3-volume-parts surfactant. The repellant formulation was applied to the ponds with a 1-gal hand sprayer. During the next several days, the ponds were examined for egg rafts which were counted but not collected. Three days after applying the repellent, larval counts were also made by taking 5 dips from predetermined locations abound the perimeter of the ponds. The samples were concentrated and returned to the laboratory for counting. Egg rafts and larval counts were taken several times each week for several weeks. The first experiment consisted of 12 ponds, 3 with 150-ppm nonanoic acid, 3 with 75-ppm nonanoic acid, 3 being untreated check ponds at the same rate as the 150-ppm ponds, and 3 being untreated check ponds. In the second experiment, 9 ponds were tested, 3 with 50-ppm nonanoic acid, 3 with 25-ppm nonanoic

acid, and 3 being solvent check ponds at the same rate as the 50-ppm ponds.

Results of these experiments showed that complete ovipositional repellency could be obtained for 1 day at 25 ppm, 2 days at 50 ppm, about 5 days at 75 ppm, and about 6 to 7 days at 150 ppm. At higher concentrations, nonanoic acid could be seen as a thin layer on the water surface. The surfactants were effective in obtaining an even surface distribution of the repellent.

It is possible that ovipositional repellents could someday become incorporated into a mosquito control program. In an area with multiple breeding sites, many could be treated with an ovipositional repellent, while leaving a few sites for mosquito oviposition. These few sites could then be treated periodically with conventional insecticides. Another method that could be employed is to mix the insecticide and repellent together. Spraying a breeding site with this mixture would kill larvae and pupae already present and delay any future egg-laying for some time, thus decreasing the total number of applications each year.

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# A NEW TRIAZINE INSECT GROWTH REGULATOR FOR FLY CONTROL ON POULTRY RANCHES


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Numerous studies on the efficacy of insect growth regulators (IGRs) for the control of filth breeding flies have been conducted. Ables et al. (1975) obtained significant reduction in adult house flies emerging from Dimilin treated manure. Wright (1974) obtained 90% control of house flies at a cattle feed lot and waste water treatment plant with Dimilin treatments. Miller et al. (1975) feeding Dimilin to chickens reported complete inhibition of emergence of *Musca domestica*. Breeden et al. (1975) found good inhibition of house fly emergence 3 days after initiation of feed through with methoprene.

When CGA-72662, a triazine type IGR, was sprayed onto chicken manure, Williams and Berry (1980) found at least a 70% reduction in emergence of native house flies at some of the application rates tested. When feeding CGA-72662 to chickens they found that 1.5 ppm and 5 ppm in feed gave up to 100% mortality of house flies. Hall and Foehse (1980), using CGA-72662 as topical manure sprays, effectively reduced emergence of muscoid flies in poultry, bovine and swine manure. When fed to poultry at 1.5 ppm in feed, they found it produced a high level of inhibition of house fly emergence.

In studies presented here, CGA-72662 (*N*-cyclopropyl-1-1, 3,5-triazine-2,4,6-triamine) an experimental IGR was evaluated under "typical" field-ranch conditions for the control of flies breeding in poultry manure on commercial caged poultry ranches. Application of the IGR was field oriented with the evaluation work taking place in the laboratory.

METHODS AND MATERIALS.—Four separate trials were conducted wherein the 1st two entailed application of the IGR as a topical surface spray to manure beneath cage rows. In the other two trials, the IGR was mixed with the feed and fed to the chickens.

Procedures for the evaluation of the effectiveness of the treatments was the same for all trials. At weekly intervals, biased manure samples were taken for muscoid larvae and returned to the laboratory. If no muscoid larvae were found, manure that could support these larvae was sampled. No attempt was made to seek out *Fannia* larvae. 0.6 liter aliquots were removed from the samples and placed into a Berlese funnel for 24 hr larval recovery. 1.9 liter aliquots were also placed in an emergence cage, where the emerging adults were collected for 14 days to determine the extent of inhibition of

mergence. The recovery numbers of the treatments were compared to the numbers recovered within checks to determine effectiveness. The various treatments were as follows:

Trial 1.- -Surface spray application of 3.8 liters of a 2% spray per 9.3 $m^2$ (0.8 $g/m^2$ A.I.) of manure surface to plots under the chicken cages. This treatment was repeated 14 days after initial spraying.

Trial 2.--Topical spray application of 1.9 liters of a 2% spray per 9.3 $m^2$ (0.4 $g/m^2$ A.I.) of manure surface to plots under the chicken cages. Only one application was made.

Trial 3.--Feed-through of 5 ppm of the IGR in chicken feed. Fed for 42 days.

Trial 4.--Feed-through of 1.5 ppm of IGR in chicken feed. Fed for 33 days.

The data were analyzed by t-test method and significance was determined between larvae of treatment and check, or, adults of treatment and check.

RESULTS AND DISCUSSION.—Trial 1.- -A significant reduction in muscoid larvae (a mixture of *Musca domestica* and *Muscina stabulans*) was evident 6 days after the first surface treatment and lasted through 13 days after the 2nd treatment. Recovery of some larvae was evident from 27-55 days after treatment 2, but large numbers did not appear until 55 days after 2nd treatment. Significant suppression of adult muscoid emergence appeared 6 days after treatment 1 and lasted through 55 days after 2nd treatment.

A significant reduction in numbers of *Fannia femoralis* larvae did not appear until 13 days after treatment 1 and lasted through 27 days after treatment 2. A significant suppression of emerging adult *F. femoralis* was evident 6 days after treatment 1 and lasted through 34 days after treatment 2. The *Fannia* populations during the course of these studies were quite low, not indicative of the normally high populations.

Trial 2.- -A significant reduction in larval recovery of *Musca domestica* was evident at the 6 day posttreatment period and continued through 27 days posttreatment. The sampling periods from 34 days up to 62 days (last sample taken) also indicated significantly fewer larvae in the treatment than the check plots. Even though significantly less than in the check plots, the numbers recovered from the treatment plots were high. Because of these high numbers, larval effectiveness could be said to have been lost between 27 and 34 days after spraying. Suppression of emerging adult *M. domestica* was almost 100% at 6 days after spraying and continued at that level through 41 days after spraying. At 48 days after spraying and beyond, large numbers of adults began to emerge.

Again, as in the 1st trial, the *Fannia* population was low, however, a significant reduction in *F. femoralis* larvae appeared 6 days after spraying and lasted through 34 days posttreatment. Emergence of adult *F. femoralis* was significantly depressed 6 days after treatment and lasted through 27 days after treatment.

Trial 3.- -In the 1st of two feed-through trials, a poultry house containing 38,000 chickens was divided down the middle, one-half being fed CGA-72662 treated feed (at 5 ppm) and the other half used as the check.

A significant reduction in *M. domestica* was evident 6 days after the feeding was initiated, and continued through the 42 days of feeding the treated feed. By 28 days into the feeding, the larvae were completely eliminated and this condition lasted the remainder of the feeding period (to 42 days). After 42 days of feeding the ration containing the IGR it was replaced with non-treated feed. One week after cessation of treated feed no larvae were evident. At two weeks after cessation some larvae became evident and by 21 days after replacement, large numbers of larvae were found in the manure.

Adult emergence of *M. domestica* was significantly suppressed 6 days after initiation of feeding of the treated feed and lasted through the treatment period (42 days) and up to 3 weeks after replacement of the treated feed with non-treated feed.

The population density of *F. femoralis* on this ranch at the time of this trial was very low. Little information as to the effect of the treatment could be ascertained from the low level of *Fannia* recovery.

Trial 4.- -In this trial, another feed-through experiment was established. Here, an entire ranch was fed 1.5 ppm CGA-72662 in feed. Another ranch located in the same area, fed ration without the IGR, was used as a check.

No significant reduction in *M. domestica* larvae appeared throughout the entire feeding period of 33 days. For 21 days after the treated feed was replaced with non-treated feed, no reduction in larval density was noted. However, emergence of adult *M. domestica* was significantly suppressed 12 days after initiation of the feeding, during the remaining feeding period, and lasting for 14 days after the treated feed was replaced.

This treatment of 1.5 ppm in chicken ration had no effect on *F. femoralis* larvae in the manure or altering adult emergence from the manure.

In summary, CGA-72662 was highly effective in suppressing the development of muscoid larvae in manure, and/or in preventing these flies from emerging as adults when applied as topical surface sprays. When added to chicken ration at 5 ppm, effectiveness lasted throughout the feeding period and for 2-3 weeks after replacement of the treatment feed with non-treated feed. At the low dosage feed-through (1.5 ppm) muscoid larvae were not suppressed but adult emergence was inhibited.

*Fannia femoralis* was also adversely affected by this IGR, but to a lesser extent than the muscoid flies.

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# THE BREEDING OF *CULEX QUINQUEFASCIATUS* WITHIN THE FRESNO URBAN STORM DRAIN SYSTEM

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INTRODUCTION.–Mosquito breeding in urban areas has long been associated with street catch basins. Almost 40 years ago Herms and Gray (1944) described an operational control method with oil applied to catch basins using 3-wheeled motorcycles. Various materials and methods have been tested or used to control two major catch basin breeders, *Culex pipiens* L. and *Cx. quinquefasciatus* Say. Treatments have included oils (Kimball and Perruzzi 1970 and Pfunter 1978), chlorinated hydrocarbons and organophosphorus insecticides (Dill and Roberts 1975), insect growth regulators (Stewart 1977), bacterial agents (Mulligan et al. 1981) and flushing out the system with sea water (Schoeppner 1977).

There are two basic types of catch basin designs. The dead well type allows water to stand below the level of the exit pipe, whereas no water is retained in the full-flushing, sloped-botton design. Preference, from an abatement viewpoint, of the latter over the former was readily apparent to Herms and Gray (1944).

In the city of Fresno, CA., these sloped-bottom catch basins have been incorporated into complex drainage systems, storm drains. Catch basins are connected via large (up to 1.5m diam), underground trunk lines, to flood control ponds, often 5km distance. The Fresno Mosquito Abatement District (FMAD) began receiving complaints of mosquitoes from houses near storm drain lines. The drain lines had been constructed 2 years previously. Since the catch basins were being routinely treated, inspections were made of the manhole chambers. Many sections of the trunk lines were found to hold standing water and contained larvae and adults of *Cx. quinquefasciatus*. A regular schedule of larviciding the water standing in manhole chambers was initiated by the FMAD; however the complaints continued.

This study originated with intent to determine the extent of mosquito breeding in storm drain lines and to investigate control methods within this habitat. The practicality of eliminating mosquito breeding in catch basins by converting dead well types to sloped bottoms was also studied.

MATERIALS AND METHODS.–Drain Line Study Site. - - A 1.6km section of storm drain line (4.8 km total length), including the origin, was chosen as a principal study site. Because this section included the origin, and water flow was unidirectional, the effects of chemical treatments could be controlled. The main trunk lines consisted of 1.1m diam pipes. Manhole chambers (manholes) varied in shape and depth and were located on the average of 2 blocks apart along the trunk line. Side lines, of 0.6m diam pipe and 1 block in length, joined the trunk at 2 locations. Twenty sloped-bottom catch basins were scattered throughout this section; some joined the trunk lines at the manholes, while others were between manholes.

Breeding Within Drain Line. - - The adult populations were monitored at 5 locations within the trunk line section. Miniature CDC-type light traps, Bio-Quip #2802 EVS, were placed in the manholes ca. 1m above the bottom, if dry, or above the surface of standing water. Each trap contained 1kg of dry ice and had fresh batteries at each use. The traps were operated overnight, about 17h for each collection time. Three traps were also placed outside of houses within a 1 block area of a manhole that was immediately connected to 2 catch basins. Traps were also placed within a separate drain line that was being routinely treated by the FMAD.

