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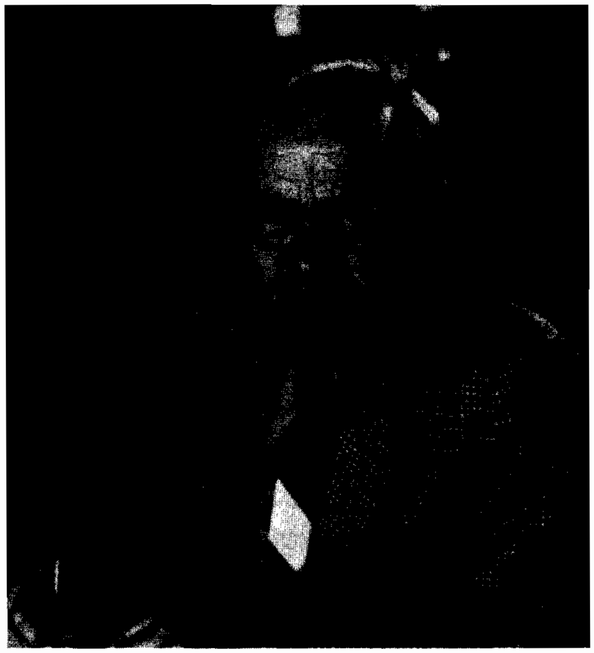
Dedicated To The Memory Of



JACK H. KIMBALL
1913-1983

On October 20, 1947, Jack H. Kimball was appointed District Manager of the Orange County Mosquito Abatement District, three months after the formation of the District. He was manager until March 30, 1974, over 26 years. He was a Sanitary Engineer by education and experience and brought this unique experience and an engineer's attention for precision and detail to these operations.

Jack served as President of the California Mosquito and Vector Control Association, Inc. (CMVCA) in 1950 and conceived, and had printed for 12 years, the CMVCA Yearbook. He set up a number of CMVCA Annual Conferences and two Joint National Conferences with the American Mosquito Control Association. The CMVCA elected Jack to Honorary Membership in 1976 and the AMCA awarded him a Meritorious Service Award in 1974.

Jack Kimball was widely recognized for his administrative and program planning abilities. He served as a consultant to the Malaria Eradication Division of the World Health Organization in Guatemala and Nicaragua and helped in preparation of the "Manual on Larval Control Operations in Malaria Programmes" in Geneva. The Orange County Mosquito Abatement District was reviewed by the State Health Department in 1972 and it concluded, "that certain elements of the program of the OCMAD must be rated outstanding and that all elements of the program are clearly above standard."

He was a native Californian, born in Burlingame on June 22, 1913. Jack graduated from the University of California, Berkeley in 1935 with a Bachelor of Science Degree in Civil Engineering. He used this education in working for the City of Palo Alto, California, State Bureau of Sanitary Engineering, and U.S. Public Health Service at which he attained the rank of Major.

Mr. Jack H. Kimball passed away on June 5, 1983.
–Gilbert L. Challet

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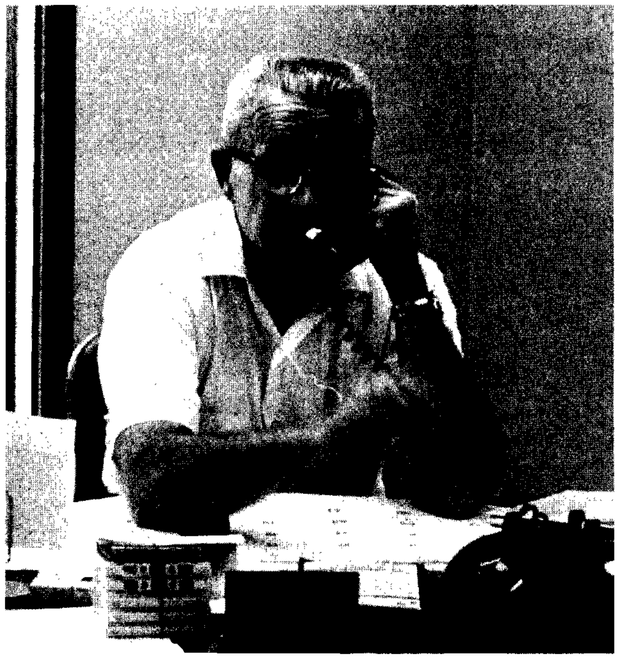

GORDON SMITH
1918-1983

Gordon Smith was graduated from the University of California, Berkeley, with a BS degree in entomology and then served two years with the 14th Malaria Control Detachment, U.S. Army, South Pacific during World War II.

In 1946 Gordon joined the Kern Mosquito Abatement District as District Entomologist. Gordon was widely recognized for his technical abilities in the development of pesticide programs and mosquito ecology studies. While at Kern Mosquito Abatement District, he was very active in the California Mosquito Control Association (CMCA) affairs, serving as chairman on the CMCA entomology and pesticide committees. During the 1950's he was instrumental in developing the need for a comprehensive mosquito research program and served as chairman of the CMCA mosquito research committee from 1963 to 1969; the committee had been formed in 1959 to implement the research effort.

In 1957 Gordon became manager of the Eastside Mosquito Abatement District and retired in 1973. He was President of CMCA in 1959 and was elected as an honorary member of CMVCA in 1977.–Earl W. Mortenson

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TABLE OF CONTENTS

PUBLIC HEALTH AND DISEASE

BIOLOGICAL CONTROL

BIOLOGICAL & ECOLOGICAL STUDIES

CHEMICAL CONTROL

GENERAL

California Mosquito and Vector Control Association, Inc.

Volume 51 — January 23-26, 1983

STUDIES TOWARDS THE MANAGEMENT OF ARBOVIRAL EPIDEMICS

I. OPERATIONAL ASPECTS AND INSECTICIDE SUSCEPTIBILITY DURING 1982[1]

G. Yoshimura[2], W.K. Reisen[3], M.M. Milby[3] and W.C. Reeves[3]

Mosquito abatement districts throughout California are responsible for keeping mosquito population levels below the threshold required for arbovirus transmission to man. The most common method employed is larval control, since it is the most cost effective approach when considering the area requiring treatment. Based on this strategy, previous aerial low-volume application evaluations in California were targeted against larvae in rice fields (Washino et al. 1972). Although a reduction in larval population density was demonstrated, no apparent reduction occurred in the post-treatment adult population.

When larvaciding methods fail, strategies for the containment of arbovirus outbreaks in urban/periurban areas emphasize the elimination of infected vectors through adulticide spraying (Breeland et al. 1980). This approach seems valid since during recent epidemics, the number of new human cases and the virus isolation rate from vector mosquito populations have declined coincidentally with aerial utra-low volume adulticiding in Canada (Mahdy et al. 1979) and Texas (Hopkins et al. 1975). The present paper reports on attempts to experimentally suppress adult populations using three replicate sprays at three day intervals over a 260 ha (one mi.²) study area.

Culex tarsalis Coquillett females egress from diurnal resting sites shortly after sunset with the peak periods of biting activity occurring within the first few hours thereafter (Nelson and Spadoni 1972). As our aircraft was not equipped for night flying, the adulticides were applied as close to sunset as possible, anticipating that some particles would remain in the treated area to contact the egressing mosquitoes. Dispersal studies concluded that populations may move up to one mile every three days during summer (Dow et al. 1975) and ultra-low volume adulticiding trials in Texas (Mitchell et al. 1970) noted that *Cx. tarsalis* populations recovered to pre-treatment levels after 48 hours. Thus we anticipated that 260 ha would be the minimum sized study area for which populations suppression could be documented prior to replacement by infiltration. The treatment area was later enlarged with the spray zone extended into the prevailing wind to counteract drift.

MATERIALS AND METHODS.—**Insecticides.** Insecticides were selected from those that were registered for adulticide use in California. As resistance to malathion was already known in Kern County through operational failures, it was decided that chlorpyrifos (Dursban® Mosquito Cold Fogging Concentrate) should be tried. Resistance to chlorpyrifos was encountered at the John Dale study site prompting a search for alternative compounds. A limited amount of bendiocarb (Ficam ULV®), a carbamate, became available in August and was applied once at John Dale by nonthermal aerosol generators. Following the bendiocarb trial, resmethrin (SBP-1382® MF"Z") was tested. The use of all three insecticides was approved by the local County Agricultural Commissioner as bendiocarb is not registered for mosquito control and both Dursban® Mosquito Cold Fogging Concentrate and resmethrin have no labels for aerial application in California.

Equipment and calibration. An Ayres Thrush Commander, used for applying chlorpyrifos and resmethrin, was equipped with 8 UniJet® No. 80005 flat fan nozzles pointed 90° down. The output rate of the nozzles was measured by using external ground spray equipment and the swath width was determined by using the bioassay method. *Cx. tarsalis* adults from the Breckenridge 1980 (*Br80*) colony were placed in sentinel cages (Townzen and Navtig 1973) that were spaced over a 230 m line that was set perpendicular to the prevailing wind direc-

[1]These studies were supported, in part, by Research Grant AI3028 from the National Institute of Allergy and Infectious Diseases, General Research Support Grant I-SOI-FR-0441 from the National Institutes of Health and by special state funds for mosquito control research appropriated annually by the California Legislature.

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tion. The aircraft sprayed the standard line into the wind at an altitude of ca. 9 m and at a speed of 193 kph. The spray pressure was 40 psi with an output rate of 1.65ℓ/min. Post-treatment mortality after 24-h was greater than 90% over a 65 m swath, yielding a calculated dosage rate of 56.1 g ai/ha.

The two Microgen ED2-20A cold foggers were calibrated with bendiocarb diluted at a 1:1 ratio with Klearol® (Witco Chemical Co.) and the output rate was measured by collecting the diluted insecticide in a graduated cylinder for one minute. Problems developed whereby the flow rate was much less than expected and the in-line filters were removed to allow the clay-based bendicarb formulation to flow. The first cold fogger was measured at a flow rate of 236 mℓ/min. and the second was measured at a flow rate of 252.2 mℓ/min.

Operational evaluation. Two types of sentinels, 1-3 day old *Br80* and target population mosquitoes of unknown age, were placed in bioassay cages in four annuli within the 260 ha study area to document insecticide coverage and efficacy under field conditions. Twelve standards were positioned within the spray zone and four comparison standards were located outside the spray zone border. Sentinel cages were also sequestered within the spray zone to ascertain spray penetration through vegetative canopy. Sequestered cages at Poso West were placed amongst tules and in pipe traps (Nelson 1980) which simulated animal burrows. At John Dale, cages were placed under trees and vegetative understory within the duck club.

The locations of the sentinel cages during the cold fogging trial was modified from those used in the aerial applications. Transect A, laid out in an E-W direction, bisected the northern half of the study site and consisted of 16 sentinel cages spaced over the 1.67 km length. Transect B, also laid out in an E-W direction, was located in the SW quadrant of the section and consisted of 10 sentinel cages spaced over 0.6 km. The N-S transect was located along the eastern border of the section and consisted of five sentinel cages spaced along 1.67 km. The four sequestered cages were placed amongst the trees within the duck club.

Susceptibility test methodology. Adult susceptibility was tested using the contact filter paper method (Georghiou and Gidden 1965). The adults from the *Br80* colony were 1-3 days old when tested, whereas adults from the target popula tion were of unknown age and were obtained using CDC traps. These females were sorted under chloroform and allowed to recover for a day before being tested.

RESULTS.–**Poso West - chlorpyrifos.** Poso West, an oil-field area situated in the Sierra Nevada foothills ca. 16 km north of Bakersfield, was chosen for study because the target population was semi-isolated, rarely pressured by insecticide spraying and the mosquito population has been intensively monitored since 1975. The application of chlorpyrifos was done on 3, 6 and 9 June. During each trial, the insecticide was applied at an altitude of 9-15 m due to the rolling terrain and power poles located within the spray area. A total of 22.7ℓ of chlorpyrifos was applied over 260 ha during trials 1 and 2 respectively, yielding a dosage rate of 62 g ai/ha on both trials. On trial 3, a total volume of 26.5ℓ was sprayed over 323 ha giving a dosage rate of 59 ai/ha.

Sentinel mortality results were corrected by using Abbott's formula and no significant difference in mortality was noted between the Poso West and *Br80* adults. Mortality of the Poso West sentinels set on standards was 98% and 95% during trials 1 and 2, respectively. No sentinels were exposed on trial 3, since too few adults were collected in the CDC traps. Mortality of the *Br80* sentinels was greater than 90% during the 3 consecutive trials.

The Poso West sequestered sentinel mortality during trial 2 was 32% for cages set within the pipe traps and 75% for cages set amongst the tules, with an average mortality of 50%. *Br80* sentinel mortality during trial 2 was 20% in the pipe traps and 63% in cages set within tules, for an average mortality of 42%. *Br80* mortality during trial 3 was 13% in pipe traps and 61% in tules, for an average mortality of 41%.

John Dale - chlorpyrifos. The John Dale study site, ca. 17 km south of Bakersfield, was situated on the floor of the San Joaquin Valley and consisted of peripheral mixed agriculture and a centrally situated duck club. This typical agro-ecosystem was selected for study because of the recurrent mosquito control problems that were consistently associated with Western equine encephalomyelitis virus activity. Chlorpyrifos was applied by air on 14, 17 and 21 July at an altitude which varied from 9 m over the crop land to ca. 30 m over the trees and houses. Trial 1 covered an area of 344 ha, whereas trials 2 and 3 covered an area of 349 ha. A total volume of 26.5ℓ of chlorpyrifos was used during each trial, yielding dosage rates of 55, 54 and 54 g ai/ha, respectively.