After collection, the adults were separated by sex and counted. Where numbers were very high the sample was weighed, a subsample counted and the total calculated. Sampling was done on a weekly basis and semi-weekly through July and August at the houses.

Treatments of Drain Line. - - Treatments of the entire section were made on 3 occasions with 3 different equipment modifications. A jeep-mounted mist blower was modified by connecting three 2.1m lengths of 15cm diam, flexible aluminum tubing and attaching them to the discharge. The tubing was extended into the trunk lines via the manholes. This modification was used on 8-15-80 to apply 0.45% (wt/vol) malathion at 4.08 atm (60 psi). The duration of application for each subsection of trunk line was determined by detection of odor at the distal end (maximum run 5 min).

A second application, of 4.8% (wt/vol) malathion, was made with another modification of the mist blower on 8-29-80. Air from the blower was passed through a 6.1m long, 15cm diam, fabric tube to the spray nozzle, which was detached from the blower. A separate 1.3cm ID hose connected the nozzle to the pesticide tank and the extended nozzle assembly was manipulated by use of an attached, telecoping aluminum pole. The average fogging run down a subsection was 2-3 min (maximum run 5 min).

The principles of the second mist blower modification were also used to modify a jeep-mounted LECO Model HD, ULV generator. The atomizing head was placed on the end of a 6.1m long, 7.6cm diam, flexible hose, which attached to the blower. A separate 0.6cm polyethylene line carried 91% Cythion concentrate to the head, which was manipulated with an aluminum pole. Application to the drain line section was

made on 9-12-80. The average fogging run down a sub-section was 2 min (maximum run 4 min).

Catch Basin Retrofit. - - Dead well type catch basins in the city of Kerman were retrofitted to eliminate standing water and mosquito breeding. The basins had held water throughout the previous summer (1979) with resultant mosquito breeding (Mulligan et al. 1981). Ready mix cement was added to level with the bottom of the exit pipe and then sloped 10cm to the basin walls. A grade was formed which sloped down to the exit pipe. Seven basins were converted with an equal number left as controls. The basins, 53cm diam and 80-120cm deep, were connected to trunk lines by 15cm diam exit pipes.

RESULTS AND DISCUSSION.—Breeding Within Drain Line. - - Underground storm drain trunk lines were found to provide excellent harborage for adult mosquitoes. During the summer and fall, temperatures in the manholes maintained a near constant 27°C (80°F) during both days and nights, in spite of ambient air temperatures which were often above 38°C (100°F) in the summer and below 15°C (60°F) in the fall. Relative humidity within the lines was generally around 70-80%.

Large numbers of adult mosquitoes were trapped throughout the study. Individual trap night counts above 3,000 adults were not uncommon; the largest single catch yielded 8,180 adults. In the larger samples there was apparent equality in numbers between the sexes; males and females were equally attracted to the traps.

Miura (1980) demonstrated estimation of total larval population in a confined area by sequential removal of large samples. An attempt to utilize this method to estimate the total adult population in a manhole yielded respective captures of 2,670, 2,609, 4,708 and 3,101 adults on 4 consecutive nights. Failure to obtain reduced numbers indicate that the samples captured were not large enough to dent the population reservoir.

Data obtained through the first 6 weeks was used to group the 5 manhole trapping sites by analysis of variance and paired t tests (P=0.05). Two groupings were used and weekly means were derived for each (Figure 1). One group of 2 trap sites (X) consistently produced means well over 1,000 adults/trap night, while the means for the other group of 3 trap sites (Z) were less. Grand means for the initial 6 week pre-treatment period were 3,310 and 383 adults/trap night, respectively -- a near 10X difference.

The sex ratios of adults trapped also differed between the two manhole groups. The group with higher mean numbers produced parity of sexes; 0.522:0.478, female:male (difference not significant). Whereas, in the other group, there were significantly more females; 0.631:0.369, female:male.

Not only did the adult collection quantity and quality differ, but the physical environment of the manhole groups differred as well. Trunk lines of the former manhole group (X) had constant, deep (>0.5m), standing water throughout the summer. Water depth in the latter group (Z) was <3cm and intermittently dry. Breeding was primarily concentrated in

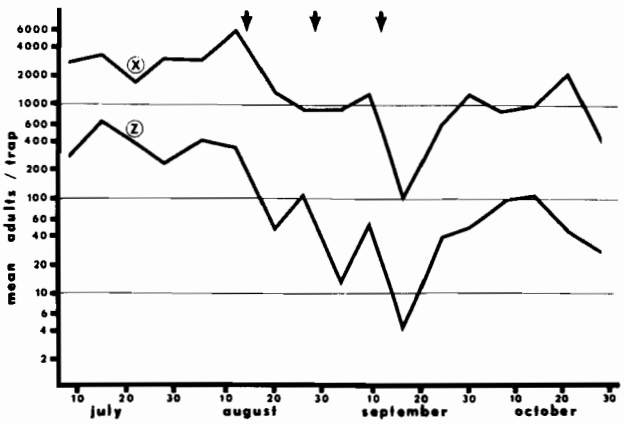


**Figure 1.** Mean numbers of adult mosquitoes (*Culex quinquefasciatus)* collected in miniature CDC traps placed in deep water (X) and shallow water (Z) manholes of a storm drain trunk line in Fresno, CA. Arrows denote applications of adulticide to the trunk line.

the deeper water areas as indicated by the large total numbers and the equal numbers of both sexes. While limited breeding occurred in the shallow water areas, it was apparent from the greater ratio of females that there was significant dispersal from deeper water areas throughout the trunk lines.

Females trapped from the manholes readily took blood from mice in the laboratory and a self-mating colony was started. Such females did not oviposit without a blood meal. Since no autogenous reproduction could be induced in the laboratory, the mosquitoes were regarded as pure *Cx. quinquefasciatus*. No other species was identified from manhole collections.

Of several thousand females collected from manholes and examined, only 1 was found to be blooded. While this showed that gravid females were re-entering the drain line, such individuals were masked by the total production from the breeding source. The probable avenues of exit and entrance were the street catch basin openings.

The numbers of blooded females were much greater in collections from yard traps -- ca. 4% were blooded (out of 350 females). Males comprised 2% of the yard collections and only 2 mosquitoes other than *Cx. quinquefasciatus* were caught (1 *Cx. tarsalis* Coquillett and 1 *Culiseta inornata* (Williston), both females). During the entire study period the overall mean was 29 adults/trap/night, with individual per night means ranging from 7 to 91 adults/trap (Figure 2).

Three manholes in a separate drain line were monitored on three nights (7-27, 8-11 and 8-19-80) and produced means of 1,869, 3,184 and 1,854 adults/trap/night. These manholes, with constant standing water, were known to produce mosquitoes and had been routinely treated by the MAD with larvicide oil from the beginning of summer. Evidently the larviciding treatments were not spreading through the trunk lines to any great extent, as indicated by adult collections as great as from the untreated line.

Treatments of Drain Line. - - Application of malathion on 8-15-80 (Figure 1, first treatment) resulted in a reduction (al-

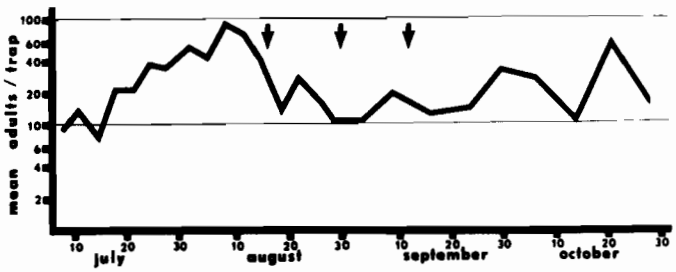


**Figure 2.** Mean numbers of adult mosquitoes *(Culex quinquefasciatus)* collected in miniature CDC light traps placed at 3 houses adjacent to a storm drain line in Fresno, CA. Arrows denote applications of adulticide to the storm drain line.

most 10X) in the adults collected from both manhole groups. After application an increase in adults trapped occurred in shallow water manholes (Z), but not in deep water manholes (X); however, this resurgence did not reach former levels. This first equipment modification had several drawbacks: the aluminum tubing was not adequately flexible or durable, the fittings leaked spray and a method for directing the tubing was needed. However, the main problem was condensation of the spray in the tubing, which resulted in runoff.

To correct the problems, the mist blower was modified again and a second malathion application made on 8-29-80. Again there was an almost 10X adult reduction in the shallow water manholes. No coinciding decrease was apparent in the deep water manholes, although there was no increase either. This second modification was easier to maneuver and transport on an operational basis, although there were still spray dispersal and detection problems. Droplet sizes of the mist were too large to be blown through any considerable length of trunk line. Only the odor was carried through the lines and detection was by odor alone, with inherent difficulty.

The modified Leco cold fogger produced a fine particle fog which readily carried long distances (3 blocks) in the line and was easy to detect visually. The equipment was durable and practical, but two or more operators were required for efficient use, as for the other modifications. It was necessary to cover catch basin inlets with plastic sheeting to prevent leakage of fog, which followed the path of least resistance.

Adult populations decreased > 10X following the Cythion treatment with Leco unit (Figure 1, last treatment). As was the general case after each adulticide application, the numbers of adults trapped increased within two weeks of treatment although there was an apparent cumulative reduction in numbers caused by the three consecutive applications. After the third application trapping continued. Adult numbers were high, but generally did not reach pre-treatment levels. A decline in numbers occurred in the latter part of October.

Although adulticide applications reduced adult populations in the storm drain line, the treatments were never completely effective and the effects were short-lived. The population proved to be highly resilient. Such dosing of a confined population with concentrate pesticide would most probably lead to rapid development of resistance. High levels of resistance to a broad spectrum of organophosphorus insecticides have developed in *Cx. quinquefasciatus* of the San Joaquin Valley, CA (Georghiou et al. 1975).

Preliminary insecticide susceptibility tests with the colony obtained from the drain lines indicates a moderate to high degree of organophosphorus-resistance is present in this population. This indicates a need to determine the susceptibility of this population to other chemical types, e. g. resmethrin. The FMAD can expect considerable control difficulties if organophosphorus compounds are used routinely against these populations.

Thus chemical treatment should not be considered as more than a limited tool. Other more permanent control strategies, e.g. physical control methods, are called for in existing lines. In the future every effort must be made to construct new trunk lines at an adequate grade for drainage and pumping stations should be added and operated if necessary, to prevent water from standing in the lines.

Catch Basin Retrofit. - - The retrofitted catch basins in Kerman did not hold water nor breed mosquitoes. Water continued to stand in the control basins though, and mosquito larvae were found in them, necessitating larvicidal treatment. On one occasion drain water backed up in the trunk lines into the retrofitted basins; however, the cause was a float failure at the pumping station. By retrofitting the old-style basins, the need for routine chemical and oil treatments was eliminated. Only occasional spot checks to determine line blockage or pumping station failure were required.

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# DIEL RHYTHMS IN SUSCEPTIBILITY OF *SIMULIUM ARGUS* WILLISTON TO ABATE®

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ABSTRACT

The diel susceptibility rhythms in *Simulium argus* Williston larvae were studied in the laboratory. Penultimate and ultimate larvae were exposed to a fixed concentration of Abate® (0.025 ppm) for 1-h and then held for 24-h after which mortality was assessed. The larvae were treated at 1800, 2400, 0600 and 1200 h in these experiments. Maximum mortality among *S. argus* larvae (77%) was produced in the 2400-h treatment. The average mortalities produced in 1800, 0600 and 1200-h treatments were not significant from each other but were all highly significant from the average mortality of the 2400-h treatment.

Recently diel rhythmic susceptibility to insecticides has been documented in a number of medically important insects such as *Blatella germanica* (L.), *Musca domestica* Linnaeus and *Aedes aegypti* (L.) (Beck 1963, Halbert et al. 1974, Roberts et al. 1974). Under laboratory conditions, Roberts et al. (1974) noted that 4th instar larvae of *A. aegypti* were more susceptible to Dursban® treatment during the dark period (2100-0100h) than during the light period (0900-1300h). The establishment of diel rhythms of susceptibility in many pestiferous and disease vector arthropods will enable us to apply the least amount of insecticides at the proper time to obtain the highest level of control. This paper presents the results of a series of bioassays to determine the diel rhythms in susceptibility of *Simulium argus* Williston larvae to the commonly used larvicide Abate®.

MATERIALS AND METHODS.–The diel rhythms in susceptibility of *S. argus* larvae to Abate 50% EC [*0,0,0,0*,-tetramethyl-*0-0*thiodi-*p*-phenylene phosphorothirate] was studied in the laboratory employing the flushing bioassay system developed by Lacey and Mulla (1977) and following the procedure described by Mohsen and Mulla (1981a). Abate was selected for these tests because it is the most commonly used larvicide and it shows good activity against *S. argus* ($LC_{50}$= 0.201, $LC_{90}$= 0.0383 ppm/lh) in the laboratory and was found selective in the field. (Mohsen and Mulla 1981a, 1981b). It is currently used in area-wide control programs of Onchocerciasis in west Africa (Walsh et al. 1979) and for *Simulium* control elsewhere.

Four replicates of 35-40 field-collected penultimate and ultimate *S. argus* larvae were exposed to 0.025 ppm of Abate for 1-h 4 times during a day at 1800, 2400, 0600 and 1200 h. Each treatment including check was replicated 4 times. The experiments were conducted on 3 different occasions utilizing larvae from 3 different populations breeding in an artificial creek located at Thousand-Palms Canyon, CA. Water temperature in the laboratory was maintained at 20±1°C. Throughout the period of these tests no specific photophase-scotophase cycle was followed. However, during the day (14h) larvae received natural light through laboratory windows; during the night (10h) the laboratory was kept dark but fluorescent light was turned on only for approximately 1.5h, the period required to carry out the 2400 h treatments.

RESULTS AND DISCUSSION.–Fig. 1 shows the average mortality of *S. argus* larvae exposed for 1 h to 0.025 ppm of Abate applied at 1800, 2400, 0600 and 1200 h. In the 3 ex-

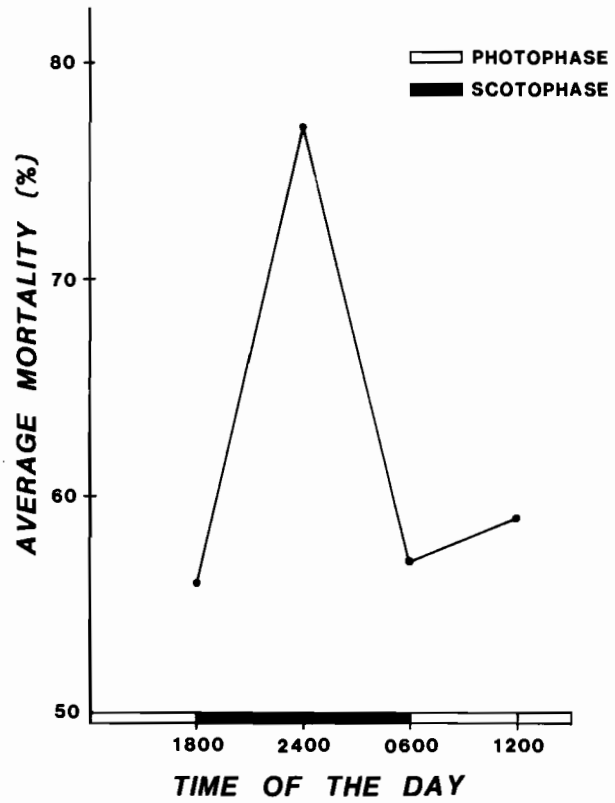


**Figure 1.** Average percent mortality of *S. argus* larvae exposed to 0.025 ppm of Abate in 24-h period study.

periments, the highest mortality in *S. argus* larvae was produced in midnight treatment (Fig. 1). The average mortality in this treatment was 77%, while the average mortalities produced at other times of the day 1800, 0600 and 1200 h were 56%, 57% and 59% respectively. These mortalities were not significantly different from each other, but they were highly significant from the average 2400 h mortality at 0.01 probability level.

Since the test larvae were exposed to the same dosage of Abate, the differential susceptibility to this larvicide among *S. argus* reported here may be attributed to some larval endogenous factors. It is believed that differential susceptibility of insects to insecticides at different circadian times reflects some endogenous biochemical oscillations which may be phase-controlled by external phenomena such as photophase-scotophase cycles (Saunders 1976). In Simuliidae, some other physiological phenomena of this sort have been described such as larval drift (Disney 1972), pupation and adult eclosion (Disney 1969, Hunder 1977) and oviposition (Hunter 1977). Susceptibility rhythms in *S. argus* to Abate as evidenced in this study may be added to those already reported rhythmic or cyclic phenomena.

This study clearly indicates that efficacy of Abate larviciding may be maximized if this chemical is applied at midnight time. However, field trials are necessary to confirm these laboratory findings.

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# FATTY ACIDS AS OVIPOSITION REPELLENTS FOR MOSQUITOES

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Volatile chemicals, which are produced by the decomposition of organic matter in natural habitats, are important factors influencing the ovipositional behavior of gravid female mosquitoes. In our previous work, the ovipositional activity of various organic infusions was quantitatively studied in laboratory olfactometers against *Culex quinquefasciatus* Say and *Cx. tarsalis* Coquillett. In addition, the production of oviposition-modifying chemicals in the organic infusions was determined to be due to the action of microorganisms (Kramer and Mulla 1979). Subsequent chemical investigations resulted in the isolation and identification of six aliphatic carboxylic acids from an infusion of Purina Laboratory Chow. They included acetic ($C_2$), propionic ($C_3$), isobutyric (*iso*-$C_4$), butyric ($C_4$), isovaleric (*iso*-$C_5$), and caproic ($C_6$) acids, which singly or in combination showed significant ovipositional repellency against ovipositing females of *Cx. quinquefasciatus* (Hwang et al. 1980). The repellency of these lower aliphatic carboxylic acids was later quantified, and the ovipositional repellency of butyric acid against other mosquito species, such as *Culiseta*, *Aedes*, and *Anopheles*, was also quantitatively determined (Kramer et al. 1980).

Our studies on the concentration-activity relationship showed that the lowest concentration, at which the lower aliphatic carboxylic acids demonstrated significant ovipositional repellency against gravid *Culex* mosquitoes, was 6 x $10^{-3}$% (60 ppm). This concentration seemed too high to be useful in pest management programs. In order to procure more active chemicals, we evaluated higher aliphatic carboxylic acids from $C_5$ through $C_{13}$ for their ovipositional repellency against *Cx. quinquefasciatus*, *Cx. tarsalis*, and *Aedes aegypti* L.

Laboratory olfactometers, developed in this laboratory (Kramer and Mulla 1979), were used for bioassay tests to determine ovipositional activity of the fatty acids. The olfactometer unit consisted of two Stender dishes, a 1-liter polystyrene plastic food cup, and an outlet tubing attached to the center of the bottom of the food cup. Two Stender dishes were placed side-by-side 3.5 cm apart on a piece of paper towel; one dish contained a solution or a suspension of a test chemical, and the other contained distilled water as control. The plastic food cup was inverted over the dishes so that the outlet tubing was leading upward. The outlet tubing was connected to a vacuum line through a flow meter which was set at 50 ml/min. After the olfactometer unit was set up, five gravid mosquitoes were introduced into the unit. The tests were replicated at least eight times, and the eggs or egg rafts oviposited on the treated and control dishes were counted after 24 and 48 hrs.

For testing *Ae. aegypti*, some modifications were made. A strip of paper towel or filter paper, 2.5 x 11 cm, was placed in the liquid along the inside margin of the Stender dish to provide a damp substrate for oviposition.

The results of the bioassays were expressed in terms of the oviposition activity index (OAI) which was calculated according to the following formula (Kramer and Mulla 1979):

$$OAI = \frac{N_T - N_S}{N_T + N_S}$$

wherein $N_T$ represents the number of eggs (for *Ae. aegypti*) or egg rafts (for *Cx. quinquefasciatus* and *Cx. tarsalis*) in a treated sample, and $N_S$ represents those in the control. The OAI values fall between +1 and -1; the maximum numbers, +1 and -1, represent the maximum ovipositional activity. A positive OAI indicates an attractancy, stimulancy, or a combination of both, and a negative OAI indicates a repellency, deterrency, or a combination of both. The data were analyzed by the chi-square analysis to determine the significance of all indices.

The results of our studies are shown in Figure 1 through 3,

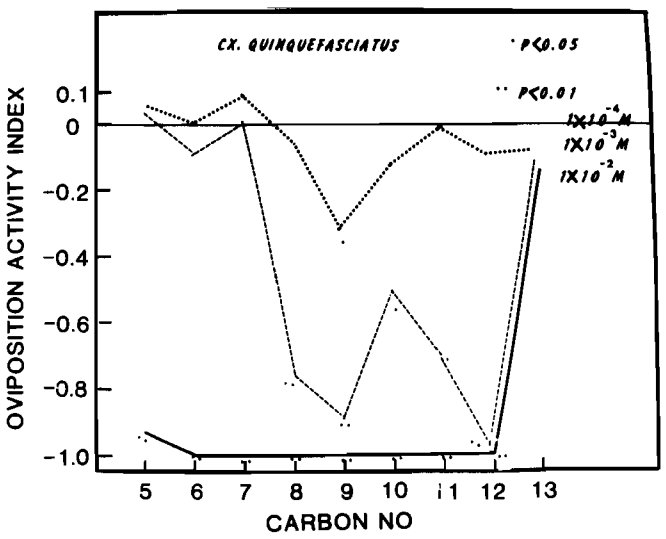


**Figure 1.** Ovipositional repellency of fatty acids against *Cx. quinquefasciatus*.

in which the number of carbon atoms in a straight-chain fatty acid is plotted against its oviposition activity index and those points with a single asterisk and double asterisks denote that the OAI's are significantly different from the control at the 5 and 1% level, respectively.