Sentinel mortality of the John Dale adults hung from standards decreased from a high of 53% in trial 1 to a low of 40% by trial 3, whereas mortality of the *Br80* sentinels remained greater than 88% throughout. A similar decrease in mortality was noted in the John Dale sequestered adults. Mortality declined from 36% on trial 1, to 9% on trial 3.

John Dale - bendiocarb. Bendiocarb was applied by two cold foggers on 17 August. The attempted swath width of the first cold fogger varied from 0.4 -0.6 km, while the swath of the second cold fogger varied from 0.4-0.8 km. The fogging commenced at 37 minutes after sunset. Both foggers traveled at a speed of 8 kph with a spray pressure of 5 psi. Air movement was minimal throughout the spray period with a maximum wind speed of 5 kph recorded at the termination of spraying. The wind direction, however, shifted from the prevailing WNW to the SE as the spraying progressed. Temperature readings taken at 3 and 9 m showed a maximum inversion of only 0.5 °C. The total volume of bendiocarb sprayed by the 2 foggers was 13.25ℓ, which yielded an average dosage rate of 10 g ai/ha.

The 12-h post-treatment mortality was 60% for John Dale and 56% for *Br80* sentinels at transect A. Mortality at transect B was 22% for John Dale and 14% for *Br80*. Mortality among the John Dale sentinels was 1% for cages under trees and 3% on the N-S transect. No mortality was observed for the *Br80* sentinels positioned at either location.

John Dale - resmethrin. The aerial application of resmethrin immediately followed the bendiocarb trial. Resmethrin was diluted in Klearol® at a 1:3 ratio and applied on 18, 21 and 24 August. A total of 364 ha was treated on each of the 3 trials. The spray altitude varied from 9-30 m as in the chlorpyrifos trials. A total volume of 8, 8.1 and 7.2ℓ of resmethrin was applied during each trial, yielding dosage rates of 8, 8 and 7 g ai/ha, respectively.

Sentinel mortality of the John Dale adults hung from standards declined from a high of 45% during trial 1 to a low of 26% during trial 3. John Dale sequestered sentinel mortality also declined from 23% to 2%, respectively. *Br80* sentinels exhibited a similar pattern with a 52% mortality on trial 1 decreasing to a 27% mortality by trial 3. Mortality among the sequestered *Br80* sentinels was 2% during trial 1 and 4% on trial 3.

Susceptibility status. The contact filter paper susceptibility tests of the *Cx. tarsalis* adults to chlorpyrifos, bendiocarb, resmethrin and resmethrin plus piperonyl butoxide in a 1:3 ratio gave the following pooled results. The chlorpyrifos LD_{50} value declined by 2X for Poso West adults collected during pre- and post-spray periods, whereas the John Dale adults were 16X more resistant than the *Br80* adults during the pre-spray tests and increased to 23X for the post-spray comparison.

Comparisons of the LD_{50} values to bendiocarb between the *Br80* and post-spray John Dale adults indicated that the John Dale strain is 3X more tolerant. However, with resmethrin, the *Br80* adults were 2.8-5.4X more resistant than the John Dale adults. The addition of piperonyl butoxide lowered the LD_{50} of the post-spray John Dale adults by 4.6X and the *Br80* LD_{50} by 7.9X when compared to the LD_{50} values obtained with resmethrin alone.

DISCUSSION.–Evaluation of the equipment used in the adulticiding trials demonstrated a clear preference for aerial application methods. The aircraft is able to obtain a more uniform coverage of the insecticide on the target zone. By comparison, the cold fogger requires optimum weather conditions for good insecticide coverage, is limited to the area that could be covered during a limited period of time, and is restricted to the type of terrain over which it could be used. The main drawback with our aircraft was that it was unable to fly at night during the peak flight activity period of the vector species.

The comparison of mortalities observed between the sentinel populations and the adult susceptibility tests indicated that there was a good correlation between the two methods of insecticide exposure. Although the *Br80* colony was slightly resistant to chlorpyrifos, it served as a reasonable base-line for comparisons with the native populations, except in the case of resmethrin.

The sequestered sentinels always exhibited a lower mortality than those hung from standards in the open. Presumably, mortality among sequestered sentinels was indicative of what was actually occurring in the field at the time of application. There was some exposure of resting adults to the insecticide, but effective penetration was too poor to suppress the population. Resistance of the John Dale adults to the insecticides tested further complicated attempted suppression.

Further studies are indicated to suggest insecticides useful for aerial application against *Cx. tarsalis* in intensive agricultural areas.

ACKNOWLEDGEMENTS.–The authors wish to thank the staff of the Arbovirus Field Station and the Kern Mosquito Abatement District for their assistance; Mr. K.W. Boyce, Delano Mosquito Abatement District, for his participation and loan of a Microgen cold fogger; and P.A. Gillies and C.M. Myers, California Department of Health Services, for providing the bendiocarb and participating in the cold fogging trial.

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STUDIES TOWARDS THE MANAGEMENT OF ARBOVIRAL EPIDEMICS

II. DYNAMICS AND AGE STRUCTURE OF THE TARGET POPULATION[1]

W.C. Reeves[2], W.K. Reisen[2], M.M. Milby[2], G. Yoshimura[3] and R.P. Meyer[2]

There is a need to develop strategies for effective programs to control epidemics of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE). Such programs can be initiated in anticipation of an epidemic or to interrupt an epidemic. In the event of an epidemic in California, the principal target of an emergency control program will be to kill the older adult female *Culex tarsalis* that are infected with virus and are, or soon will be, capable of transmitting infection to avian hosts, humans and equines. The previous paper (Yoshimura et al. 1983) described the background and operational aspects of a collaborative program developed to evaluate our current capacity to reduce the population of adult *Cx. tarsalis* to threshold levels below which viral infection in the basic cycle and effective transmission to human populations will not occur. The principal method evaluated was ULV application of insecticides by fixed wing, single engine aircraft as close as possible to dusk when adult vectors become active. The present paper describes the monitoring of the relative abundance, age structure and viral infection rates of the target population as compared with populations in adjacent untreated areas during the study period. This is a preliminary report and details will be presented in a subsequent publication.

METHODS.–The studies were carried out at two sites--Poso West, a semi-isolated non-agricultural foothill area 16 km N of Bakersfield, and a site on the valley floor (John Dale) with diversified agricultural development 21 km south of Bakersfield.

In each test, mosquitoes were sampled over a 23 day period and the adulticide applications were made on days 9, 12 and 15. Host-seeking females were collected by 12 CDC light traps augmented with 1-2 kg of dry ice that were located within the spray zone and four more traps positioned more than 0.8 km outside the perimeter to provide a comparison. The index of control was based on the ratio of the number of *Cx. tarsalis* females collected per trap night in the central traps (traps 1-4) and peripheral areas of the spray zone (traps 5-12) as compared to the surrounding zone (traps 13-16). Previous epidemiological analyses (Olson et. al 1979) had indicated that a weekly average of less than one *Cx. tarsalis* female per New Jersey light trap night would support WEE virus transmission in its basic cycle in rural California and that the seasonal index should be less than ten per trap night to prevent human disease. The vector levels required for SLE viral activity are slightly higher. Since CDC light traps augmented with dry ice generally collect three times or more the number of *Cx. tarsalis* females captured in New Jersey light traps (Milby et al. 1978), we considered that CDC light trap indices would have to be reduced to less than 30 females per trap night to assure that WEE virus would not be transmitted to a susceptible human population.

Resting mosquitoes were collected from the central spray zone concurrently with early morning light trap pickups. At Poso West where most mosquitoes rested in rodent burrows, mosquitoes were collected from five small red boxes, eight pipe traps and eight egress (cone) traps placed in the mouth of rodent burrows. At the John Dale site, where mosquitoes rested in vegetation, collections were made from natural shelters and along tree-row understory for timed periods using a battery powered aspirator. Additional specimens were collected from 8-10 cardboard box shelters placed in vegetation. Resting abundance was expressed as total specimens collected per day per sampling unit and thus was comparative within experiments.

Forty *Cx. tarsalis* females trapped from each of the three spray and comparison zones were dissected to determine parity status. Since autogeny seriously alters the parity status of the host-seeking population that is sampled by dry ice augmented light traps, the incidence of autogeny was estimated from samples of pupae collected from representative breeding sites within or adjacent to the spray zone. Fifty emerging females from each collection were offered 10% sucrose for 7-10 days and then dissected to determine the state of ovarian development.

Extensive breeding occurred at Poso West and immature abundance was monitored by taking 20 dips along each of three transects within the central spray zone. Similar sites could not be found at John Dale.

In each test, *Cx. tarsalis* adults from the spray zone and surrounding areas were marked with identifying fluorescent dusts and released in the center of the study areas four days and one day before the first insecticide application. The rate of recapture of surviving marked adults of known origin in the spray zone before and after adulticiding provided one measure of control success.

The incidence of arboviral infection in the target population was monitored by viral tests of at least ten pools of 50 *Cx. tarsalis* females each collected before and after spraying.

[1]These studies were supported in part by Research Grant AI3028 from the National Institute of Allergy and Infectious Diseases, General Research Support Grant I-S01-FR-0441 from the National Institues of Health and by special state funds for mosquito control research appropriated annually by the California Legislature.

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RESULTS.–Adequate spray coverage by all three adulticides resulted in a suppression of the ratio of adults in the central spray zone as compared with the unsprayed zone to less than one. The degree of suppression and the rate of decline was related to the insecticide susceptibility status of the target population. At Poso West, where the target population was susceptible to chlorpyrifos, the degree of control reached a ratio below 0.75 after the first spray; while at the John Dale site, where resistance to all three insecticides was encountered, repetitive applications were required to attain detectable suppression. In all trials, the suppression was extremely short-term and population indices rapidly recovered to pre-spray levels after spraying terminated. The transient nature of the suppression was especially evident at the John Dale site where the population index recovered appreciably the night following application.

The mean number of *Cx. tarsalis* collected per trap night in the central zone decreased significantly during the spray period in all trials. However, the trap counts never dropped below the average of 30 females per trap night desired to control viral activity. Suppression of the adult population in shelters paralleled the degree of suppression of the light trap indices. The reduction in resting populations persisted into the post-spray periods in all trials.

Parity rates remained high in all trials and did not decrease appreciably after spraying. The high parity rates in both areas reflected the high autogeny rates of 84% and 88 to 100% at Poso West and the John Dale site respectively.

Larval control was good at Poso West as the prespray counts of 50 larvae and two pupae per dip declined to one larva and no pupa per dip after the first spray and did not recover during the study period.

The recapture rates of dusted *Cx. tarsalis* adults reflected the insecticide resistance of the local population which had been marked and released. At Poso West few females were recaptured after the first spray, while the decline in the recapture rates was not accelerated from the expected survivorship curves (Nelson et al. 1978) during the bendiocarb/resmethrin trials at the valley site.

Despite the minimal level of control by the application of bendiocarb and resmethrin, the minimum WEE infection rate decreased from 10.7 to 2.1 infected *Cx. tarsalis* per 1,000 concurrently with the spray applications. The infection rates increased at the same time in nearby areas. There was no significant reduction in Hart Park and Turlock viral infection rates coincidental to the control program.

DISCUSSION.–The rapid recovery of light trap indices and consistently high parity rates could be explained by a rapid immigration into sprayed areas of host-seeking females that had recently oviposited autogenous eggs. Prior mark-release studies (Dow et. al 1965) and earlier ULV applications of malathion for adult *Cx. tarsalis* control in Texas in areas as large as 65 km^2 (Hayes et at. 1971; Mitchell et al. 1969; Mitchell et al. 1970) had indicated this could be a problem. It was hoped to overcome this problem in the present studies with three spray applications at three day intervals and the selection of the semi-isolated Poso West area as one study site. At Poso West, the target population was isolated by dry hills on three sides and all major breeding sites that could be found were located within the spray zone. Despite essentially a 100% kill of the larval population and an anticipated 98% kill of the resident adult population, female abundance in the central spray zone continued to range from 22 to 72 females per trap night. This is sufficient for the persistence of arboviral transmission. Future evaluation studies will have to be targeted at completely isolated populations or must encompass large enough areas to document population reduction and interruption of viral transmission prior to massive immigrations. It probably would add to the effectiveness of adulticiding programs if spray applications could begin 30 minutes after sunset when adult *Cx. tarsalis* begin their maximum flight activity. Unfortunately, presently available aircraft were not adaptable to this schedule.

The difficulties in documenting population suppression were compounded by an inability to demonstrate shifts in age structure of females collected in traps.

The previous paper (Yoshimura et al. 1983) gave details of the problems encountered with insecticide resistance at the John Dale site. If this *Cx. tarsalis* population is representative of the southern San Joaquin Valley it can be anticipated that interruption of arboviral transmission by adulticiding with any of the compounds tested will be marginally effective and that it will not be cost effective. In addition, it can be anticipated that repetitive adulticiding pressures will rapidly enhance the level of resistance of the population. It is encouraging that adulticiding may have reduced WEE viral activity in the present study.

In conclusion, the persistent high level of arboviral activity in vector populations in close proximity to centers of human population continues to pose a potential public health problem in California and resistance of *Cx. tarsalis* populations to all major groups of commercially available adulticides continues to be documented. It must be concluded that the establishment of effective protocols for management of WEE and SLE epidemics remains unresolved and must have a high priority in future research.

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SURVEILLANCE FOR ARTHROPOD-BORNE VIRAL ACTIVITY AND DISEASE IN CALIFORNIA DURING 1982

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This summary continues the series of annual reports to the California Mosquito and Vector Control Association and is the thirteenth annual report of the series since 1969.

During 1982, there were 210 patients suspected of having encephalitis who were tested for western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) at the California Department of Health Services' Viral and Rickettsial Disease Laboratory (VRDL) or at one of the six county public health laboratories which provide this testing service (Table 1.). In addition to the serologic diagnostic tests, 13 human brain samples were tested for arboviruses by inoculation into suckling mice.

One case of WEE was detected during the year: a 14 year old boy from San Bernardino County who became ill July 21 while in Florida. He was hospitalized July 23 in Ft. Lauderdale. Studies there ruled out Eastern equine encephalomyelitis (EEE), Venezuelan equine encephalomyelitis (VEE), SLE, California encephalitis (CE), dengue, and various other viruses, but no tests were done for WEE. The patient was transferred to San Bernardino County in late August and tests by the San Bernardino County public health laboratory showed a complement-fixing (CF) antibody titer to WEE of 1:64 (single blood). Subsequently, tests by the State VRDL, using serum samples collected in Florida July 24 and August 3, and in San Bernardino August 28, confirmed rising WEE antibody titers as follows: CF - <1:8 - 1:16 - 1:32; IFA - <1:8 - 1:2048 - ≥ 1:8192; and IFA/IGM - <1:8 - ≥1:8192 - 1:128. The boy had traveled extensively in the month prior to onset of illness: the Colorado River near Parker, Arizona; Ft. Worth, Texas; back to Big River, San Bernardino County on the California side of the Colorado River opposite Parker, Arizona; then to Miami and Ft. Lauderdale, Florida, where he became ill. It cannot be stated with certainly where infection was acquired, but the Colorado River area is the most probable site.

Twenty-five clincially suspect equine cases of WEE from 16 California counties and one Arizona county were tested serologically during the year. In addition, 12 equine brain samples were tested in suckling mice. Four cases were diagnosed as WEE, three from California, and one from Arizona: (1) a seven year old female quarterhorse from Fresno with onset June 14 of fever, posterior paresis lasting several days, and other signs typical of WEE, then complete recovery; the horse had not been vaccinated during the past two years, but prior to that had been vaccinated several times; the WEE CF antibody titer rose from 1:32 on June 14 to ≥1:64 on July 1, and indirect immunofluorescence antibody titers were 1:8 and

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Table 1. Number of humans tested serologically for WEE and SLE by county and month of onset, 1982.

COUNTY	TOTALS	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	UNK
Alameda	3					1	1				1			
Butte	8				3	1		1		1	1			1
Contra Costa	2			1			1							
El Dorado	13						1	3	4	2	3			
Fresno	8								1	5	2			
Humboldt	3	1					1	1						
Imperial	5				2	1	1	1						
Inyo	3			1		1		1						
Kern	8						1	2	1	1	1			2
Kings	3					1		1		1				
Lake	1				1									
Los Angeles	19					1	2	5	3	4	1	3		
Mendocino	1									1				
Merced	10							4	2	1		1		2
Napa	1								1					
Nevada	1							1						
Riverside	2				1		1							
San Benito	1				1									
San Bernardino	43					2	1	20	7	6	2	4		1
San Diego	29					3	6	3	7	3	3	4		
San Francisco	3							2		1				
San Joaquin	5							1	1	1	1	1		
San Luis Obispo	10			1	2	1	1	1	1	2				1
Santa Clara	3				1	1		1						
Santa Cruz	8			1		1	1	1	1		2			1
Shasta	4				1			1	1					1
Sierra	1					1								
Sutter	3					1		1	1					
Tehama	3								2	1				
Tulare	1						1							
Ventura	3				2						1			
Yolo	2								2					
TOTALS	210	1		4	14	16	19	51	35	30	18	13		9

>1:512, respectively; the horse had not been out of the area for two years; (2) a five month old colt from near Blythe, on the Colorado River Indian Reservation, Yuma County, Arizona, was ill June 20-22, but recovered uneventfully. The WEE CF antibody titer rose from 1:16 on June 24 to 1:256 on July 8, and the WEE IFA antibody titer rose from <1:32 to 1:128; (3) an eight year old mare from Blythe, Riverside County, with onset of illness August 3, had a high stationary titer (1:64) of WEE CF antibody and (1:512) of WEE-IFA antibody (sera taken August 16 and September 10); the horse recovered completely; there was no history of vaccination for at least two years; (4) a six month old unvaccinated colt from Bakersfield, Kern County, had onset September 7 of fever, depression and ataxia, and blood samples taken September 21 and October 5 showed rising WEE CF antibody titers of <1:8 to 1:32, and WEE IFA titers of <1:8 to 1:128. Recovery was complete.

A fifth horse from Ojai, northern Ventura County, which expired after an encephalitis-like illness, had a single serum sample tested which had WEE CF antibody titer of >1:64 and WEE IFA antibody titer of 1:512. However, the horse had a WEE booster vaccination in March, 1982, so the significance of the antibody titers is uncertain, and the case is not considered to be valid. An autopsy brain sample from this horse was inoculated into suckling mice but failed to yield virus. Several other suspect equine cases also had stationary or single sample WEE antibody titers which were considered most likely to be due to previous vaccination, so they also are not included as presumptive or confirmed cases.

Research continues in the VRDL to develop an indirect FA test for WEE IgM antibody in equines which will distinguish between current and past infections, analogous to the utility of such a test for diagnosing current human cases of various viral diseases.

Table 2. Number of mosquitoes and pools tested during 1982 by the Viral and Rickettsial Disease Laboratory Section by county and species.

County	*C. tarsalis* Mosquito	Pools	*C. pipiens* Mosquito	Pools	*C. peus* Mosquito	Pools	*A. melanimon* Mosquito	Pools	Other Species Mosquito	Pools	Total Mosquito	Total Pools
Butte	4,669	95					745	17			5,414	112
Colusa	1,568	32					27	1			1,595	33
Fresno	95	2									95	2
Glenn	1,170	24							15a	1	1,185	25
Imperial	6,675	153	734	23					50b	1	7,459	177
Kern	39,336	802	794	17			7,980	162			48,110	981
Kings	420	9									420	9
Los Angeles	96	2									96	2
Marin	884	19							300b	6	1,184	25
Merced	628	15	150	3			250	5			1,028	23
Orange	171	5	352	10							523	15
Placer	228	5									228	5
Riverside	12,071	248	3,926	80	2,260	49					18,257	377
Sacramento	5,073	113	243	6							5,316	119
San Bernardino	847	23	51	2							898	25
San Joaquin	628	13									628	13
Shasta	290	7									290	7
Stanislaus	1,521	36			19	1	71	2			1,611	39
Sutter	6,119	125									6,119	125
Tehama	241	6									241	6
Tulare	14,727	319	159	6			134	4			15,020	329
Ventura	310	7									310	7
Yolo	3,154	65									3,154	65
Yuba	3,265	68	30	1							3,295	69
Arizona	22	2									22	2
	104,208	2,195	6,439	148	2,279	50	9,207	191	365	8	122,498	2,592

[a]*Culex erythrothorax*
[b]*Aedes dorsalis*

The mosquito surveillance program was supported again in 1982, by the University of California School of Public Health, Arbovirus Research Unit, by assignment of Mr. Charles Cravens to assist in the VRDL. Mosquitoes were collected from 24 California counties and one Arizona county, and 122,498 mosquitoes in 2,592 pools were tested for virus (Table 2). Sampling was concentrated on *Culex tarsalis*, *Culex pipiens* complex, and *Aedes melanimon* (98% of the pools). There were 477 viral isolates from the mosquito pools: (Tables 3 - 5): 227 WEE, 109 Hart Park, 125 Turlock, and 16 California encephalitis group. As usual, most viral activity was in the southern half of the State. The low level of SLE virus activity was reflected in the failure to isolate any SLE viruses, and a low seroconversion rate in sentinel chickens.

Sentinel chicken flocks were located at 25 sites in the State (Table 6). Seroconversions for WEE antibody (IFA technique) were detected in 11 flocks. High seroconversion rates were concentrated in the southern half of the State. Only 3/24 (13%) of chickens in one flock in Imperial County seroconverted to SLE.

Individual reports of surveillance findings were made promptly by telephone, and a weekly surveillance bulletin (25 issues) was mailed to program participants. In addition, summaries of mosquito surveillance results tabulated according to Mosquito Abatement District boundaries were prepared semi-monthly by the California Department of Health Services' Center for Health Statistics and were distributed to those participating groups.

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Table 3. Number of viral isolates from mosquitoes tested during 1982 by the Viral and Rickettsial Disease Laboratory section by mosquito species, county, and agent isolated.

SPECIES	COUNTY	WEE	TURLOCK	CALIFORNIA GROUP	HART PARK	TOTAL
Cx. tarsalis	Butte		2		1	3
	Imperial	30	3			33
	Kern	83	81		82	246
	Kings	4	2			6
	Orange		1			1
	Riverside	7	7		3	17
	Sacramento		3			3
	San Bernardino	1	3			4
	Stanislaus		1		1	2
	Sutter		6		2	8
	Tulare	81	8		14	103
	Yolo		1		1	2
	Yuba		5		2	7
Cx. tarsalis		206	123	0	106	435
Cx. pipiens Compx	Imperial	4				4
	Kern	2				2
	Orange		1			1
	Riverside		1			1
Cx. pipiens Compx		6	2	0	0	8
Ae. melanimon	Kern	15		16	3	34
Ae. melanimon		15	0	16	3	34
		227	125	16	109	477

Table 4. Number of WEE isolates from mosquitoes tested during 1982 by the Viral and Rickettsial Disease Laboratory section by mosquito species, county, and month collected.

SPECIES	COUNTY	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	TOTAL	MIR/1000[a]
Cx. tarsalis	Imperial	2	28					30	4.49
	Kern			2	42	38	1	83	2.11
	Kings				4			4	9.52
	Riverside			1	6			7	0.58
	San Bernardino		1					1	1.18
	Tulare			8	58	15		81	5.50
Cx. tarsalis		2	29	11	110	53	1	206	
Cx. pipiens Compx	Imperial		4					4	5.45
	Kern				2			2	2.52
Cx. pipiens Compx		0	4	0	2	0	0	6	
Ae. melanimon	Kern				5	7	3	15	1.88
Ae. melanimon		0	0	0	5	7	3	15	
		2	33	11	117	60	4	227	

[a]Minimum infection rate/1000 mosquitoes.

Table 5.—Viral isolates from mosquito pools tested during 1982 by the Viral and Rickettsial Disease Laboratory Section.

Collected Month	Day	Pool Number	County	Place	Species	Mosq.	Agent(s)	Isolated
4	28	Chlv17	Riverside	Mecca	*Cx. tarsalis*	50	Turlock	.
5	11	Kern14	Kern	Old River	*Ae. melanimon*	50	Calif. Grp.	.
	13	Kern5036	Kern	Bakersfield	*Cx. tarsalis*	50	Hart Park	.
	18	Kern21	Kern	Oildale	" "	50	Turlock	.
	18	Kern22	Kern	Oildale	" "	50	Turlock	.
	18	Kern23	Kern	Oildale	" "	50	Turlock	.
	23	Impr46	Imperial	Yuma Test Statn.	" "	50	Turlock	.
	24	Impr22	Imperial	Heber	" "	33	WEE	.
	24	Impr163	Imperial	Calexico	" "	50	WEE	.
	25	Kern29	Kern	Wasco	" "	29	Hart Park	.
	25	Kern5057	Kern	Buttonwillow	*Ae. melanimon*	50	Hart Park	.
	25	Chlv28	Riverside	Mecca	*Cx. tarsalis*	50	Hart Park	.
	25	Chlv30	Riverside	Mecca	" "	50	Hart Park	.
	25	Chlv37	Riverside	Mecca	" "	50	Turlock	.
	25	Chlv49	Riverside	Mecca	" "	50	Turlock	.
	26	Kern5065	Kern	Bakersfield	" "	50	Turlock	.
6	1	Kern5071	Kern	Bakersfield	" "	50	Turlock	.
	1	Kern5072	Kern	Bakersfield	" "	50	Turlock	.
	1	Kern5076	Kern	Bakersfield	" "	50	Hart Park	.
	1	Kern5077	Kern	Bakersfield	" "	50	Hart Park	Turlock
	1	Kern5078	Kern	Bakersfield	" "	50	Hart Park	Turlock
	1	Kern5079	Kern	Bakersfield	" "	50	Turlock	.
	1	Kern5083	Kern	Delano	*Ae. melanimon*	50	Hart Park	.
	1	Kern5084	Kern	Delano	" "	50	Hart Park	.
	4	Impr11	Imperial	Heber	*Cx. tarsalis*	50	WEE	.
	4	Impr27	Imperial	Heber	" "	50	WEE	.
	4	Impr28	Imperial	Heber	" "	50	WEE	.
	4	Impr30	Imperial	Heber	" "	50	WEE	.
	4	Impr165	Imperial	Heber	" "	50	WEE	.
	4	Impr166	Imperial	Heber	" "	50	WEE	.
	4	Impr167	Imperial	Heber	" "	50	WEE	.
	4	Impr168	Imperial	Heber	" "	50	WEE	.
	4	Impr170	Imperial	Heber	" "	50	WEE	.
	4	Impr173	Imperial	Heber	" "	50	WEE	.
	7	Kern5116	Kern	Bakersfield	" "	50	Turlock	.
	7	Dlan8	Tulare	Teviston	" "	50	Hart Park	.
	7	Dlan9	Tulare	Teviston	" "	45	Hart Park	.
	7	Dlan10	Tulare	Earlimart	" "	45	Hart Park	.
	8	Kern32	Kern	Wasco	" "	50	Hart Park	.
	8	Kern44	Kern	Arvin	" "	50	Hart Park	.
	8	Kern45	Kern	Arvin	" "	50	Hart Park	.
	8	Kern49	Kern	Greenfield	" "	50	Hart Park	.
	8	Kern5123	Kern	Bakersfield	" "	50	Hart Park	Turlock
	8	Kern5119	Kern	Bakersfield	" "	50	Hart Park	.
	8	Kern5127	Kern	Bakersfield	" "	50	Hart Park	.
	8	Kern5128	Kern	Buttonwillow	" "	50	Hart Park	.
6	8	Kern5139	Kern	Delano	*Ae. melanimon*	50	Calif. Grp.	.
	14	Dlan11	Kern	Pond	*Cx. tarsalis*	50	Hart Park	.
	14	Dlan12	Kern	Pond	" "	50	Hart Park	.
	15	Kern55	Kern	Wasco	" "	50	Hart Park	.
	15	Kern60	Kern	Rosedale	" "	50	Turlock	.
	15	Kern62	Kern	Bakersfield	" "	50	Hart Park	.
	15	Kern63	Kern	Bakersfield	" "	50	Turlock	.
	15	Kern64	Kern	Bakersfield	" "	50	Hart Park	.
	15	Kern65	Kern	Bakersfield	" "	50	Hart Park	.
	15	Kern66	Kern	Bakersfield	" "	50	Hart Park	.
	15	Kern67	Kern	Bakersfield	" "	35	Turlock	.
	15	Kern5151	Kern	Bakersfield	" "	50	Turlock	.
	15	Trlk4	Stanislaus	Newman	" "	28	Turlock	.
	17	Kern5157	Kern	Bakersfield	" "	50	Hart Park	.
	17	Kern5159	Kern	Bakersfield	" "	50	Hart Park	Turlock
	17	Kern5160	Kern	Bakersfield	" "	50	Turlock	.
	17	Kern5161	Kern	Bakersfield	" "	50	Turlock	.
	17	Kern5165	Kern	Bakersfield	" "	50	Turlock	.
	17	Kern5166	Kern	Bakersfield	" "	50	Turlock	.
	17	Kern5169	Kern	Bakersfield	" "	50	Turlock	.
	17	Kern5171	Kern	Bakersfield	" "	50	Hart Park	.
	17	Kern5172	Kern	Bakersfield	" "	50	Hart Park	.