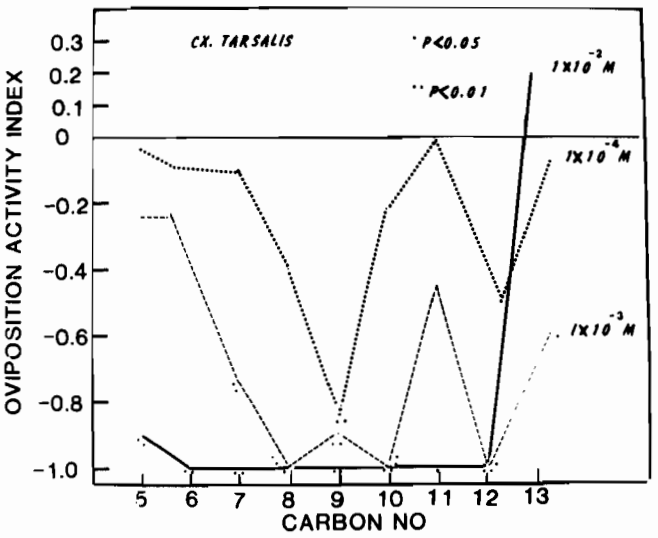


**Figure 2.** Ovipositional repellency of fatty acids against *Cx. tarsalis*.

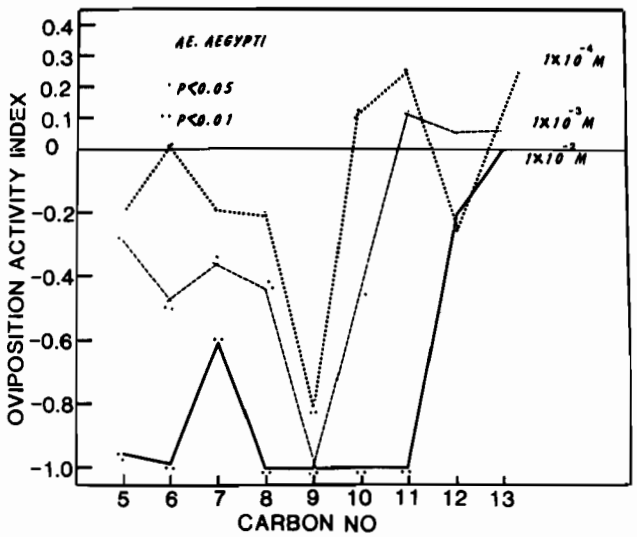


**Figure 3.** Ovipositional repellency of fatty acids against *Ae. aegypti.*

When tested against *Cx. quinquefasciatus* (Figure 1), pentanoic ($C_5$), hexanoic ($C_6$), heptanoic ($C_7$), octanoic ($C_8$), nonanoic ($C_9$), decanoic ($C_{10}$), undecanoic ($C_{11}$), and dodecanoic ($C_{12}$) acids showed almost 100% repellence (OAI=-1.0) at the $10^{-2}$M concentration. At the $10^{-3}$M concentration, only $C_8$, $C_9$, $C_{10}$, $C_{11}$, and $C_{12}$ acids displayed significant repellency. $C_9$ acid showed an OAI of -0.35 even at the lowest $10^{-4}$M concentration. Among the carboxylic acids evaluated, $C_9$ acid seemed to be the most active against *Cx. quinquefasciatus*.

The carboxylic acids from $C_5$ through $C_{12}$ exhibited almost complete repellency at $10^{-2}$M against *Cx. tarsalis* (Figure 2), however, at $10^{-3}$M, only $C_7$, $C_8$, $C_9$, $C_{10}$, and $C_{12}$ acids were significantly repellent. At $10^{-4}$M, only $C_9$ acid showed significant repellency. Therefore, $C_9$ acid was also the most active against *Cx. tarsalis*.

The evaluations of these carboxylic acids against *Ae. aegypti* showed that $C_5$ through $C_{11}$ acids were significantly repellent at $10^{-2}$M (Figure 3). At $10^{-3}$M, $C_5$ through $C_{10}$ acids were repellent. At $10^{-4}$M, only $C_9$ acid was significantly repellent. Further investigations on $C_9$ acid revealed that this acid demonstrated significant repellency against *Ae. aegypti* even at $10^{-5}$M.

In our laboratory evaluations, we therefore confirmed that nonanoic acid was the most active acid in suppressing ovipositional responses in all three species of mosquitoes. This acid was repellent at concentrations between $10^{-4}$ to $10^{-5}$M against the three mosquito species.

Based on the data presented, dosage-response curves of the acids were drawn on log-probit paper, and the concentrations showing 90% repellency ($R_{90}$) were obtained by extrapolation for comparative purposes. $C_6$ and $C_9$ acids showed the lowest $R_{90}$ values against *Cx. quinquefasciatus*. Their $R_{90}$ values were about $3.0 \times 10^{-3}$M. $C_8$, $C_9$, and $C_{10}$ acids showed $R_{90}$ of $5.0\text{-}9.1 \times 10^{-4}$M against *Cx. tarsalis*. *Ae. aegypti* females were most repelled by $C_9$ acid which showed an $R_{90}$ of $7.4 \times 10^{-4}$M.

The laboratory studies reported here establishes a working basis for field evaluations. Detailed results of our semi-field and full-scale field investigations of the fatty acids as mosquito oviposition repellents are reported elsewhere.

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# POSSIBLE INTEGRATION OF MICROBIAL AND GENETIC CONTROL OF *CULEX TARSALIS*[1]

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INTRODUCTION.–During the 1979 sterile-male release for *Culex tarsalis* control (Reisen et al., 1981.), larval samples from the field revealed the presence of a microsporidian infection. The microsporidian, originally discribed as *Thelohania californica* (Kellen and Lipa 1960), was later disignated *Amblyospora californica* as the type species for a new genus (Hazard and Oldacre 1975).

*A. californica* has two developmental sequences in the host. Octospores are produced in oenocytes of male larvae resulting in a fatal infection usually in the third or fourth instar. Infected females do not show signs of infection, but pass the parasite trans-ovarially to their male and female progeny. Thus the surviving progeny of infected females can be expected to be almost entirely female. The speculation is that if the prevalence rate of this infection can be increased in the sterile-male release area, the ratio of wild males to native females will be reduced considerably giving the released sterile males a numerical advantage. One purpose of this paper is to explore various mechanisms by which the prevalence rate of the parasite could be increased.

Unlike most microsporidian infections in insects, the spores produced in male *Cx. tarsalis* larvae are apparently not directly infectious for this mosquito species. Thus the mechanism of horizontal transmission, if there is one, is unknown. Kellen and Lipa (1960) reported soil and water from a field site, where infected larvae had been taken, was infectious for healthy *Cx. tarsalis*. They also reported transmitting the infection to healthy larvae using spores which had been alternately dried and rehydrated. These results have not been confirmed by other studies, and subsequent authors have emphasized the apparent absence of horizontal transmission (Andreadis and Hall 1979).

Recent advances in the genetics of the microsporida have prompted speculation that an alternate host may be involved (Hazard et al. 1979). Octospores found in male larvae of the species *Culex salinarius* are uninucleate and haploid, meiosis having been observed in the sporonts. Thus these spores are different from the binucleate spores of other genera (e.g. *Nosema*), which are infectious. Meiosis has not been observed in *Nosema*, thus there is still some question about the ploidy of the nuclei in the spores. Since there are two, however, they are called "minimally diploid". Spores found in female *Cx. salinarius* are not formed in octospore arrangements and are diploid. No meiosis has been observed in the formation of these spores. The question then is: do these uninucleate haploid octospores found in the male larvae of *Cx. salinarius* have a function, or are they merely a biological dead end? Hazard et al. (1979) speculate that since the spores are produced in such large numbers and since it is difficult to imagine how the infection could be maintained in nature without horizontal transmissions (see also Andreadis and Hall 1979), the spores may have biological significance perhaps as gametes which fuse in an alternate host.

The unnamed microsporidian in *Cx. salinarius* is slightly different from *A. californica* in *Cx. tarsalis*, although both are Type I infections in which octospore formation is suppressed in females (Kellen et al. 1965). A significant number of infected *Cx. salinarius* eggs fail to hatch and there is some recovery among females. No reduction of egg hatch or recovery of females has been noted in *Cx. tarsalis* infected with *A. californica*. Therefore, the possibility exists that *A. californica* sustains itself in nature through transovarial transmission alone. Whether *A. californica* infection in *Cx. tarsalis* is similar to infection of the undescribed microsporidian in *Cx. salinarius* is not certain, but the many similarities between the two suggest that it is. It is worthwhile, therefore, to continue searching for possible alternate hosts.

A colony infected with *A. californica* is easily maintained in the laboratory by crossing infected females to laboratory normal-type males. We are investigating the biology of these infected females to increase our understanding of the ecology of the microsporidia in nature. Thus, another purpose of this paper is to present our preliminary findings in this regard.

MATERIALS AND METHODS.–A colony of *Cx. tarsalis* infected with *A. californica* was established in October, 1980 from material collected at the experimental field site near Bakersfield in Kern Co., CA. Larvae and adults were maintained in a Percival environmental chamber at a constant temperature of 80°F and a photoperiod of 12 light and 12 dark hours. Lar-

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vae were fed a mixture of liver extract powder and rat chow. Adults were maintained in 1 gallon paper containers with raisins and a cup of water for moisture and oviposition. Blood meals were provided in the form of mice anesthetized with diabutal, which were placed on the top screened section of the cages.

Horizontal Transmission. - - Horizontal transmission of *A. californica* was attempted by using 5 different techniques: 1. Infected cadavers were gently macerated in a mortar and pestle and filtered through a cotton plug to remove cell debris. The resulting spore suspension was examined under phase contrast to determine whether intact spores were present and undamaged by the isolation procedure. Healthy neonate larvae were exposed to high concentrations of these spores (approx. $10^4$ spore/ml) continuously throughout their larval development. 2. An aliquot of the partially purified spore preparation was divided among a number of plastic petri plates and the water was evaporated leaving a dry film of spores. These were placed directly in pans of water containing neonate larvae which were exposed to the rehydrated spores throughout larval development. 3. Infected adult female cadavers were collected, macerated and placed in water containing neonate larvae as above. 4. Healthy neonate larvae were reared in pans of water and accumulated debris which had previously contained a generation of infected larvae. 5. Healthy and infected larvae were also reared together in the same pan.

In each of the five experiments outlined above the larvae were reared to maturity and observed for infection by making wet mounts and geimsa-stained smears of selected individuals using the techniques of Kellen and Lipa (1960). Sex ratios were compared the healthy control families reared under identical conditions.

The laboratory strain used in the above horizontal transmission trials was PWC-79, a strain originating near the same geographic area where the infected individuals had been obtained, and which had been in colonization for approximately 2 years. In addition, the few males that survived infection in the infected colony (approximately 0.5%) were bred to healthy females and the progeny were backcrossed to additional survivor males. The progeny from the backcross were challenged by methods 1) and 2) above, and observed for infection as previously indicated.

Longevity Related to Blood Meals. - - Two one-gallon paper containers were set up with 125 infected females and 40 PWC-79 males per cage. Blood meals were provided regularly (3 times per week) for one container but withheld from the other. Mortality was scored periodically and compared to similar studies which had been done previously with healthy adults.

Fitness Studies. - - To determine whether individuals infected with the microsporidian are as fit as those not harboring the infection, larval survival tests and adult feeding experiments were done with both infected and uninfected groups. In replicated studies ten larvae from a healthy stock and 10 from the infected stock were placed together in a small plastic container (100 ml, 5 cm. deep) and reared to the adult stage. The resulting sex ratio as adults was compared to 2 separate cups containing either 20 healthy or 20 infected controls.

Two one-gallon paper containers were set up with 100 infected females and 100 healthy PWC-79 females respectively. Approximately 50 male PWC-79 adults were added to each container. One anesthetized mouse was placed on top of each container for the females to take a blood meal. After 30 minutes the mice were switched to avoid any individual differences between them. Feeding continued for 30 minutes after which the engorged females in each sample were counted.

Exposure of Infected Females to Radiation. - - Infected females were exposed to 3000 r of gamma radiation in a single exposure and then crossed to healthy males for mating and oviposition. Earlier tests had indicated that infected females could not tolerate as much radiation as normal females. The latter, for comparison studies of oviposition, were exposed to 6000 r of gamma radiation and set up in a similar manner. Blood meals were offered each group every other day. Egg rafts were collected and scored for percent hatch.

Sterile-male Mating Studies. - - To determine whether infected females would mate with radiosterilized males and produce low-hatch rafts, 82 infected and 82 healthy females colonized from the same geographic location were placed in seperate wooden cages ( 2' square) with raisins for food and a pan of water for oviposition. Sixty radiosterilized males were placed in each cage. In addition, 60 non-irradiated males from the same source were placed in the cage with healthy females since in nature uninfected populations would contain normal males while infected ones would not. Both were blood-fed with anesthetized mice approximately every other day. Egg rafts were collected and scored for percent hatch.