continued -

Table 5 - continued

Collected Month	Day	Pool Number	County	Place	Species	Mosq.	Agent(s)	Isolated
6	22	Impr50	Imperial	Calexico	*Cx. tarsalis*	30	WEE	.
	22	Impr60	Imperial	Calexico	" "	23	WEE	.
	22	Impr92	Imperial	Calapatria	" "	50	WEE	.
	22	Impr93	Imperial	Calapatria	" "	50	WEE	.
	22	Impr95	Imperial	Calapatria	" "	50	WEE	.
	22	Impr112	Imperial	Calapatria	" "	50	Turlock	.
	22	Impr66	Imperial	Heber	*Cx. pipiens* Compx	50	WEE	.
	22	Impr102	Imperial	Calapatria	" "	50	WEE	.
	22	Impr104	Imperial	Brawley	" "	50	WEE	.
	22	Kern5183	Kern	Bakersfield	*Cx. tarsalis*	50	Turlock	Hart Park
	22	Kern5184	Kern	Bakersfield	" "	50	Hart Park	.
	22	Kern5185	Kern	Bakersfield	" "	50	Turlock	.
	22	Kern5187	Kern	Bakersfield	" "	50	Turlock	.
	22	Kern5188	Kern	Bakersfield	" "	50	Turlock	.
	22	Kern5189	Kern	Bakersfield	" "	50	Turlock	.
	22	Kern5190	Kern	Bakersfield	" "	50	Hart Park	.
	22	Kern5191	Kern	Bakersfield	" "	50	Hart Park	.
	22	Kern5193	Kern	Bakersfield	" "	50	Turlock	.
	22	Kern5194	Kern	Bakersfield	" "	50	Turlock	.
	22	Kern5196	Kern	Bakersfield	" "	50	Hart Park	Turlock
	22	Kern5197	Kern	Bakersfield	" "	50	Hart Park	Turlock
	22	Kern5198	Kern	Bakersfield	" "	50	Turlock	.
	22	Sanb22	San Bernard	Needles	" "	50	Turlock	.
	22	Sanb23	San Bernard	Needles	" "	50	Turlock	.
	22	Sanb29	San Bernard	Needles	" "	34	WEE	.
	23	Impr82	Imperial	Palo Verde	" "	43	WEE	.
	24	Impr52	Imperial	Heber	" "	18	WEE	.
	24	Impr74	Imperial	Heber	" "	56	WEE	.
	24	Impr89	Imperial	Heber	" "	50	WEE	.
	24	Impr90	Imperial	Heber	" "	50	WEE	.
	24	Impr94	Imperial	Heber	" "	50	WEE	.
	24	Impr76	Imperial	Heber	" "	50	WEE	.
	24	Impr79	Imperial	Heber	" "	55	WEE	.
	24	Impr96	Imperial	Heber	" "	50	WEE	.
	24	Impr110	Imperial	Heber	" "	50	WEE	.
	24	Impr114	Imperial	Heber	" "	58	WEE	.
	26	Impr69	Imperial	Seely	" "	50	WEE	.
	26	Impr86	Imperial	Seely	" "	50	WEE	.
	26	Impr70	Imperial	Seely	*Cx. pipiens* Compx	49	WEE	.
	28	Dlan16	Tulare	Teviston	*Cx. tarsalis*	35	Hart Park	.
	29	Kern5212	Kern	Bakersfield	" "	50	Turlock	.
	29	Kern5213	Kern	Bakersfield	" "	50	Hart Park	.
	29	Kern5218	Kern	Bakersfield	" "	50	Hart Park	.
	29	Kern5220	Kern	Bakerfield	" "	50	Turlock	.
	29	Kern5222	Kern	Bakersfield	" "	50	Turlock	.
7	1	Kern5228	Kern	Bakersfield	" "	50	Turlock	.
	1	Kern5230	Kern	Bakersfield	" "	50	Turlock	.
	1	Kern5231	Kern	Bakersfield	" "	50	Turlock	Hart Park
	6	Kern5244	Kern	Bakersfield	" "	50	Turlock	Hart Park
	6	Kern5245	Kern	Bakersfield	" "	50	Turlock	.
	6	Kern5246	Kern	Bakersfield	" "	50	Turlock	Hart Park
	6	Kern5247	Kern	Bakersfield	" "	50	Turlock	.
	6	Kern5249	Kern	Arvin	" "	50	Hart Park	.
	6	Kern5250	Kern	Arvin	" "	50	Hart Park	.
	6	Kern5253	Kern	Arvin	" "	50	Hart Park	.
	6	Kern5254	Kern	Arvin	" "	50	Turlock	Hart Park
	6	Kern5260	Kern	Arvin	" "	50	Hart Park	.
	6	Kern5255	Kern	Arvin	*Ae. melanimon*	50	Calif. Grp.	.
	6	Suya5277	Sutter	Yuba City	*Cx. tarsalis*	50	Turlock	.
	7	Chlv61	Riverside	Mecca	" "	50	Turlock	.
	7	Chlv63	Riverside	Mecca	" "	50	WEE	.
	7	Suya27	Yuba	Marysville	" "	50	Turlock	.

Table 5 - continued

Collected Month	Day	Pool Number	County	Place	Species	Mosq.	Agent(s)	Isolated
7	7	Suya34	Yuba	Olivehurst	*Cx. tarsalis*	50	Turlock	.
	10	Kern5284	Kern	Arvin	" "	50	Hart Park	.
	10	Kern5285	Kern	Arvin	" "	50	Hart Park	.
	10	Kern5286	Kern	Arvin	" "	50	Hart Park	.
	10	Kern5287	Kern	Arvin	" "	50	Hart Park	.
	10	Kern5288	Kern	Arvin	" "	50	Hart Park	.
	10	Kern5292	Kern	Arvin	" "	50	Hart Park	.
	11	Kern5295	Kern	Arvin	*Ae. melanimon*	50	Calif. Grp.	.
	12	Nwst75	Riverside	Norco	*Cx. tarsalis*	50	Hart Park	.
	12	Suya41	Sutter	Sutter	" "	50	Turlock	.
	12	Dlan18	Tulare	Teviston	" "	44	Hart Park	.
	12	Sacr14	Yolo	Knights Landing	" "	50	Turlock	.
	12	Sacr31	Yolo	Woodland	" "	50	Hart Park	.
	13	Kern69	Kern	Rosedale	" "	50	Hart Park	.
	13	Kern71	Kern	Rosedale	" "	50	Hart Park	.
	13	Kern72	Kern	Rosedale	" "	38	Turlock	.
	13	Kern81	Kern	Old River	" "	50	Hart Park	.
	13	Kern84	Kern	Old River	" "	50	Turlock	.
	13	Kern5322	Kern	Delano	*Ae. melanimon*	50	Calif. Grp.	.
	13	Kern73	Kern	Rosedale	" "	50	Calif. Grp.	.
	13	Kern74	Kern	Rosedale	" "	50	Calif. Grp.	.
	13	Suya49	Yuba	Olivehurst	*Cx. tarsalis*	50	Turlock	.
	13	Suya57	Yuba	Marysville	" "	50	Turlock	.
	14	Kern5317	Kern	Buttonwillow	" "	50	Hart Park	.
	14	Kern5326	Kern	Bakersfield	" "	50	Hart Park	.
	14	Kern5329	Kern	Bakersfield	" "	50	Turlock	Hart Park
	14	Kern5331	Kern	Bakersfield	" "	50	Turlock	.
	14	Kern5332	Kern	Bakersfield	" "	50	Hart Park	.
	14	Kern5333	Kern	Bakersfield	" "	50	Turlock	.
	14	Kern5335	Kern	Bakersfield	" "	50	Turlock	.
	14	Kern5336	Kern	Bakersfield	" "	50	Turlock	.
	19	Dlan22	Kern	Pond	" "	37	Hart Park	.
	19	Sacr38	Sacramento	Franklin	" "	50	Turlock	.
	20	Kern5352	Kern	Buttonwillow	" "	50	Turlock	.
	20	Kern88	Kern	Old River	" "	50	Turlock	.
	20	Kern91	Kern	Old River	" "	50	Turlock	Hart Park
	20	Kern93	Kern	Greenfield	" "	50	Hart Park	.
	20	Kern94	Kern	Greenfield	" "	50	Hart Park	.
	20	Kern5357	Kern	Delano	*Ae. melanimon*	50	Calif. Grp.	.
	21	Kern5364	Kern	Bakersfield	*Cx. tarsalis*	50	Turlock	.
	21	Kern5367	Kern	Bakersfield	" "	50	Turlock	.
	21	Kern5368	Kern	Bakersfield	" "	50	Turlock	.
	22	Kern5370	Kern	Bakersfield	" "	50	Turlock	.
	24	Kern5371	Kern	Arvin	" "	50	Hart Park	.
	24	Kern5372	Kern	Arvin	" "	50	Hart Park	.
	24	Kern5373	Kern	Arvin	" "	50	Turlock	.
	24	Kern5374	Kern	Arvin	" "	50	Turlock	.
	24	Kern5375	Kern	Arvin	" "	50	Hart Park	.
	24	Kern5378	Kern	Arvin	" "	50	Hart Park	.
	24	Kern5379	Kern	Arvin	" "	50	Hart Park	.
	24	Kern5380	Kern	Arvin	" "	50	Hart Park	.
	24	Kern5382	Kern	Arvin	" "	50	Turlock	.
	24	Kern5383	Kern	Arvin	" "	50	Hart Park	.
	26	Sacr65	Sacramento	Rio Linda	" "	50	Turlock	.
	26	Suya80	Sutter	Live Oak	" "	50	Hart Park	.
	26	Dlan23	Tulare	Earlimart	" "	50	WEE	.
	26	Dlan24	Tulare	Earlimart	" "	50	WEE	.
	26	Dlan26	Tulare	Earlimart	" "	49	Hart Park	.
	26	Dlan29	Tulare	Earlimart	" "	50	WEE	.
	26	Dlan32	Tulare	Earlimart	" "	50	WEE	.
	26	Dlan34	Tulare	Teviston	" "	50	WEE	.
	26	Dlan35	Tulare	Teviston	" "	50	WEE	.
	26	Dlan36	Tulare	Teviston	" "	50	WEE	.
	26	Dlan37	Tulare	Teviston	" "	41	WEE	.
	27	Kern97	Kern	Old River	" "	50	Hart Park	.
	27	Kern99	Kern	Old River	" "	50	WEE	.
	27	Kern101	Kern	Rosedale	" "	50	Turlock	.
	27	Kern102	Kern	Rosedale	" "	50	Turlock	Hart Park
	27	Kern103	Kern	Rosedale	" "	50	Turlock	.
	27	Kern106	Kern	Rosedale	" "	50	Hart Park	.

Table 5 - continued

Collected Month	Day	Pool Number	County	Place	Species	Mosq.	Agent(s)	Isolated
7	27	Kern5395	Kern	Buttonwillow	*Cx. tarsalis*	50	WEE	.
	27	Kern5396	Kern	Buttonwillow	" "	50	Hart Park	.
	27	Orco2	Orange	Irvine	" "	50	Turlock	.
	27	Tlre9	Tulare	Corcoran	" "	50	Hart Park	.
	28	Kern5404	Kern	Bakersfield	" "	50	Hart Park	.
8	2	Sacr78	Sacramento	Elk Grove	" "	42	Turlock	.
	2	Dlan38	Tulare	Teviston	" "	50	Turlock	Hart Park
	2	Dlan39	Tulare	Teviston	" "	50	WEE	.
	2	Dlan40	Tulare	Teviston	" "	50	WEE	.
	2	Dlan41	Tulare	Teviston	" "	50	WEE	.
	2	Dlan42	Tulare	Teviston	" "	50	WEE	.
	2	Dlan43	Tulare	Teviston	" "	50	WEE	.
	2	Dlan44	Tulare	Teviston	" "	50	WEE	.
	2	Dlan45	Tulare	Teviston	" "	50	WEE	.
	2	Dlan46	Tulare	Teviston	" "	50	WEE	.
	2	Dlan48	Tulare	Earlimart	" "	50	WEE	.
	2	Dlan49	Tulare	Earlimart	" "	50	Hart Park	.
	2	Dlan56	Tulare	Earlimart	" "	50	Hart Park	.
	2	Dlan59	Tulare	Earlimart	" "	50	WEE	.
	2	Dlan61	Tulare	Earlimart	" "	50	WEE	.
	3	Kern108	Kern	Old River	" "	50	Hart Park	.
	3	Kern110	Kern	Old River	" "	50	Hart Park	.
	3	Kern 117	Kern	Arvin	" "	50	WEE	.
	3	Kern118	Kern	Arvin	" "	50	Turlock	.
	3	Kern120	Kern	Arvin	" "	50	WEE	.
	3	Kern122	Kern	Arvin	" "	50	Turlock	.
	3	Kern123	Kern	Arvin	" "	50	WEE	.
	3	Kern125	Kern	Arvin	" "	50	WEE	.
	3	Kern126	Kern	Arvin	" "	50	WEE	.
	3	Kern5410	Kern	Delano	" "	50	WEE	.
	3	Kern5421	Kern	Buttonwillow	" "	50	WEE	.
	3	Kern5422	Kern	Buttonwillow	" "	50	WEE	.
	3	Kern112	Kern	Old River	*Ae. melanimon*	50	Calif. Grp.	.
	3	Kern5412	Kern	Delano	" "	50	Calif. Grp.	.
	3	Kern5425	Kern	Buttonwillow	" "	50	WEE	.
	3	Kern5428	Kern	Buttonwillow	" "	50	WEE	.
	4	Chlv72	Riverside	Mecca	*Cx. tarsalis*	50	Turlock	.
	4	Chlv73	Riverside	Mecca	" "	50	WEE	.
	4	Chlv74	Riverside	Mecca	" "	50	WEE	.
	4	Chlv75	Riverside	Mecca	" "	50	WEE	.
	4	Chlv77	Riverside	Mecca	" "	50	Turlock	.
	4	Chlv80	Riverside	Mecca	" "	50	Turlock	.
	4	Chlv81	Riverside	Mecca	" "	50	WEE	.
	4	Chlv82	Riverside	Mecca	" "	50	WEE	.
	4	Chlv83	Riverside	Mecca	" "	35	WEE	.
	9	Suya119	Sutter	Yuba City	" "	50	Turlock	.
	9	Suya122	Sutter	Yuba City	" "	50	Turlock	.
	9	Dlan67	Tulare	Teviston	" "	50	WEE	.
	9	Dlan68	Tulare	Teviston	" "	50	WEE	.
	9	Dlan71	Tulare	Teviston	" "	50	WEE	.
	9	Dlan72	Tulare	Teviston	" "	43	WEE	.
	9	Dlan73	Tulare	Earlimart	" "	50	WEE	.
	9	Dlan74	Tulare	Earlimart	" "	50	WEE	.
	9	Dlan75	Tulare	Earlimart	" "	50	WEE	.
	9	Dlan76	Tulare	Earlimart	" "	50	WEE	.
	9	Dlan77	Tulare	Earlimart	" "	50	Turlock	.
	9	Dlan79	Tulare	Earlimart	" "	50	WEE	.
	9	Dlan92	Tulare	Teviston	" "	50	WEE	.
	10	Buco54	Butte	Nord	" "	50	Turlock	.
	10	Kern128	Kern	Wasco	" "	50	WEE	.
	10	Kern134	Kern	Arvin	" "	50	WEE	.
	10	Kern135	Kern	Arvin	" "	50	WEE	.
	10	Kern137	Kern	Arvin	" "	50	Hart Park	.
	10	Kern138	Kern	Arvin	" "	50	Hart Park	.
	10	Kern139	Kern	Arvin	" "	50	WEE	.
	10	Kern141	Kern	Arvin	" "	50	Hart Park	.
	10	Kern5495	Kern	Buttonwillow	" "	50	WEE	.
	10	Kern5497	Kern	Buttonwillow	" "	50	Turlock	.
	10	Kern5499	Kern	Buttonwillow	" "	50	WEE	.
	10	Kern5500	Kern	Buttonwillow	" "	50	WEE	.

Table 5 - continued

Collected Month	Day	Pool Number	County	Place	Species	Mosq.	Agent(s)	Isolated
8	10	Kern5501	Kern	Buttonwillow	*Ae. melanimon*	50	Calif. Grp.	.
	10	Kern5506	Kern	Buttonwillow	" "	50	Calif. Grp.	.
	10	Trlk23	Stanislaus	Patterson	*Cx. tarsalis*	18	Hart Park	.
	10	Tlre13	Tulare	Tulare	" "	50	Turlock	.
	10	Tlre14	Tulare	Tulare	" "	50	WEE	.
	10	Tlre15	Tulare	Tulare	" "	50	Turlock	Hart Park
	10	Tlre16	Tulare	Tulare	" "	43	WEE	.
	10	Suya131	Yuba	Marysville	" "	50	Turlock	.
	11	Kern5481	Kern	Arvin	" "	50	WEE	.
	11	Kern5483	Kern	Arvin	" "	50	WEE	.
	11	Kern5484	Kern	Arvin	" "	50	WEE	.
	11	Kern5485	Kern	Arvin	" "	50	Hart Park	.
	11	Kern5486	Kern	Arvin	" "	50	WEE	.
	11	Kern5487	Kern	Arvin	" "	50	WEE	.
	11	Kern5488	Kern	Arvin	" "	50	WEE	.
	12	Kern5471	Kern	Arvin	" "	50	Hart Park	.
	12	Kern5472	Kern	Arvin	" "	50	WEE	.
	12	Kern5474	Kern	Arvin	" "	50	Turlock	.
	12	Kern5475	Kern	Arvin	" "	50	WEE	.
	12	Kern5477	Kern	Arvin	" "	50	WEE	.
	12	Kern5478	Kern	Arvin	" "	50	WEE	.
	12	Kern5480	Kern	Arvin	" "	50	WEE	.
	12	Kern5515	Kern	Bakersfield	" "	50	Hart Park	.
	16	Dlan98	Tulare	Teviston	" "	50	WEE	.
	16	Dlan100	Tulare	Teviston	" "	50	WEE	.
	16	Dlan102	Tulare	Teviston	" "	50	WEE	.
	16	Dlan104	Tulare	Pixley	" "	50	WEE	.
	16	Dlan106	Tulare	Pixley	" "	50	WEE	.
	16	Dlan107	Tulare	Pixley	" "	50	WEE	.
	16	Dlan108	Tulare	Pixley	" "	50	WEE	.
	16	Dlan109	Tulare	Pixley	" "	50	Turlock	.
	16	Dlan115	Tulare	Teviston	" "	50	WEE	.
	16	Dlan116	Tulare	Teviston	" "	50	Turlock	.
	16	Dlan117	Tulare	Teviston	" "	50	Hart Park	.
	16	Dlan118	Tulare	Teviston	" "	50	WEE	.
	16	Dlan119	Tulare	Earlimart	" "	50	WEE	.
	16	Dlan122	Tulare	Earlimart	" "	25	WEE	.
	17	Kern142	Kern	Arvin	" "	50	WEE	.
	17	Kern143	Kern	Arvin	" "	50	Hart Park	.
	17	Kern144	Kern	Arvin	" "	50	WEE	.
	17	Kern146	Kern	Old River	" "	50	WEE	.
	17	Kern148	Kern	Old River	" "	50	WEE	.
	17	Kern150	Kern	Old River	" "	50	Hart Park	.
	17	Kern5518	Kern	Delano	" "	50	WEE	.
	17	Kern5526	Kern	Buttonwillow	" "	50	Turlock	.
	17	Kern5527	Kern	Buttonwillow	" "	50	WEE	.
	17	Kern5529	Kern	Buttonwillow	*Ae. melanimon*	50	WEE	.
	17	Tlre25	Tulare	Tulare	*Cx. tarsalis*	35	WEE	.
	17	Tlre28	Tulare	Tipton	" "	50	WEE	.
	17	Tlre29	Tulare	Tipton	" "	50	WEE	.
	17	Tlre30	Tulare	Tipton	" "	50	WEE	.
	17	Tlre31	Tulare	Tipton	" "	27	Turlock	.
	18	Kngs1	Kings	Lemoore Nas	" "	50	WEE	.
	18	Kngs2	Kings	Lemoore Nas	" "	50	Turlock	.
	18	Kngs4	Kings	Lemoore Nas	" "	50	WEE	.
	18	Kngs5	Kings	Lemoore Nas	" "	50	Turlock	.
	18	Kngs7	Kings	Lemoore Nas	" "	50	WEE	.
	18	Kngs8	Kings	Lemoore Nas	" "	50	WEE	.
	18	SanB37	San Bernard	Needles	" "	50	Turlock	.
	21	Kern5539	Kern	Arvin	*Cx. pipiens* Compx	50	WEE	.
	21	Kern5550	Kern	Arvin	*Ae. melanimon*	50	Calif. Grp.	.
	23	Suya135	Sutter	Sutter	*Cx. tarsalis*	50	Turlock	.
	23	Dlan124	Tulare	Earlimart	" "	50	WEE	.
	23	Dlan128	Tulare	Teviston	" "	50	WEE	.
	23	Dlan130	Tulare	Teviston	" "	50	WEE	.
	23	Dlan131	Tulare	Teviston	" "	50	WEE	.
	23	Dlan133	Tulare	Teviston	" "	50	WEE	.
	23	Dlan134	Tulare	Teviston	" "	50	WEE	.
	23	Dlan135	Tulare	Teviston	" "	50	WEE	.
	23	Dlan138	Tulare	Teviston	" "	50	WEE	.
	23	Dlan139	Tulare	Teviston	" "	12	Hart Park	.