RESULTS AND DISCUSSION.–All attempts at horizontal transmission were unsuccessful. Either environmental conditions were not suitable, spores were not at the infectious stage, or the host was not at the susceptible stage. Further tests will explore other possibilities with the concept of a alternate host playing a vital role. The longevity studies indicated that infected females do not live as long as uninfected females but do live long enough to serve as vectors of encephalitis virus (Table 1). The acquisition of a blood meal does not affect the

**Table 1.** Lifespan of normal adult *Culex tarsalis* females and females infected with *Amblyospora californica*.

| | % mortality | | |
|---|---|---|---|
| | infected | | healthy* |
| Day | Blood Fed | Unfed | Blood Fed |
| 7 | 7 | 3 | 9 |
| 9 | 18 | 11 | 9 |
| 14 | 80 | 81 | 27 |
| 17 | 90 | 93 | 43 |
| 26 | 98 | 99 | No data |

*Healthy and infected tests done at different times

longevity of infected females. Fertile egg rafts were also obtained from both infected and uninfected females that had not yet received a blood meal, indicating that autogeny is not limited to normal uninfected females of *Cx. tarsalis*. The rate of autogeny between the 2 groups, however, was not compared in this study.

When healthy and infected larvae were reared together in a confined space, they were found to be equally competitive for survival (Table 2). This, plus the observation that developmental times are similar for both healthy and diseased instars, indicated that in the larval stage infected and healthy females are competitive for limited resources.

As adults, however, the females infected with the microsporidian appear to be more vigorous in blood feeding than are healthy ones. Eleven percent more females from the infected colony became engorged in a 60 minute time period than did females of the same age from the healthy colony.

**Table 2.** Larval competition for limited sources.

| No. of replicates | No. of larvae per cup | Surviving Adults (Average) females | males |
|---|---|---|---|
| 3 | 20 healthy | 1.3 | 3.3 |
| 3 | 20 infected | 6.7 | 0 |
| 9 | 10 healthy and 10 infected | 3.8 | 1.8 |
| | (Expected) | 4.0 | 1.7 |

Regarding exposure to irradiation, the data clearly indicated that uninfected females are considerably less sensitive to gamma radiation than are infected ones (Table 3). This may indicate that there are more radiosensitive processes occurring in young infected females, which in turn may indicate more vigorous ovigenesis.

The latter point was demonstrated in matings of both infected and uninfected females to males irradiated by 6000 r. Table 4 illustrates that both groups mated with these sterilized males and produced low hatch rafts. However, the number of egg rafts obtained from 82 infected females over a 30 day period was significantly higher than the number of rafts obtained from equal number of females from the uninfected line. The percent hatch for eggs from the two groups was similar, as was the average egg raft size (138 for infected, and 149 for healthy). The data are consistent with the hypothesis that infected female adults are more vigorous than are healthy females in blood feeding and oviposition. If, as the data suggest, infected females are more reproductively fit, infected individuals may have a competitive advantage in nature, in which case releases of infected material may actually reduce the number of healthy females by competitive exclusion. This would be another method of increasing the prevalance rate of the disease in nature. In addition, preliminary tests indicate that infected females are incompetent as vectors of WEE virus, thus a population replacement might have additional advantages. Such an approach would have to be thoroughly tested in cages and with modeling simulations before field releases were attempted.

**Table 4.** Oviposition of uninfected and infected *Culex tarsalis* females mated to sterilized males.

| | No. Rafts After 30 Days | Rafts Per Female | Percent Hatch |
|---|---|---|---|
| 82 uninfected females | 5 | 0.06 | 2.5 |
| 82 infected females | 41 | 0.5 | 2.8 |

In some orders of insects, microsporidia have been implicated with the production of substances which slow down or prevent pupation and decrease susceptibility to diapause. In some cases substances analogous to juvenile hormone have also been identified (Weiser, 1976). Juvenile hormones in insects are relevant to the development of ovarian follicles, the initiation of sexual behavior and biting behavior (Meola and Petralia 1980). If infected females are more reproductively fit than uninfected females as our data suggest, the hypothesis that juvenile hormone analogues are produced as a result of infection would be consistent with observations in other insect orders.

In the search for possible alternate hosts, the following observations in research with other microsporidia should also be considered. In one study, *Nosema algerae* infecting *Anopheles stephensi*, was fed to *Biomphalaria glabrata* snails that were in turn infected with *Schistosoma mansoni*. The results indicated that the trematode, *S. mansoni*, became infected rather than the snail which was its host. In searching the habitat of *Cx. tarsalis* attention should be paid not only to the invertebrates found there, but also any possible predators or other parasites of *Cx. tarsalis*.

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**Table 3.** Effect of exposure to gamma irradiation on oviposition.

| | Co-60 Dose | Egg Rafts No. | Percent Hatch | Days(post-irradiation) |
|---|---|---|---|---|
| Healthy females | 6000 r | 6 | 25 | 9 |
| Infected females | 3000 r | 2 | 0 | 30 |

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# THE RELATIONSHIP OF THE LONGEVITY OF IRRADIATED *CULEX TARSALIS* MALES TO MATING BEHAVIOR[3]

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## ABSTRACT

Releasing radiosterilized *Culex tarsalis* males into isolated field populations is part of a study evaluating the use of sterile males for the control of this species. The success of the program depends in part on the selection of a dose of gamma radiation which maximizes damage to sex cells while minimizing damage to somatic cells. Previous work indicated that laboratory reared males exposed to 6000 r in a single exposure of 160 r/min. were competitive with laboratory males for laboratory females. These sterilized males, however, were less competitive against wild males for wild females, and competitiveness decreased with time. Two hypotheses consistent with this observation are that radiation reduces male longevity or the ability of sterile males to inseminate females. Experiments reported here tested these hypotheses.

Laboratory reared males were exposed to 5000-7000 r at 160-200 r/min. There was no difference in longevity between irradiated and unirradiated males, whether or not they were caged with females, or whether they were irradiated at 0-24 or 24-28 hours post-eclosion. Thus an exposure of approximately 6000r in a single dose does not appear to affect longevity of the male *Cx. tarsalis*.

Males exposed in the same manner were placed with females of the same strain at male to female ratios of 1:1, 1:2, and 1:5. After 1 week the first harem of females was removed and replaced with a second. Females of both harems that failed to oviposit were dissected to determine whether they were inseminated. Data indicated that there was no difference in the inseminating ability of irradiated males compared to unirradiated males at any of the sex ratios, or between the first and second harems.

The decrease in competitiveness of laboratory reared males against wild males for wild females may involve assortative mating or other biological factors instead of a simple reduction in mating ability of sterile males due to somatic damage.

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# THE DEVELOPMENT OF PSEUDOHOMOZYGOTE TRANSLOCATION STRAINS FOR GENETIC CONTROL OF *CULEX TRASALIS*[1]

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## ABSTRACT

Translocation pseudohomozygotes are fertile individuals heterozygous for two very similar translocations. Methods to produce and capture pseudohomozygotes have been explored. Self-perpetuating stocks containing pseudohomozygotes have been colonized for more than ten generations.

The goal of this research was to isolate strains of chromosomal variants for use in the suppression of *Culex tarsalis* poplations. Translocations are chromosomal rearrangements with autocidal properties (Asman, McDonald and Prout 1981). In an individual heterozygous for a translocation, gametes that are duplicate for some genetic information and deficient for other genetic information are produced. These gametes cause the death of a substantial proportion of offspring.

Translocation heterozygotes with desirable characteristics cannot be maintained as such in laboratory colonies. Instead translocation homozygotes which produce heterozygote offspring when outcrossed are maintained to furnish the needed heterozygotes. The homozygotes produce no duplication-deficiency gametes, are fertile and breed true.

In general, translocation homozygote strains have proved to be unsuitable for laboratory colonization because they are highly inbred. Thus, pseudohomozygote strains that would be less inbred and yet essentially homozygous for a translocation rearrangement have been sought. These strains would be heterozygous for two very similar translocations and produce gametes with such insignificant duplications and deficiencies that no mortality resulted. These fertile strains would show heterosis since they resulted from the combination of two translocation stocks.

MATERIALS AND METHODS.—In the first stage of investigation the question of how similar two translocations would have to be in order to combine into a fertile pseudohomozygote was raised. Two autosomal and mutant-marked translocations of similar structure were interbred. The T(2;3)15*A* had a pseudolinkage between carmine eye(*car*), a recessive of linkage group III black eye (*ble*), a recessive of linkage group II, of 5.3 crossover units (McDonald et al; 1978). The T(2;3)16A had a pseudolinkage between *car* and *ble* of 1.6 crossover units. In the experimental cross individuals heterozygous for both the translocations were crossed to normals. In the control, normals were inbred. The hatch of rafts derived from the crosses were categorized as high (>80%) or reduced (<80%).

In the second stage of investigation a crossing scheme to capture a pseudohomozygote was followed (Figure 1). In the first generation an established *car ble*-marked translocation homozygote stock was crossed with an irradiated (2500 rads) wild type normal stock. In the second generation the normal *car ble* stock was crossed with individual F-1 progeny. Only in the case where a pseudohomozygote F-1 was produced would fertility of a cross be high.

All the progeny of a highly fertile pseudohomozygote would be expected to be semisterile heterozygtes. The members of families descendant from a suspected pseudohomozygote were crossed with normal *car ble* stock and the fertility of the rafts determined. Families with some tested members of

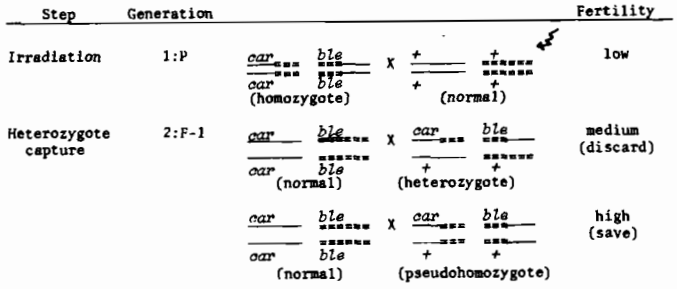


**Figure 1.** A method to screen for pseudohomozygotes by selecting high fertility progeny from the cross of an established homozygote with an irradiated normal.

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high fertility were classified as descendants of heterozygotes rather than pseudohomozygotes.

RESULTS AND DISCUSSION.--In the initial stage of investigation the combined T(2;3)15*A*/T(2;3)16*A* individuals were tested and on outcross produced 13 of 20 rafts with high hatch. The control had 4 of 4 rafts with high hatch, as expected. The finding that two translocations which were dissimilar to the extent of 3.7 crossover units could combine to yield fertile and presumably pseudohomozygote progeny became the stimulus for further investigations into creation of pseudohomozygote combinations.

In the second stage of investigation, out of 374 rafts a total of 17 fertile rafts from presumed pseudohomozygotes were recovered. The 17 families from these rafts were classified according to fertility of their members. Only 12 families were reproductive and all of them had some fertile members and thus were descendants of heterozygotes rather than pseudohomozygotes. It was unexpected that the high hatch rafts resulted from heterozygote rather than pseudohomozygote matings. Perhaps, in these cases, the expression of mortality (ordinarily during prehatch development) was delayed to a posthatch time.

Through the use of methods similar to those presented here three pseudohomozygote lines have been isolated and colonized for several generations (Asman, McDonald and Stoddard). The T(2;3)5*A*/T(2:3)16*A* line illustrates the features of the pseudohomozygote stocks, with regard to genetics and reproductive characteristics. A polymorphic system was created and included the two homozygotes which when interbred generate the pseudohomozygote. This system consisted of T(2;3)5*A* homozygotes of wild phenotype, T(2;3)16*A* homozygotes of *car ble* phenotype and the T(2;3)5*A*/T(2;3)16*A* of wild phenotype.