Table 5 - continued

Collected Month	Day	Pool Number	County	Place	Species	Mosq.	Agent(s)	Isolated
8	23	Dlan149	Tulare	Pixley	“ ”	19	WEE	.
	23	Dlan143	Tulare	Teviston	“ ”	50	WEE	.
	23	Dlan151	Tulare	Pixley	“ ”	50	WEE	.
	24	Buco69	Butte	Gridleyer	“ ”	50	Turlock	.
	24	Kern151	Kern	Arvin	“ ”	50	Turlock	.
	24	Kern152	Kern	Arvin	*Cx. tarsalis*	50	WEE	.
	24	Kern154	Kern	Arvin	“ ”	50	WEE	.
	24	Kern158	Kern	Shafter	“ ”	50	Turlock	.
	24	Kern161	Kern	Shafter	“ ”	50	WEE	.
	24	Kern5566	Kern	Delano	“ ”	50	WEE	.
	24	Kern5567	Kern	Delano	“ ”	28	WEE	.
	24	Kern5570	Kern	Buttonwillow	“ ”	50	WEE	.
	24	Kern5571	Kern	Buttonwillow	*Ae. melanimon*	50	Calif. Grp.	.
	24	Kern5572	Kern	Buttonwillow	“ ”	28	WEE	.
	24	Suya150	Sutter	Yuba City	*Cx. tarsalis*	50	Turlock	Hart Park
	24	Tlre35	Tulare	Tipton	“ ”	50	WEE	.
	24	Tlre38	Tulare	Tulare	“ ”	50	WEE	.
	24	Tlre42	Tulare	Tulare	“ ”	50	WEE	.
	24	Tlre47	Tulare	Pixley	“ ”	50	WEE	.
	24	Tlre48	Tulare	Pixley	“ ”	50	WEE	.
	24	Suya149	Yuba	Marysville	“ ”	41	Hart Park	.
	26	Kern5577	Kern	Arvin	“ ”	50	Turlock	.
	26	Kern5590	Kern	Arvin	“ ”	50	Hart Park	.
	26	Kern5593	Kern	Arvin	“ ”	50	WEE	.
	26	Kern5594	Kern	Arvin	“ ”	50	WEE	.
	26	Kern5600	Kern	Arvin	“ ”	50	WEE	.
	26	Kern5574	Kern	Arvin	*Cx. pipiens* Compx	50	WEE	.
	30	Nwst169	Riverside	Norco	“ ”	50	Turlock	.
	30	Dlan164	Tulare	Earlimart	*Cx. tarsalis*	50	WEE	.
	30	Dlan167	Tulare	Teviston	“ ”	50	WEE	.
	30	Dlan170	Tulare	Teviston	“ ”	50	Hart Park	.
	30	Suya162	Yuba	Marysville	“ ”	42	Hart Park	.
	31	Kern164	Kern	Old River	“ ”	50	Turlock	.
	31	Kern167	Kern	Rosedale	“ ”	50	WEE	.
	31	Kern168	Kern	Rosedale	*Ae. melanimon*	50	WEE	.
	31	Tlre58	Tulare	Pixley	*Cx. tarsalis*	50	WEE	.
	31	Tlre64	Tulare	Pixley	“ ”	50	WEE	.
9	1	Impr145	Imperial	Seely	“ ”	26	Turlock	.
	1	Kern5621	Kern	Buttonwillow	“ ”	50	WEE	.
	1	Kern5623	Kern	Buttonwillow	“ ”	50	WEE	.
	1	Kern5625	Kern	Buttonwillow	“ ”	50	WEE	.
	1	Kern5629	Kern	Delano	“ ”	50	WEE	.
	1	Kern5626	Kern	Buttonwillow	*Ae. melanimon*	50	WEE	.
	1	Kern5627	Kern	Buttonwillow	“ ”	50	WEE	.
	1	Orco12	Orange	Irvine	*Cx. pipiens* Compx	30	Turlock	.
	7	Tlre73	Tulare	Tulare	*Cx. tarsalis*	50	WEE	.
	7	Dlan177	Tulare	Earlimart	“ ”	50	WEE	.
	7	Dlan178	Tulare	Earlimart	“ ”	50	WEE	.
	7	Dlan180	Tulare	Earlimart	“ ”	50	Turlock	.
	7	Dlan181	Tulare	Earlimart	“ ”	50	WEE	.
	7	Dlan182	Tulare	Earlimart	“ ”	50	WEE	.
	7	Dlan193	Tulare	Teviston	“ ”	50	WEE	.
	7	Dlan194	Tulare	Teviston	“ ”	50	WEE	.
	7	Dlan197	Tulare	Teviston	“ ”	50	WEE	.
	8	Buco91	Butte	Gridley	“ ”	50	Hart Park	.
	8	Kern170	Kern	Old River	“ ”	50	WEE	.
	8	Kern172	Kern	Rosedale	“ ”	50	WEE	.
	8	Kern177	Kern	Bakersfield	“ ”	50	Hart Park	.
	8	Kern175	Kern	Rosedale	*Ae. melanimon*	50	Calif. Grp.	.
	8	Kern176	Kern	Rosedale	“ ”	35	WEE	.
	9	Kern5650	Kern	Buttonwillow	*Cx. tarsalis*	50	WEE	.
	9	Kern5651	Kern	Buttonwillow	“ ”	50	WEE	.
	9	Kern5653	Kern	Buttonwillow	“ ”	50	WEE	.
	9	Kern5656	Kern	Delano	“ ”	50	WEE	.
	9	West2	Kern	Taft	“ ”	50	WEE	.
	9	Kern5654	Kern	Buttonwillow	*Ae. melanimon*	50	WEE	.
	9	Kern5655	Kern	Buttonwillow	“ ”	50	WEE	.
	13	Dlan200	Tulare	Earlimart	*Cx. tarsalis*	50	WEE	.
	13	Dlan217	Tulare	Teviston	“ ”	50	WEE	.

Table 5 - continued

Collected Month	Day	Pool Number	County	Place	Species	Mosq.	Agent(s)	Isolated
9	14	Kern181	Kern	Shafter	" "	50	Turlock	.
	14	Kern182	Kern	Shafter	" "	50	WEE	.
	14	Kern184	Kern	Shafter	" "	50	WEE	.
	14	Kern187	Kern	Shafter	" "	50	Turlock	.
	14	Kern188	Kern	Shafter	" "	50	WEE	.
	14	Kern195	Kern	Arvin	" "	50	WEE	.
	14	Kern196	Kern	Arvin	" "	50	WEE	.
	14	Tlre94	Tulare	Tulare	" "	50	WEE	.
	14	Tlre96	Tulare	Tulare	*Cx. tarsalis*	27	WEE	.
	15	Kern5667	Kern	Delano	" "	32	WEE	.
	15	Kern5670	Kern	Buttonwillow	" "	50	WEE	.
	15	Kern5671	Kern	Buttonwillow	" "	50	WEE	.
	15	Kern5672	Kern	Buttonwillow	" "	50	Turlock	.
	15	Kern5676	Kern	Buttonwillow	" "	50	WEE	.
	15	Kern5678	Kern	Buttonwillow	*Ae. melanimon*	50	WEE	.
	15	Kern5679	Kern	Buttonwillow	" "	50	WEE	Calif. Grp.
	20	Dlan229	Kern	Delano	*Cx. tarsalis*	30	WEE	.
	20	Kern221	Kern	Cawelo	" "	50	WEE	.
	20	Kern226	Kern	Cawelo	" "	50	WEE	.
	20	Kern227	Kern	Cawelo	" "	50	WEE	.
	20	Kern228	Kern	Cawelo	" "	50	WEE	.
	20	Dlan224	Tulare	Teviston	" "	50	WEE	.
	20	Dlan225	Tulare	Teviston	" "	50	WEE	.
	21	Kern206	Tulare	Arvin	" "	50	Turlock	.
	21	Kern208	Kern	Arvin	" "	50	Tulrock	.
	21	Kern217	Kern	Wasco	" "	50	WEE	.
	21	Tlre105	Tulare	Tulare	" "	24	WEE	.
	22	Kern5691	Kern	Buttonwillow	" "	50	WEE	.
	22	Kern5693	Kern	Buttonwillow	" "	50	WEE	.
	22	Kern5695	Kern	Buttonwillow	" "	50	WEE	.
	22	Kern5696	Kern	Buttonwillow	" "	50	WEE	.
	22	Kern5697	Kern	Buttonwillow	" "	50	WEE	.
	22	Kern5700	Kern	Buttonwillow	" "	50	Turlock	.
	22	Kern5701	Kern	Delano	" "	50	WEE	.
	22	Kern5703	Kern	Bakersfield	" "	50	WEE	.
	24	Kern230	Kern	Cawelo	" "	50	WEE	.
	24	Kern232	Kern	Cawelo	" "	50	Turlock	.
	24	Kern233	Kern	Cawelo	" "	50	WEE	.
	24	Kern237	Kern	Cawelo	" "	50	Turlock	.
	27	Kern5706	Kern	Bakersfield	" "	50	WEE	.
	28	Kern5720	Kern	Buttonwillow	" "	50	WEE	.
	28	Kern5721	Kern	Buttonwillow	" "	50	WEE	.
10	6	Kern5738	Kern	Buttonwillow	" "	50	Turlock	Hart Park
	6	Kern5740	Kern	Buttonwillow	" "	50	Turlock	.
	6	Kern5741	Kern	Buttonwillow	" "	50	WEE	.
	6	Kern5747	Kern	Buttonwillow	*Ae. melanimon*	50	WEE	.
	6	Kern5748	Kern	Buttonwillow	" "	50	WEE	.
	6	Kern5750	Kern	Buttonwillow	" "	50	WEE	.

Table 6. Serological conversions to WEE and SLE viruses in sentinel chickens, California, 1982.*

COUNTY	LOCATION	NO.(%) POSITIVE			♀♀*CX.TARSALIS* PER TRAP NIGHT	
		WEE	SLE	DATE 1st POSITIVE	SEASON AVERAGE	HIGHEST (date)
Shasta	MAD Office	0	0		1.7	10.3(8-4)
Tehama	MAD Office	1(4)	0	8-31	8.1	27.4(8-27)
Butte	Chico	0	0		0.7	3.3(8-3)
Butte	Grey Lodge	0	0		47.9	283.4(9-28)
Glenn	MAD Office	0	0		21.1	91.4(7-29)
Yuba	Marysville	0	0		2.8	16.0(7-28)
Sutter	Dean's	0	0		49.8	256.0(7-14)
Sacramento	Elk Grove	0	0		6.1	32.6(8-25)
San Joaquin	Escalon	0	0		6.9	20.9(8-25)
Turlock	Sportsman's Club	0	0		15.0	71.6(5-27)
Merced	Pereira Dairy	0	0		15.8	77.7(7-29)
Fresno	Lost Lake	0	0		0.7	3.1(9-9)
Fresno	Mendota Refuge	13(93)	0	9-2	36.9	147.1(9-1)
Kings	Riverview Ranch	19(79)	0	8-5	29.9	353.9(7-20)
Tulare	Rocky Hill	0	0		0.7	2.6(8-12)
Kern	Teviston	12(52)	0	8-3	1.7	16.9(7-14)
Kern	Wasco	11(46)	0	8-30	0.4	2.5(7-14)
Kern	Oildale	3(14)	0	10-4	0.2	1.0(9-22)
Kern	F.C. Tracy	18(90)	0	8-30	5.7	16.0(6-16)
Kern	Buttonwillow	16(94)	0	8-30	2.3	15.5(6-3)
Kern	John Dale	19(95)	0	8-2	26.7	126.0(6-23)
Riverside	Corona	0	0		4.1	17.1(10-21)
Riverside	Mecca	0	0		7.8	51.4(10-19)
Imperial	Palo Verde	22(92)	3(13)	6-30 WEE 7-28 SLE	2.3	8.0(7-20)
Imperial	Corda Ranch	15(83)	0	6-10	3.1	22.3(9-28)

*Results from FA tests; SLE positive chickens were confirmed by neut tests.

COMPARATIVE VECTOR COMPETENCE OF *CULEX QUINQUEFASCIATUS* SAY TO SEVEN CALIFORNIA STRAINS OF ST. LOUIS ENCEPHALITIS VIRUS[1]

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ABSTRACT

Since the early 1950's, the number of reported human cases of St. Louis encephalitis (SLE) virus in California has decreased significantly from 99 cases in 1954 to only one or two cases a year during the 1970's. The recent decline in the number of human cases can be partially attributed to statewide mosquito control efforts or possibly to a temporal change in the transmissibility of indigeous SLE viral strains.

In order to test the latter hypothesis, we evaluated the transmissibility of seven California strains of SLE virus that had been isolated from pools of *Culex* mosquitoes during the years when laboratory-confirmed cases of SLE were recorded in California. The strains tested, listed chronologically by year along with the number of reported human cases, were BFS 314 (1950 - 69 cases), BFS 1584 (1952 - 45 cases), BFS 2035 (1954 - 99 cases), FMS 2108 (1959 - 40 cases), FMS 4823 (1963 - 13 cases), BFN 3252 (1971 - 2 cases), and IV 824 (1978 - 1 case).

Although *Culex quinquefasciatus* Say is a poorer vector of SLE virus than *Cx. tarsalis* Coquillett in California, the species is a much more sensitive indicator than *Cx. tarsalis* for detecting differences in the transmissibility of different SLE viral strains. A field population of *Cx. quinquefasciatus* was collected as pupae from Ashe Sewer Farm located near Bakersfield, Kern County, California. When 4 - 7 days old post-emergence, females were infected by feeding on viremic chicks that had been inoculated with each viral strain. Blood-fed individuals were incubated at 25 ± 1 °C for 2 - 3 weeks before transmission and infection rates were determined.

The transmission rates (data combined for 2 - 3 weeks post-infection) for the strains isolated from 1950 to 1978 were 38, 40, 33, 19, 20, 9, and 8 percent respectively. When the transmission rate was compared with the number of human cases for each year and viral strain, there was a significant correlation ($r = .91$, $p<0.01$) between the number of human cases reported and the transmissibility of each viral strain. These findings demonstrate that transmission rates varied among the viral strains, but more importantly, that transmissibility of the strains we tested has decreased progressively since the 1950's. These findings may also explain to some extent, the lower incidence of human cases in California during the 1970's as compared to the 1950's. However, additional strains of SLE virus and *Cx. quinquefasciatus* will have to be tested before any valid conclusions can be reached. We were unable to demonstrate any relationship ($r = .66$ $p> 0.05$) between mosquito infection rates for each strain of SLE virus and the number of human cases.

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FIELD EVALUATION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS, LAGENIDIUM GIGANTEUM*, AND *ROMANOMERMIS CULICIVORAX* IN CALIFORNIA RICE FIELDS

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INTRODUCTION.–Problems associated with chemical control of mosquito populations such as persistence of toxic metabolites, deleterious effects on nontarget organisms, and development of pesticide resistance can often be mitigated by use of microbial insecticides and biological control agents. Field evaluations were made in rice fields in the Central Valley of California during the 1982 growing season of the microbial insecticide *Bacillus thuringiensis* var. *israelensis* (BTI, serotype H-14), the mermithid nematode *Romanomermis culicivorax*, and the oomycetous fungus *Lagenidium giganteum*.

MATERIALS AND METHODS.–**BTI** - Teknar®, a liquid formulation of BTI, was obtained from Sandoz, Inc. Three rice fields in Yolo County near Sacramento, CA, were used as test sites, with one aerial application and two ground applications made. For the aircraft application, ten 15 m swaths were made over a distance of 0.8 km for a total of 30 acres covered. Three gallons (11.34 liters) of Teknar diluted 50% with water were applied, and droplet size monitored using nigo slides. Technical details of application methods for both aerial and ground applications are included elsewhere in this volume (Akesson et al.).

A mist blower from the Sacramento Yolo Mosquito Abatement District was used in the first ground application of Teknar®. Two passes were made on the upwind side of the field along a 0.5 mi stretch at 5 mi/hr (8 km/hr). A total of 3.25 gallons of Teknar® was diluted 50% with water and sprayed using four hollow core nozzles. Sacramento Yolo MAD also supplied a cold fogger for the second ground trial. Six passes were made at 5 mi/hr along a 0.5 mile distance to disseminate four gallons of the BTI formulation diluted 50% with water. For both ground trials 10 nigo slides were placed between 15 and 215 m from the application point to monitor drop size and distribution.

Sentinel buckets (Case and Washino, 1979) with mesh sides and bottom containing 20 second instar *Culex tarsalis* larvae were placed without tops at various intervals in the rice fields six hours prior to BTI application. Lids were placed on the sentinel buckets one hour after each was sprayed. For the aerial application 14 series of three buckets each were placed at 10 meter intervals from a road at the south end (upwind side) of the test field, and a series of control buckets placed 0.4 km from the edge of the treated area. For the ground applications the sentinel buckets were placed between 0 and 250 m in the downwind direction from the road used for BTI spraying. The control series was 600 m from the point of application.

Only the field used for the mist blower evaluation had sufficient larvae for pre- and posttreatment dipping. Using one pint dippers five parallel transects each separated by 30 m were sampled at 10 m intervals at 25 different stops. Each individual took four dips at each of the 25 stops, and the number of *Anopheles freeborni* and *Culex tarsalis* collected in each dip were recorded.

Romanomermis culicivorax - Nematodes were reared at the Sutter Yuba Mosquito Abatement District using established procedures (Critchfield et al. 1982). Postparasites were applied in the field weekly from the last week in May through the first week in September during the 1982 rice-growing season. Nematodes were weighed in 2.5, 5 or 10 g lots, and applied using either a compressed air backpack sprayer or 1 m long plexiglass tubes (4 cm diam.). For tube applications tubes were placed in the benthos, postparasites poured into them, and allowed to settle into the mud for a minimum of five minutes to minimize predation by various aquatic arthropods. Plexiglass tubes were placed every 10 m, and the position of every third tube was marked by a wooden stake. Transects used for spray applications were marked by stakes as necessary to allow subsequent assessment of nematode activity.

Nematodes were applied using plexiglass tubes in linear transects beginning ca. 5 m from and following the contour of rice field checks near the town of Sutter, CA. Transects used for spray applications usually paralleled the check contours 15 to 20 m from transects used for tube inoculations.

Three to four weeks after inoculation sentinel cages containing 10 laboratory-reared *An. freeborni* and 10 *Culex pipiens* second instar larvae were placed at staked positions in the rice fields. Positioning of the sentinel buckets was determined by sentinel larva availability, accessibility of the site, and potential for damage to the rice crop during various stages in rice plant maturation.

Sentinel larvae were placed weekly along each transect beginning three to four weeks after inoculation until a minimum of 50% of the monitored sites showed some degree of infection. This monitoring period is subsequently referred to as the establishment period. A second period of monitoring was initiated a minimum of seven weeks after inoculation of the nematode along a given transect to verify persistence of *R. culicivorax*, and is referred to as the persistence period of monitoring. After three days in the rice field, sentinel larvae were returned to the laboratory for dissection to determine infection rate.

Lagenidium giganteum - *L. giganteum* cultures were grown in 2800 ml Fernbach flasks in liquid media consisting of 1.25 g Bacto-peptone, 1.25 g Difco yeast extract, 3.0 g glucose, 2.5 g agar, and 2.0 g Mazola® corn oil per liter of deionized water, supplemented with 50 mg/l lecithin (type IX-E, Sigma) and 25 mg/l cholesterol. Each flask containing one liter of media was inoculated with 7-day old cultures of *L. giganteum* grown on the same media as above, but with 12 g/l agar, and incubated for 5 days at 80 rpm on a rotary shaker.

Seepage ditches ca. 1 meter wide associated with rice fields in Sutter County, CA, were inoculated with the 5-day old cultures at a rate of three liters per 100 m. The fungus was applied using a compressed air backpack sprayer, and 10 sentinel cages containing 20 second instar *Cx. tarsalis* were placed in each 100-150 m section. Surviving larvae were collected after 60 hours and returned to the laboratory for determination of sentinel mortality. Persistence of the fungus was monitored at intervals ranging from two to eight weeks following introduction of the fungus.

RESULTS.—**BTI** - Sentinel larvae mortality in the field treated by air ranged from 8% to 100% among individual sentinel cages, with an overall mortality of 75 ± 24% (Table 1). The observed variation in mortality among closely spaced sentinel buckets could be due to differences in rice canopy height, failure to deliver a uniform spray, or differential drift following application.

Sentinel mortality as a function of distance from point of application for the cold fog application is summarized in Table 2. Very high mortality was observed as far as 100 m from the point of application, with an average of 94 ± 6% sentinel mortality at the nine distances out to that point. Sentinel larvae were killed at levels exceeding control mortality as far as 250 m from the application point (Table 2). Using the mist blower, 97 ± 3% mortality of sentinel larvae was achieved out to 100 m from the point of application (Table 3). Mortality significantly greater than control mortality was observed out to 250 m.

Pretreatment dipping in the field used for the mist blower evaluation yielded sufficient numbers of larvae to merit post-treatment monitoring of the field mosquito population. Control of the indigenous larval population of both *Cx. tarsalis* and *An. freeborni* was exerted out to 100 m from the application point (Figure 1), complementing the high sentinel mortality observed out to that distance. The occurrence of first instar anopheline larvae inside of 100 m from the point of BTI application 36 hours after spraying (Figure 1) indicates that this BTI formulation has little or no persistence in the rice field against *An. freeborni* larvae.

Table 1. Sentinel mortality as a function of distance from a reference point in a rice field following aerial application of BTI. Three replicates with 20 second instar *Cx. tarsalis* per sentinel bucket were used at each distance.

Distance from reference point (m)	Sentinel Mortality (%) (Mean ± Std. Dev.)
0	71 ± 29
10	41 ± 30
20	74 ± 12
30	51 ± 34
40	43 ± 40
50	93 ± 7
60	43 ± 50
70	53 ± 24
80	92 ± 8
90	100 ± 0
100	94 ± 5
110	90 ± 10
120	100 ± 0
130	100 ± 0
600 (control)	11 ± 7

Table 2. Sentinel mortality as a function of distance from point of BTI application using an aerosol generator (cold fogger). Three replicates with 20 second instar *Cx. tarsalis* per sentinel bucket were used at each distance.

Distance from point of application (m)	Sentinel Mortality (%) (Mean ± Std. Dev.)
0	90 ± 10
5	100 ± 0
10	100 ± 0
20	100 ± 0
30	100 ± 0
40	87 ± 11
50	98 ± 2
75	78 ± 22
100	89 ± 11
125	52 ± 3
150	27 ± 4
175	50 ± 18
200	44 ± 25
250	60 ± 36
600 (control)	6 ± 6

Table 3. Sentinel mortality as a function of distance from point of BTI application using a mist blower. Three replicates with 20 second instar *Cx. tarsalis* per sentinel bucket were used at each distance.

Distance from point of application (m)	Sentinel Mortality (%) (Mean ± Std. Dev.)
0	100 ± 0
5	100 ± 0
10	100 ± 0
20	100 ± 0
30	98 ± 2
40	100 ± 0
50	94 ± 6
75	98 ± 2
100	83 ± 8
125	79 ± 21
150	54 ± 27
175	46 ± 41
200	60 ± 27
250	22 ± 16
600 (control)	6 ± 6

Distance from Point of Inoculation (m)	PRETREATMENT A	B	C	D	E	36-HOUR POST TREATMENT A	B	C	D	E
0	4C1A				1C2A		1A*			
10	2A									
20	1C1A	2A			2A					
30		2A								3A*
40	2A	2A	1A		2A			1A*		
50				1A	1A	1A				
60		1A	1A				1A			
70	3A	1A	3A	1A	1A					4A*
80	1C		1A		1A		2A			2A*
90	1C	1C2A	1A	1A	2A		1A			
100	2C1A	1A	3A	1A	1A					
110	2C1A	2A	1A		4A	1C2A		4A		2A
120	2A		2A			1C*1C	1C		1A	1A
130	1C1A	1A	2A		1A	2C2A	2A*3A			
140	2C	1A	1C		2A	1C	2A	1A	2A	
150		1A	1A			3C	1A	2A		1A
160	3A	6A	1C2A	4A	1A		5A	1C3A		1A
170	3A	1A			2A	1A*	5A			
180	3A	1A	2A	1A		1A	1A	1C1A		3A
190	1C	2A	3A	1A		1C*				1A1A*
200	1A		1C1A			2A	2A	1A		
210		7A				2C*1A*	1A	1C		
220	1A			3A		1C3A1A*		1A	1A	4A
230	1A	6C1A			3C1A	3A	2C	1C	1A	
240	2A	9C4A			1A	1C*	4A*		1A*	1A

Figure 1. Distribution of mosquito larvae in a rice check prior to and 36 hours after mist blower application of BTI. A-E indicate positions of samplers at 30 m intervals. 1-25 indicate positions of stops made by samplers at 10 m intervals. C indicates total number of *Cx. tarsalis* larvae collected in 4 dips. A indicates total number of *An. freeborni* larvae collected in 4 dips. A*, C* indicates total number of first instar *An. freeborni* or *Cx. tarsalis*, respectively, collected during the posttreatment dipping.

Romanomermis culicivorax - Fourteen individual rice checks in three rice fields encompassing ca. 25 acres were inoculated with *R. culicivorax* postparasites during the 1982 season (Table 4). Sentinel infection ranged from 2% to 51% during the establishment monitoring period, and from 14% to 50% during the persistence evaluations (Table 4). *An. freeborni* larvae, which are very susceptible to *R. culicivorax* preparasite infection, were preferentially parasitized. The infection figures are an underestimation of the actual infection rate since heavily parasitized sentinel larvae are killed immediately and decay in the field. Because of this phenomenon we included both *An. freeborni* larvae, a sensitive indicator of preparasite activity, and *Cx. pipiens* larvae, a less susceptible host, in each sentinel cage.

Overall sentinel mortality, with the exception of the persistence evaluation of the 50 g/100 m spray applications, was comparable for all application rates whether applied by tubes or spraying (Figure 2). Failure to document a definite increase

Table 4. Infection of Sentinel Larvae by *Romanomermis culicivorax* during the 1982 Season.

Site Designation		Monitor Period[1]	No. Sentinel sites / No. of Sites with infected larvae[2]	Method of Inoculation / Application rate (g)[3]	% infected *An. freeborni*[4]	% infected *Cx. pipiens*[5]	% infected larvae
Brown	#1A,B	E (5/6)	12/3	tubes/25	4 (2/54)	1 (1/84)	2
		P (7/8)	13/9		36 (5/14)	24 (21/86)	26
Brown	#2A,C	E (5/6)	8/5	tubes/25	24 (7/29)	5 (2/38)	13
		P (8/9)	16/5		25 (5/20)	9 (1/11)	19
Brown	#2B,D	E (7/8)	7/4	spray/25	30 (15/50)	3 (1/40)	18
		P (8/9)	13/9		66 (23/35)	24 (5/21)	50
Brown	#3A	E (5/6)	12/10	spray/100	39 (26/66)	25 (17/68)	32
		P (7/8)	15/13		57 (31/54)	29 (19/65)	42
Brown	#4A	E (4/5)	8/5	tubes/100	43 (19/44)	40 (19/47)	42
		P	--------				
Brown	#5A	E (4)	11/10	tubes/50	62 (31/50)	34 (22/65)	46
		P	--------				
Thomasen	#1A	E (3)	15/9	tubes/50	29 (20/68)	18 (19/108)	22
		P (8)	12/12		79 (38/48)	30 (27/90)	47
Thomasen	#1B	E (3)	7/7	tubes/25	67 (4/6)	47 (21/45)	49
		P (7)	7/4		47 (14/30)	41 (22/54)	43
Thomasen	#1C	E (3)	11/7	spray/25	41 (7/17)	23 (17/73)	27
		P (7)	6/5		33 (11/33)	7 (3/43)	18
Thomasen	#2A	E (3)	13/8	tubes/25	18 (2/11)	32 (20/62)	30
		P (7)	6/3		55 (6/11)	6 (1/16)	26
Thomasen	#2B	E (4/5)	10/7	tubes/50	52 (14/27)	24 (11/46)	34
		P (7)	5/2		47 (8/17)	11 (2/18)	29
Thomasen	#2C	E (4/5)	10/7	spray/50	25 (6/24)	20 (13/66)	21
		P (7)	10/5		32 (12/38)	2 (1/54)	14
Thomasen	#3A	E (4)	10/8	tubes/50	43 (3/7)	52 (33/63)	51
		P (7)	6/1		50 (6/12)	0 (0/4)	38
Thomasen	#3B	E (4)	7/5	tubes/100	36 (9/25)	11 (4/36)	21
		P	--------				
Thomasen	#3C	E (4)	4/2	spray/100	50 (3/6)	15 (3/20)	23
		P	--------				
Thomasen	#4A	E (4)	13/11	tubes/50	44 (17/39)	28 (27/98)	32
Thomasen	#4B	E (4)	12/8	spray/50	38 (18/47)	17 (12/70)	26
		P	--------				
Lemenager	#1A	E (4)	10/5	tubes/100	27 (13/49)	8 (3/36)	19
		P	--------				
Lemenager	#1B	E (4)	7/2	spray/100	11 (3/28)	0 (0/42)	4
		P	--------				

1 E: Initial monitoring period to ascertain establishment of *R. culicivorax* along a given transect. Figure in parenthesis is the number of weeks after inoculation that the corresponding sentinel mortality occurred.
P: Second monitoring period to determine the degree of persistence of *R. culicivorax* preparasites in inoculated rice fields. Figure in parentheses is the number of weeks after inoculation that the corresponding sentinel mortality occurred.