In the genetic analysis of the stock, the frequency of recombinant phenotypes (*car* or *ble*) remained low after 10 generations, indicating that the translocations were not lost (Table 1). Once more, the presence of the *car ble* homozygote at a significant and low frequency after 10 generations was evidence that a balanced polymorphic system had been established. A pseudohomozygote fitness greater than that of either homozygote would account for this.

**Table 1.** Phenotypes and hatchability of rafts from the strain derived by combining T(2;3)5*A* with T(2;3)16*A*. The phenotypes of T(2;3)5*A* homozygotes and T(2;3)5*A*/T(2;3)16*A* pseudohomozygotes are wild and that of T(2;3)16*A*, *car ble*.

| Generations | Phenotypes scored | | | | | Hatch of rafts | | |
|---|---|---|---|---|---|---|---|---|
| | wild | car ble | car or ble | %car ble | Reduced (<70%) | High (>70%) | %High |
| 1 - 3 | 1510 | 116 | 7 | 7.1 | 139 | 10 | 6.7 |
| 11 -13 | 1704 | 85 | 2 | 4.7 | 245 | 10 | 3.9 |

When the hatches of rafts were classified for early and later generations a decline in the proportion of high hatch rafts was seen (Table 1). In the future, it is hoped that in this or one of the other pseudohomozygote lines the processes of segretation and recombination will enrich the stock, yielding a pure breeding strain that has high reproductive vigor.

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# A SECOND EVALUATION OF IRRADIATED MALE *CULEX TARSALIS* FOR MODIFICATION OF FIELD POPULATIONS

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## ABSTRACT

*Culex tarsalis* males were field-collected as pupae, radiosterilized with 6000 R from a $Co^{60}$ source, marked with fluorescent dust and released into a semi-isolated field population at a foothill site east of Bakersfield in Kern County, California. Analysis of recapture data showed the released males had an a average daily survival rate of 72% and mixed well with males from the native population. The use of field-collected males circumvented the loss of mating competitiveness observed in previous release experiments wich utilized males from laboratory colonies. The proportion of sterile rafts recovered from native females increased significantly during the release period, indicating the sterile males competed well with native males. Although 71,016 males were released between June 17 and August 28, 1980, this number was not sufficient to cause substantial reduction of the native population.

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# LABORATORY STUDY ON THE AGE-SPECIFIC SURVIVORSHIP OF *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE) FROM BORAX LAKE, CALIFORNIA

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## ABSTRACT

Laboratory experiments were conducted to determine the age-specific survivorship of female *Culicoides variipennis occidentalis* at constant and variable temperatures and photoperiods. Mean and maximum longevities were significantly greater for gnats held at variable 16.7/8.9°C, 11L:13D and constant 15.6°C, 14L:10D conditions compared to those at 26.7/18.3°C, 14L:10D and 32.2°C, 14L:10D respectively. Survivorship curves and partial life tables were compiled for each of the temperature and photoperiod conditions. The significant variation in gnat survival is discussed in the context of bluetongue virus epizootics.

INTRODUCTION.–Life tables of medically important insects have become a necessary tool in understanding the epidemiology of vector-borne diseases. In particular mosquito life tables for vectors of yellow fever, malaria and Japanese encephalitis have been much studied (Lansdowne and Hacker 1975, Chubachi 1979, Reisen et al. 1979, Reisen and Mahmood 1980). These investigations have contributed to both the understanding of the diseases and the effectiveness of control measures.

To date, no life tables exist for any *Culicoides* spp. of biting midge. In the United States, *C. variipennis* is the primary vector of bluetongue virus in ruminants (Foster et al. 1968). Epizootics of the virus appear most often in mid-fall. This raises the question of whether an association between seasonal occurrence of the virus and a pattern of vector survival exists.

The investigation examines and compares the variation in survivorship of an adult population of *C. variipennis* at constant and seasonal temperatures and photoperiods. Results indicate significant differences in survival which may contribute to the seasonality of bluetongue virus epizootics.

MATERIALS AND METHODS.–Pupae of *Culicoides variipennis occidentalis* were collected from the margins of Borax Lake by removing sections of the mud habitat and swirling the samples in water-filled pans. Pupae which floated to the surface were poured through a series of 5-, 40- and 80-brass mesh sieves and returned to the laboratory.

In the laboratory pupae were transferred to moistened cotton-lined, enamel pans and placed in organdy-screened cages at room temperature until the adults emerged. Because of the large numbers of pupae collected, only those adults which emerged between 0-24 hours where used for the experiment.

To reduce the chance of injury, gnats were collected with a mouth aspirator and transferred directly to 0.5 litre cardboard containers with organdy-screened tops. Two overlaid 5x5 cm pieces of rubber dam (center cut) were installed for access into the containers. Each experiment consisted of 8 replicate cohorts, with a mean and standard deviation of 55.3 ± 7.6 gnats.

Each container had resting on its screen a cotton wick, extending from a 50 ml beaker of 10% sucrose and a cotton pledget moistened in tap water. The pledgets were remoistened every 24 hours and the wicks replaced whenever sucrose was added to the beaker. For the four experiments, containers were kept in environators at temperature and photoperiod settings of: constant 32.2° ± 1.0°C, 14L:10D, 15.6° ± 2.5°C, 14L:10D; and variable 26.7/18.3° ± 1.0°C, 14L:10D; and 16.7/8.9° ± 2.5°C, 11L:13D. Variable temperature cohorts were used in an attempt to simulate seasonal conditions.

Containers were checked every 24 hours and dead adults were removed, counted and sexed. Mortality values were averaged and used to determine survivorship curves. Life table estimates (Southwood 1978) for each experiment determined the following population parameters: $l_x$, the number of adults surviving to age x (corrected for one-thousand); $d_x$, the number of adults dying during age x; and $e_x$, the expectation of life remainging for individuals of age x.

Student t-test was used to compare survivorship characteristics.

RESULTS.–Results for these experiments are shown in Figures 1 and 2 and Tables 1 through 4. Table 1 compares the age-specific survivorship of females held at variable temperatures and photoperiods. The life expectancy on day one ($e_1$) is 1.6 times greater for gnats incubated at 16.7/8.9° Gnat survival ($l_x$) under these conditions totaled 77 days compared to 41 days at 26.7/18.3°C (Figure 1).

Similar results are seen with gnats held at constant temperatures and photoperiods (Table 2). Survivorship becomes great-

ly reduced at the warmer temperature with gnats incubated at 32.2°C surviving 25 days in comparison to 73 days at 15.6°C (Figure 2). Newly-emerged females ($e_1$) have a life expectancy 4.1 times longer at the constant cooler temperature (Table 2).

Tables 3 and 4 show the life table characteristics for *C. variipennis*. Female longevity and maximum longevity were significantly greater for gnats incubated at the cooler variable (Table 3) and the constant (Table 4) temperatures. Mean daily survival for females reared in 15.6°C was significantly greater than those at 32.2°C. No significant variation in daily survival was observed at variable temperatures.

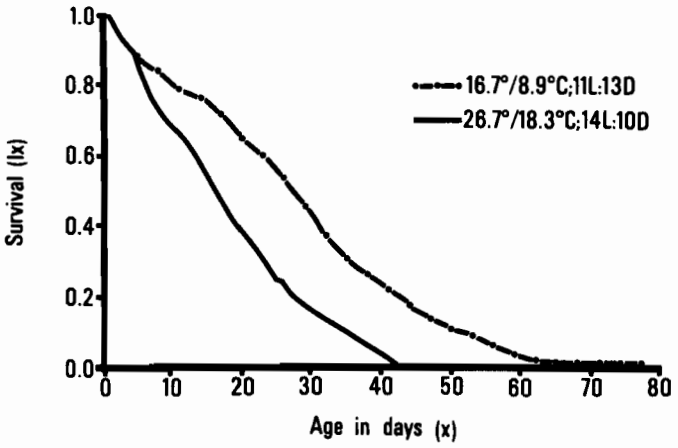


**Figure 1.** Age-specific survivorship curves for female *Culicoides variipennis* held at variable temperatures and photoperiods.

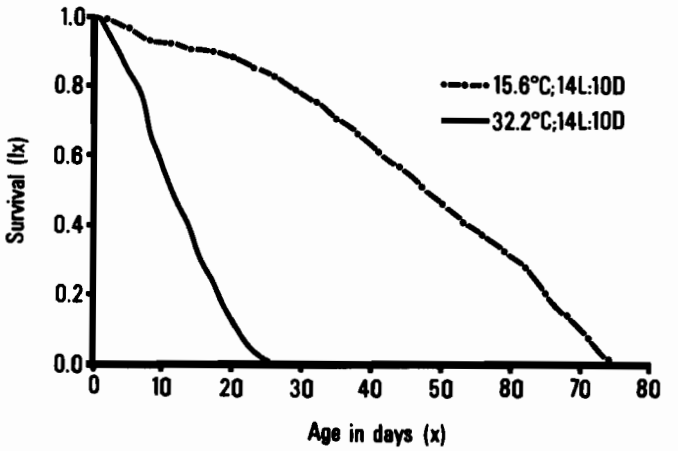


**Figure 2.** Age-specific survivorship curves for female *Culicoides variipennis* held at constant temperatures and photoperiods.

**Table 1.** Comparison of age-specific survivorship of female *Culicoides variipennis* held at 16.7/8.9°C, 11L:13D and 26.7/18.3°C, 14L:10D.

| x* | 16.7/8.9°C** | | | 26.7/18.3°C** | | |
|---|---|---|---|---|---|---|
| | $l_x$ | $d_x$ | $e_x$ | $l_x$ | $d_x$ | $e_x$ |
| 1 | 1000 | 55 | 26.7 | 1000 | 57 | 16.7 |
| 3 | 919 | 31 | 26.9 | 929 | 34 | 15.9 |
| 5 | 878 | 15 | 26.2 | 854 | 54 | 15.2 |
| 7 | 850 | 7 | 25.0 | 755 | 27 | 15.0 |
| 9 | 822 | 13 | 23.8 | 698 | 14 | 14.2 |
| 11 | 790 | 12 | 22.7 | 663 | 18 | 12.9 |
| 13 | 774 | 8 | 21.2 | 609 | 29 | 11.9 |
| 15 | 756 | 18 | 19.7 | 539 | 27 | 11.3 |
| 17 | 718 | 20 | 18.7 | 475 | 27 | 10.7 |
| 19 | 683 | 29 | 17.6 | 412 | 21 | 10.2 |
| 21 | 634 | 13 | 16.9 | 361 | 23 | 9.5 |
| 23 | 606 | 22 | 15.6 | 311 | 27 | 8.8 |
| 25 | 557 | 16 | 14.9 | 252 | 13 | 8.7 |
| 27 | 512 | 22 | 14.1 | 210 | 14 | 8.2 |
| 29 | 462 | 29 | 13.5 | 176 | 14 | 7.5 |
| 31 | 393 | 17 | 13.7 | 151 | 9 | 6.6 |
| 33 | 347 | 17 | 13.3 | 124 | 7 | 5.8 |
| 35 | 310 | 11 | 12.8 | 101 | 9 | 3.9 |
| 37 | 281 | 16 | 12.0 | 71 | 13 | 3.0 |
| 39 | 250 | 13 | 11.4 | 53 | 11 | 1.8 |
| 41 | 219 | 11 | 10.8 | 29 | 29 | 0.5 |
| 43 | 190 | 13 | 10.3 | | | |
| 45 | 164 | 11 | 9.8 | | | |
| 47 | 141 | 13 | 9.2 | | | |
| 49 | 118 | 7 | 8.8 | | | |
| 51 | 107 | 7 | 7.7 | | | |
| 53 | 94 | 10 | 6.6 | | | |
| 55 | 76 | 9 | 5.9 | | | |
| 57 | 57 | 9 | 5.6 | | | |
| 59 | 41 | 3 | 5.5 | | | |
| 61 | 31 | 6 | 4.9 | | | |
| 63 | 17 | 3 | 6.1 | | | |
| 65 | 14 | 3 | 5.3 | | | |
| 67 | 10 | 0 | 5.2 | | | |
| 69 | 7 | 2 | 4.9 | | | |
| 71 | 5 | 0 | 4.6 | | | |
| 73 | 4 | 0 | 3.5 | | | |
| 75 | 3 | 1 | 2.0 | | | |
| 77 | 1 | 1 | 1.0 | | | |

* x = age interval in days
** $l_x$ = age-specific survivorship; $d_x$ = age-specific death rate; $e_x$ = life expectancy at age x

**Table 2.** Comparison of age-specific survivorship of female *Culicoides variipennis* held at 15.6°C and 32.2°C, 14L:10D.

| x* | 15.6°C** | | | 32.2°C | | |
|---|---|---|---|---|---|---|
| | $l_x$ | $d_x$ | $e_x$ | $l_x$ | $d_x$ | $e_x$ |
| 1 | 1000 | 8 | 44.7 | 1000 | 46 | 10.8 |
| 3 | 983 | 9 | 43.4 | 912 | 40 | 9.8 |
| 5 | 965 | 10 | 42.2 | 833 | 37 | 8.6 |
| 7 | 944 | 13 | 41.1 | 760 | 98 | 7.3 |
| 9 | 922 | 2 | 40.1 | 621 | 57 | 6.7 |
| 11 | 917 | 3 | 38.3 | 570 | 50 | 5.9 |
| 13 | 912 | 4 | 36.5 | 423 | 46 | 5.0 |
| 15 | 903 | 4 | 34.9 | 322 | 47 | 4.2 |
| 17 | 895 | 3 | 33.2 | 238 | 43 | 3.4 |
| 19 | 888 | 6 | 31.4 | 156 | 37 | 2.6 |
| 21 | 871 | 8 | 30.0 | 86 | 28 | 2.0 |
| 23 | 851 | 10 | 28.7 | 36 | 14 | 1.4 |
| 25 | 836 | 11 | 27.2 | 10 | 16 | 0.5 |
| 27 | 811 | 12 | 26.0 | | | |
| 29 | 786 | 12 | 24.8 | | | |
| 31 | 763 | 12 | 23.5 | | | |
| 33 | 738 | 19 | 22.3 | | | |
| 35 | 707 | 13 | 21.2 | | | |
| 37 | 679 | 16 | 20.0 | | | |
| 39 | 646 | 16 | 19.0 | | | |
| 41 | 610 | 18 | 18.1 | | | |
| 43 | 576 | 8 | 17.1 | | | |
| 45 | 551 | 18 | 15.8 | | | |
| 47 | 515 | 19 | 14.8 | | | |
| 49 | 478 | 14 | 13.9 | | | |
| 51 | 448 | 19 | 12.8 | | | |
| 53 | 415 | 9 | 11.7 | | | |
| 55 | 391 | 19 | 10.3 | | | |
| 57 | 355 | 13 | 9.3 | | | |
| 59 | 322 | 5 | 8.1 | | | |
| 61 | 302 | 22 | 6.6 | | | |
| 63 | 257 | 23 | 5.6 | | | |
| 65 | 205 | 23 | 4.7 | | | |
| 67 | 158 | 14 | 3.8 | | | |
| 69 | 120 | 18 | 2.7 | | | |
| 71 | 79 | 25 | 1.5 | | | |
| 73 | 26 | 26 | 0.5 | | | |

* x = age interval in days

** $l_x$ = age-specific survivorship; $d_x$ = age-specific death rate; $e_x$ = life expectancy at age x

**Table 3.** Life table characteristics (Mean ± 1 S.D.) of female *Culicoides variipennis* held at variable temperatures and photoperiods.

| | 16.7/8.9°C 11L:13D | 26.7/18.3°C 14L:10D |
|---|---|---|
| Longevity | 29.9 ± 2.1** | 16.7 ± 2.5 |
| Maximum longevity | 68.4 ± 6.6** | 29.2 ± 2.3 |
| Daily survival | 0.91 ± 0.17ns | 0.89 ± 0.15 |

**P<0.001

NS not significant at P>0.05

**Table 4.** Life table characteristics (Mean ± 1 S.D.) of female *Culicoides variipennis* held at constant temperatures and photoperiods.

| | 15.6°C 14L:10D | 32.2°C 14L:10D |
|---|---|---|
| Longevity | 47.7 ± 4.9** | 12.8 ± 3.4 |
| Maximum longevity | 69.9 ± 2.2** | 22.9 ± 3.3 |
| Daily survival | 0.94 ± 0.14** | 0.81 ± 0.21 |

**P<0.001

DISCUSSION.–Information on adult *Culicoides* survivorship is limited. Akey et al. (1978) compared the effects of larval density on survival of adult *C. variipennis*. Flies, reared at low larval density, had a mean survival of 5.89 ± 0.05 and a maximum life span of 8.08 ± 0.17 days. Comparisons between these results and ours cannot be made since mortality in Akey's experiment was due to intentional starvation rather than aging.

The increased survivorship at the cooler temperature may be biologically significant when compared to the seasonality of bluetongue virus epizootics. The disease in the United States is most frequently observed in late summer and early fall (Bowne 1971). With cooling seasonal temperature increasing gnat longevity (as shown experimentally), the probability of virus transmission should increase. Jones and Foster (1971) have shown that the infection rate in gnats increases with repeated (up to three) blood meals infective for bluetongue. If all other variables are considered equal, then during fall when survivorship is greatest the probability of epizootics should be near their peak due to the presence of longer lived populations with potentially higher infection rates.

Survival under the conditions in our experiment would have to be considered approaching the optimum possible for these gnats. Under field conditions, mean longevities would be less than what was determined in this experiment. Foster and Jones (1979) have shown that the extrinsic incubation period of bluetongue virus is 14 days at 23°C. Survival to the age when the gnat is capable of transmitting the virus may not be possible during warmer seasonal temperatures when gnat longevity is so reduced.

Survivorship and fecundity studies of these gnats under field conditions are needed to confirm the laboratory study and to determine a realistic adult life budget. In addition, immature survivorship studies should be estimated to assess the effectiveness of possible control measures.

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# PANEL DISCUSSION OF COMPUTERS IN MOSQUITO CONTROL

## INTRODUCTORY REMARKS

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Mosquito control is a business. To be sure, it is government related, but it most definitely has a product--the health and welfare of the people it serves.

To accomplish the end to which we are entrusted, tools are needed. We are all acquainted with these tools--pickups, jeeps, aircraft, sprays, foggers, etc. The specific type of tool is selected by each agency based upon conditions, resultant needs, and economics of the area; therefore, there are a variety of tool (equipment) combinations from agency to agency.

Record-keeping is an integral part of our business and, depending upon the size and number of the field problems, the programs outlined by the district's management staff, the imaginative problem solving techniques, etc., one may find as many record-keeping, storing and retrieval systems in California mosquito control as there are districts.

Gathering information in the field is relatively simple. We all have personnel trained to do this. Storing this information in raw form is simple if the agency is small and storage area large. In time, however, needed space will become a problem. Retrieval of this information into a usable form for management decision purposes requires so many man hours that only certain bits and pieces of data are summarized and used. The rest are stored and/or destined for oblivion.

There is a tool -- a much maligned tool -- now being used in three MADs which can aid in making available more information more rapidly to management -- the computer. This is what we are to discuss in the next hour or so.

Once an information storage-retrieval program has been set up there is almost no limit on how this information can be of immediate use to the District. I wish to emphasize--the word *immediate*. What would take 8 to 20 hours to compile and summarize can be done in a few minutes with the computer. Much information is presently stored in MADs which is too difficult and too tedious to extract, but the computer can do it in a flash without tiring.

About saving storage space -- two or three ten inch discs can store all the raw data that my district's control technicians bring in from the field in one year. It presently takes a 12" X 16" X 10" carton to store this same material, and FWMAD is a small agency!!

Each of the next three speakers will discuss different aspects involved in the operations of a computer. Fred Roberts is to review what is necessary to do in your agency to make a computer really valuable to you. Mark Dawson is to discuss the various types of computers and how to choose one to fit your needs as well as how to make the computer work for you or for the program. Don Rohe is to review one of the important and necessary factors required to make one computer communicate with another in an understandable form--a universal code.

# WHY MOSQUITO CONTROL AGENCIES SHOULD CONSIDER AUTOMATED DATA PROCESSING

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There are a number of very good reasons why mosquito control agencies would find computers beneficial to their operations. Mosquito control agencies generally process large amounts of biological, operational and administrative data. The information is usually hand processed by one or two members of the clerical staff. Computers would handle these large amounts of information rapidly and efficiently.

Mosquito control agencies also look at data in many different ways. For example, it may be necessary to collect information about the species of mosquitoes at a particular type of source, or the number of man-hours spent at a particular source, or the man-hours spent on a particular species. A computer can be programed to provide the data in any variety of combinations.

The computer is also an extremely useful tool in mosquito control because of the complex nature of mosquito problems. Effective mosquito control in California usually requires knowledge of the life history of a number of species of mosquitoes and their numeric response to a variety of environmental parameters. The complexity is further increased by the necessity to consider the potential environmental impact of control methods and potential insecticide resistance problems. The computer offers the opportunity to have data immediately available and to process it appropriately to provide pertinent and timely information to support complex mosquito control decisions.

One of the strongest arguments for computers in mosquito control is that our problems are generally of a cyclic nature and the logic patterns re-occur. As biologists we spend much of our time learning about these cyclic patterns so that we can effect control in the future. The computer can provide us with the opportunity to simulate these cyclic phenomena. The result can be better predictions concerning the levels of mosquito populations and better decisions concerning the need and/or type of control.

The computer can be a helpful tool because mosquito control often requires accurate solutions. For example, the chemical equipment needs to be properly calibrated in order to assure that proper amounts of insecticide are being applied. The computer committee of the California Mosquito and Vector Control Association has developed a program to calibrate spray equipment for mosquito control agencies. The program eliminates the need for laborious and difficult mathematical calculations by the technician and provides immediate, accurate results.

Finally, a strong argument can be made that computers should be utilized in mosquito control because they are inexpensive and highly reliable. Just a few years ago the cost was prohibitive for most small agencies. Today, a number of highly reliable micro-computer systems are available that would meet the needs of most mosquito control agencies and would cost only a few thousand dollars.

COMPUTER APPLICATIONS IN MOSQUITO CONTROL.—If you are not yet convinced that mosquito control agencies could benefit by automated data processing, let me give some specific examples of how computers can be used in an agency. First the various kinds of data can be stored on disks or other medium. Biological records, operational data, weather data, literature and accounting information can all be stored on disks, increasing its accessibility and saving voluminous space compared to paper files. These "raw data files" can then be updated by computer programs to develop "2nd generation data files" that can be updated routinely as raw data is collected and processed. An example of a 2nd generation data file would be a file of mosquito sources that would be constantly updated to reflect the costs of mosquito control for any particular source.

The computer can also be used to generate reports. The stored data can be processed efficiently and accurately to create reports for the trustees, manager, entomologists or technicians. These reports can be particularly effective if they put current field information into the hands of employees who must make critical control decisions.

Computers can also be used to transmit data from one location to another by telephone. If an agency has more than one division, the computer can increase the efficiency of information flow. The data that is transmitted may be quantitative or textual in nature. For example, it may not be too far away that we will be reporting our light trap data to the state through a computer telephone system.

BENEFITS OF AUTOMATED DATA PROCESSING (ADP).—The computer should benefit mosquito control because better decisions can be made by employees to effect better control. Better decisions should be made because greater amounts of more accurate information can be processed faster. The result may also be a reduction of costs.

Another benefit provided by computers is the opportunity to provide administrative control. The programs that are developed for the computer can be designed to provide essential

information to supervisors, manager and entomologists concerning activities for all personnel. Decisions can then be made to adjust the employee effort to insure that goals and objectives are being met.

A final benefit that ADP can provide is to re-evaluate the logic of an agency's approach to mosquito control. The implementation of ADP in a mosquito control agency requires an analysis of information flow. The selection of the kinds of data that is necessary to make the various administrative and control decisions may well be a worthwhile re-evaluation resulting in major or minor modifications of the control program.

BEFORE YOU PURCHASE A COMPUTER.–If you are now ready to run out and buy the inexpensive miracle just described for your mosquito control agency, perhaps a few words of caution. A master plan should probably be developed to insure the efficient implementation of ADP. The potential applications of the computer could be listed, and the objectives defined for each application. Priorities could then be established for the various applications. It may also be prudent to involve as many employees as possible in the planning process.

# A PROPOSED STANDARD CMVCA ACTIVITY/TREATMENT INPUT CODE

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## ABSTRACT

The proposed standard CMVCA activity/treatment input code developed by the CMVCA Computer Committee is discussed. The code contains over 1283 coded items. The format, structure, and rationale used in the construction of the coding system is described.

In order to work with a computer, two needs must be met. First, the computer has to understand what we want done, and second, we have to understand what the computer is saying. Part of the charge received by the CMVCA Computer Committee was to develop a means of meeting these needs. The result is the Standard CMVCA Activity/Treatment Input Code. As with any coding system, this input code has two primary aims. One is to reduce useful data to parcels of manageable size so that they can be processed for storage and retrieval. The second is to provide a key which translates data into codes and then enables specific codes to be translated back into meaningful data statements.

This particular code document contains a great deal of material (39 pages) that cover a wide range of subjects, and so it is quite diverse in its structure and format (Table 1). Every code item has a numerical counterpart and all codes are made up of numbers only. This was done so that optical card readers could ultimately be used to put data into the memory of a computer in lieu of more time consuming keyborad input. The document contains more than 1283 coded items which are grouped under seven primary division headings. Table 2 shows the number of coded items in each division and the number of digits used to make up the code for each division.

With few exceptions, all categories, including category headings, have received code numbers. This was done to allow each user the flexibility to develop their own use codes which are as general or detailed as a specific agency's program requires. Choosing code numbers for each code item was largely arbitrary, but followed these guidelines:

1. Sufficient "space" should be left between code numbers to allow for future expanion and maintain the general alphabetical order of the listings.

2. Each code number should be constructed of one or more sub-codes relating to the category in which the item is listed. For example, in the five digit code for activity/action items, all physical control activity codes are found in the 40000 series, and all vegetation control items of that series are found within the 46 hundreds, and the soil sterilants within that series are prefixed with the number 463 leaving space for the listing of 99 potential compounds in that category.

**Table 1.** Contents of Standard CMVCA Activity/Treatment Input Code.

- I. Activity/Action:
  - A. Administration
  - B. Inspection
  - C. Preventive Planning
  - D. Alternate Control Actions
  - E. Physical Control
  - F. Biological Control
  - G. Chemical Control
  - H. Monitoring/Research
  - I. Support/Maintenance
- II. Source Type:
  - A. Agricultural
  - B. Domestic
  - C. Industrial/Commercial
  - D. Natural
  - E. Recreational
- III. Source Condition/Description:
- IV. Other Animal Species Observed:
  - A. Natural Control Organisms
  - B. Non-Target Organisms
  - C. Endangered Species
- V. Vector Findings:
  - A. Genus and Species/Description
  - B. Developmental Stage/Density
- VI. Measurement Method/Collection Method
  - A. Adults
  - B. Aquatic Larva
  - C. Eggs
- VII. Method of Application:
  - A. Aircraft
  - B. Boat/Hovercraft
  - C. Ground Rig
  - D. Hand
  - E. ULV

3. The code format for each division should allow for maximum flexibility of use, with special emphasis on eliminating the need for multiple line entries wherever possible. This is especially apparent in the structure of the coding format used in the developmental stage/density section under vector findings.

**Table 2.** Coded Items and Number of Digits Making up the Codes for Various Divisions of the CMVCA Input Code.

| | CODE DIVISION | CODED ITEMS | DIGITS |
|---|---|---|---|
| I. | Activity/Action | 351 | 5 |
| II. | Source Type | 333 | 4 |
| III. | Source Condition/Description | 70 | 6 |
| IV. | Other Animal Species Observed | 144 | 4 |
| V. | Vector Findings: | | |
| | A. Genus and Species/Description | 343 | 4 |
| | B. Developmental Stage/Density | | 7 |
| VI. | Measurement Method/Collection Method | 36 | 2 |
| VII. | Method of Application | 6 | 2 |

The above guidelines and the large number of items which were included, made it necessary to use large code numbers of four to seven digits in most code divisions. Although no code ever contains everything that everybody wants, most agencies would probably not need to use every item this code document contains. The size of this code system can be justified on the basis that since it represents a compilation of just about every code already used in California, every item is already important to somebody, making it important enough to be included in this document.

This code system can be viewed as a source of numbers that refer to specific things and actions. Its primary value is that it provides a means whereby the same number means the same thing to everybody, thereby greatly assisting the need for compatibility in data processing.

In actual use, the code document need not be used as as entire unit. The user can pick and choose those items which are useful to a particular program and use them in any desirable way. Also, the content of this code can be expanded, and suggested additions to the listing of coded items are invited by the committee. Copies of the complete standard activity/treatment input code will be available directly from CMVCA. Also, notices of additions to the code will be distributed as needed.

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

January 1980 - April 1981

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The success of the California Mosquito and Vector Control Association, Inc. (CMVCA) since its inception in 1930 is directly dependent upon the productivity of its various committees. Without exception, throughout the years the accomplisments of the committees have been outstanding. This year, the committees were very productive as shown by their following activities.

BUDGET AND EXECUTIVE.–This committee was assigned to develop the annual budget of the association, review expenditures, prepare an agenda for each quarterly meeting of the Board of Directors, and to coordinate and stimulate the CMVCA activities. The committee fulfilled its responsibilities admirably.

COMPUTER COMMITTEE.–This committee was established by the Board of Directors in 1980. It was assigned to review applicable computers and develop a coding system for recording field data on surveillance and suppression of vectors. A request was made to all agencies for background data for developing the coding system. Many meetings were held for the purpose of selecting a computer and studying the data submitted for the coding system. Progress reports to the Board of Directors indicate many achievements.

BIOLOGICAL CONTROL.–The long awaited "Fishes in California Mosquito Control" developed by the committee was published. The publication represents dedicated efforts by all committee members. Continuing projects studied are a non-fish biocontrol notebook and a directory of MAD facilities for rearing biocontrol agents.

CHEMICAL CONTROL.–Co-sponsored with the Division of Toxicology and Physiology, University of California at Riverside a Workshop on Insecticide Resistance. This workshop held in October was very successful as indicated by the number of persons attending. The committee continued their review and updating of promising new chemicals for mosquito control.

PHYSICAL CONTROL.–The committee critiqued a proposed Water Bank Program to establish a waterfowl area in the Sacramento Valley. This program was initiated and sponsored by the U. S. Soil Conservation Service and the California Waterfowl Association to increase waterfowl habitat in California. The committee also, provided valuable input to the Vector Biology and Control Section, California Department of Health Services toward this agency's development of a statewide Mosquito Prevention Criteria for Water Bank Programs.

COOPERATIVE PLANNING COMMITTEE.–As the name implies, this committee promotes inter-agency understanding and cooperation between the CMVCA and Federal, State, Regional and Local agencies by making the mosquito and all other vector control programs better known. To help achieve better understanding and cooperation, the committee co-sponsored with the Cooperative Extension, University of California, the Vector Biology and Control Section - California Department of Health Services, the California Department of Fish and Game, the California Department of Food and Agriculture, and the U. S. Fish and Wildlife Service the 1980 Conference on Wildlife - Mosquito Coordinated Management. This two day meeting was held in September in the City of Sacramento. As to the importance of this conference to all concerned agencies, there were well over 100 registered attendees. The last conference of this type was held in Yosemite National Park, California in October 1962. It was expressed by many attending the Sacramento Conference that this type of meeting should be held again in about 4 to 5 years.

ENTOMOLOGY COMMITTEE.–This committee sponsored its first Entomology Seminar in 1957 at the Davis Campus of the University of California. Since that year, the committee has continued to sponsor the Seminar at various locations throughout California. In 1980 and 1981 the committee co-sponsored the Seminar with the Biological Control Committee and the Society of Vector Ecologists. Both of these Seminars were very informative and had many participants.

ENVIRONMENT COMMITTEE.-The committee continued to review new Federal or State legislation and/or rules and regulations that may be imposed on mosquito and vector control agencies. Reviewed proposals by the U. S. Fish and Wildlife Service for establishing critical habitats for threatened beetle species and proposed salt marsh restoration projects in the San Francisco Bay Area.

EQUIPMENT COMMITTEE.–The committee is conducting a feasibility study of developing a Mosquitofish Handling and Transporting Equipment Manual. This Manual would describe the design, construction, and materials sources for such equipment. The committee is to report to the Board of Directors in 1981 on progress made concerning this project activity.

LEGISLATIVE COMMITTEE.–One of the many outstanding accomplishments was the guidance afforded by this committee in securing through the California Legislature,

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Senate Bill 382. This appropriation bill of $3,250,000 was administered by the Vector Biology and Control Section of the California Department of Health Services. Allocations were then made to all mosquito and vector control agencies who applied for funds. Revised the Legislative Helper for member agencies of the association. Initiated and recommended to the association was the formation of the California Conference of Managers of Vector Abatement Districts (CCMVAD). The formation of CCMVAD was unanimously passed by the Board of Directors. CCMVAD is the officially recognized affiliate to the California Conference of Local Health Officers (CCLHO). This recognition and affiliation allows the CCMVAD to more effectively and cooperatively work with CCLHO on matters of legislation, funding and delivery of governmental disease prevention services with state government. The committee also, reviewed many legislative bills having a potential impact on mosquito and vector control agencies. The committee recommendations to oppose or support the various bills was unanimously passed by the Board of Directors.

LOCAL ARRANGEMENTS AND PROGRAM COMMITTEES.–These two committees worked to select the site, provide local arrangements and develop the program for the 49th CMVCA Annual Conference, April 26-29 1981, Red Lion Motor Inn, Redding, California.

PUBLICATIONS COMMITTEE.–This committee produced the much anticipated Trustee Reference Manual. This publication was prepared for persons serving on Board of Trustees of mosquito or pest abatement and vector control agencies in California. The Manual provides basic orientation and reference material pertinent to the Trustee role of governing these agencies. Abundant accolades were received from Trustees and Managers on this fine Manual. Provided editorial comment on papers submitted for publication in the CMVCA Proceedings, and apprised the Board of Directors on the current status of all CMVCA publications.

RESEARCH COMMITTEE.–Critiqued many mosquito research proposals submitted by various researchers of the University of California. A tremendous amount of in depth study by each member of the committee on each proposal submitted was accomplished. This is an outstanding feat as observed by this writer on the voluminous research proposals submitted for the review and comment.

WAYS AND MEANS COMMITTEE.–Developed changes in CMVCA Bylaws which were approved by the Board of Directors. Implemented at the approval and direction of the Board of Directors of relocating the CMVCA business office from Visalia to Sacramento. Produced a reference summary on District Funding and Legal Opinions. The accomplishments of this committee were truly superb, because of the difficult tasks it was assigned.

The Board of Directors at its Quarterly Meeting in Anaheim, California established the Integrated Pest Management Committee. This committee is in the process of developing management programs in accordance with the defined IPM principles.