2 The sentinel cage at each site initially contained 10 *Cx. pipiens* and 10 *An. freeborni* second instar larvae.

3 Tubes: Every 10 m the appropriate dose of postparasites was dumped into 1 m long plexiglass tubes placed into the benthos.
Spray: Inoculated using a compressed air backpack sprayer.
(Application rates given in g/100 m.)

4 5 Figure in parentheses is the (number of infected sentinel larvae)/(total number of recovered sentinel larvae).

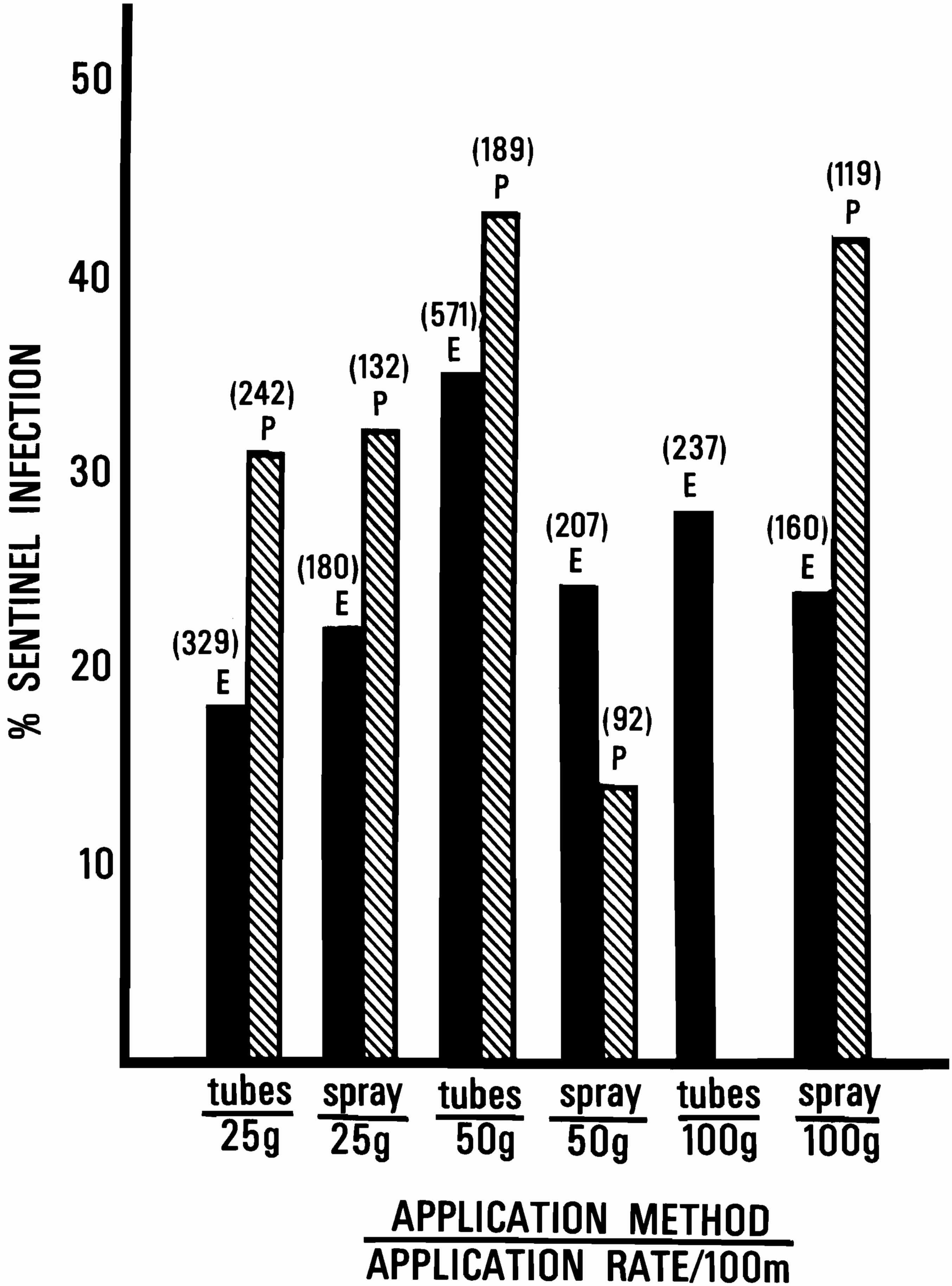

Figure 2. Summary of sentinel larvae infection by *Romanomermis culicivorax* as a function of application rate and method. E, P refer to the separate monitoring periods as described in Table 4. The figures in parentheses refer to the total number of sentinel larvae on which each mortality figure is based.

in sentinel infection with increasing application rates may be due to insufficient monitoring, variability in virulence between batches of nematodes reared at different periods over the summer, or differential rates of adult maturation and/or mating in the various rice checks. Differences may be found during subsequent seasons as we evaluate the overwintering ability of the parasite inoculated at the various rates.

Because of the low density of mosquito larvae in the inoculated field, we were unable to assess the effect of post-parasite application on the indigenous populations. Differences in the effectiveness of the various application rates may have been evident had we been able to evaluate the effect on field populations.

Lagenidium giganteum - Four roadside seepage ditches associated with rice fields in Sutter and Yuba Counties were inoculated with *L. giganteum* over a period of seven weeks, with a total of 1465 meters of ditch inoculated. Overall sentinel mortality for all ditches monitored following spraying (120 sentinel cages) was 82 ± 11% (Table 5).

The nutrient media in which *L. giganteum* is grown must be diluted in order to induce the formation of the infective stage, the zoospore (Boswell 1977). Our results indicate that zoosporogenesis can be successfully induced by applying the undiluted culture medium directly into the field.

Persistence of the asexual stage of *L. giganteum* in the inoculated seepage ditches was monitored late in the breeding season. Sentinel mortality for all four sites was 21 ± 14% (26 sentinel cages) with a range of 0 to 31% (Table 6).

DISCUSSION.–All three BTI application methods achieve comparable control of larval populations of *Cx. tarsalis* and *An. freeborni* in California rice fields. Ultimately the most cost-effective method of BTI dissemination will be used by mosquito abatement agencies. The mist blower in its present configuration is superior to the aerosol generator since it is able to deliver material at the higher rates necessary for BTI to reach toxic concentrations in the rice fields. Either method of ground application is limited by the distance over which BTI formulations will drift, in this series of trials approximately 200 m. Even if access roads are present every 200 m over the length of a rice field, which is usually not the case, the expense of multiple runs at such short intervals may be prohibitive. Aircraft spraying of BTI may be the best method since relatively large amounts can be delivered quickly over large distances. Further discussion is presented by Akesson et al. elsewhere in this volume.

For the *R. culicivorax* study, persistence monitoring resulted in consistently higher infection rates than was obtained for the initial establishment evaluations. It appears that a single application of postparasites will exert partial control of mosquito larvae breeding in rice fields for the entire growing season. If relatively high levels of nematode activity remain in inoculated sites over two or more growing seasons, *R. culicivorax* may prove to be a cost-effective alternative to more established control methods.

The asexual stage of *L. giganteum* grown *in vitro* under appropriate conditions can effectively control populations of *Cx. tarsalis* in rice fields in the Central Valley. Previous work in this laboratory showed this fungus to be effective in controlling *An. freeborni* under comparable conditions (Westerdahl and Washino, unpublished).

Because of the low density of larvae breeding in the inoculated sites (less than 0.1 larva/dip), it was surprising that zoosporogenesis persisted at a reduced rate, at least six weeks

Table 5. Sentinel mortality in seepage ditches associated with rice fields in Sutter and Yuba Counties, CA, following application of the asexual stage of *L. giganteum*.

Inoculation Site	Date of Inoculation	Distance Inoculated (m)	Sentinel Mortality(%)[1]
Clements	23 July	150	93 (10)
Clements	30 July	150	85 (10)
Clements	6 August	130	100 (10)
W. Catlett (north side)	13 August	425	80 (30)
W. Catlett (south side)	20 August	300	64 (20)
S. Butte	27 August	210	77 (30)
Clements	3 September	100	90 (10)

Total Sentinel Mortality[2] : 82 ± 11%

[1]Number of sentinel cages used for each evaluation is enclosed in parentheses. Each sentinel cage initially contained 20 second instar *Cx. tarsalis.*

[2]Generated by treating each contiguous group of 10 sentinel cages as a single data point.

Table 6. Sentinel mortality in seepage ditches associated with rice fields in Sutter and Yuba Counties, CA., evaluating the persistence of the asexual stage of *L. giganteum*.

Inoculation Site	Number of Weeks following Application[1]	Sentinel Mortality (%)[2]
Clements	4 - 6	25 (8)
W. Catlett (north side)	3	0 (5)
W. Catlett (south side)	2	31 (5)
S. Butte	1	26 (8)

Overall Sentinel Mortality[3] : 21 ± 14%.

[1]All of the persistence evaluations were carried out from 3 September to 6 September, 1982.

[2]Number of sentinel cages used for each evaluation is enclosed in parentheses. Each sentinel cage initially contained 20 second instar *Cx. tarsalis*.

[3]Generated by treating each site as a single data point.

in one case, following application. Larval density may have been sufficient due to oviposition after inoculation of a given site for secondary transmission to occur. Alternatively, because it is a facultative parasite, the fungus could have continued growing saprophytically following its application. All of the inoculation sites were filled with algae and aquatic plants, and may have contained a sufficient concentration of free phytosterols to induce zoosporogenesis.

Although *in vitro* culture of the asexual stage of *L. giganteum* is a great improvement over *in vivo* culture, the ephemeral nature of this stage limits its use to relatively small mosquito breeding areas. Future efforts will involve field application of the sexual (oospore) stage of this fungus.

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EVALUATION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* SEROTYPE H-14 FOR MOSQUITO CONTROL[1]

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ABSTRACT

Several formulations of *Bacillus thuringiensis* var. *israelensis* (Serotype H-14) which included corn-based granular materials, emulsifiable solutions, wettable powders, wettable powders bound to sand grains, and compacted material in the form of large and small pellets were applied to various mosquito breeding sources. In general, good to excellent control was achieved at recommended dosage levels with most of the formulations in the majority of field situations tested. The wettable powder bound to sand proved very effective in sources with substantial emergent vegetation. A high efficacy was obtained with this latter formulation at rates as low as .25 kg of wettable powder per hectare.

INTRODUCTION.–Investigations over the last six years with *Bacillus thuringiensis* var. *israelensis* (Serotype H-14) (BTI) have demonstrated its broad and general effectiveness against a variety of mosquito species in many parts of the world. (Goldberg and Margalit 1977, Garcia and DesRochers 1979, Garcia et al. 1980, Garcia and DesRochers 1980, Mulla et al. 1980, Garcia et al. 1981, Nugud and White 1982, Van Essen and Hembree 1982).

Its selectivity for mosquitoes and blackflies and little else is one of its most desirable attributes (Colbo and Undeen 1980, Garcia et al. 1980, Miura et al. 1980). However, field evaluations of this pathogen are still necessary to further delineate situations in which it will be most effective. The kind and location of the mosquito breeding source, the biology of the species involved, the timing and dosage of the application, and the type of formulation are problem areas where research contributions are still needed. The studies reported here examined the effectiveness of different formulations of Bti in diverse mosquito breeding habitats that have posed control problems in the past. Special emphasis in these studies was placed on the control of floodwater *Aedes* spp.

MATERIALS AND METHODS.–The selection and dimensions of the test plots for these studies were dictated generally by the extensiveness of the mosquito breeding source, the larval age and density, the formulation and dosage rates to be applied, and the similarity of conditions within and among the plots. Consequently plot size ranged from 50 M^2 to 1000 M^2, however, where feasible the smaller size was avoided. A standard mosquito dipper was used to assess population density. The number of dips used to sample a population in any particular test series varied in relation to the surface area of the plot and the density of larval populations as determined by general observations. For example, in plots where populations appeared low, the number of dips was

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increased to reduce variability. The number of pre-treatment and post-treatment dips per plot and their corresponding standard deviations are given with each table in the Results and Discussion section. The mean number of larvae per dip was calculated from the total number of larvae dipped for all replicates divided by the number of dips in the treatment. The percentage reduction of larvae for any treatment was calculated from the differences of these means.

Each treatment was replicated three to five times with one exception. In an irrigated pasture in Contra Costa County there were only two treatments at the low dosage tested as only two 1000 M^2 plots were available. At least three or more control plots were used in each test series.

Emulsifiable solutions and wettable powders were mixed with approximately five liters of either tap or water collected at the site. Solutions were sprayed on the surface of the water using a Solo® backpack sprayer[1] at a delivery rate of approximately one liter per minute. Granular formulations were dispensed with an Ortho® whirlybird hand seeder[2] except for one test series which used a Cyclone® seeder.[3] Both machines dispensed materials in swaths of up to about eight meters; however, a four meter swath was employed for most tests reported here. The orifice for the whirlybird seeder was set at 1 and the Cyclone seeder at 2.5 for delivery of the sand/Bti mixture. All other solid formulations were dispensed with the whirlybird at a setting of 1 or 2, depending upon the size of the granular material. A steady even pace in a back-and-forth pattern of four-meter swaths, requiring about two minutes for a 400 M^2 plot, generally gave good coverage. The following formulations were tested during the 1982 mosquito season:

Trade Name	Formulation	AA Units/Mgm
Vectobac®[4]	Emulsifiable Solution (ES)	1000
	Wettable Powder (WP)	2000
	Granules (corn-based)	300
	Pellets (slow release)	250
Bactimos®[5]	Flowable Concentrate (FC)	1000
	Wettable Powder (WP)	3500
	Granule (corn-based)	160
	Briquet (slow-release 'donut')	400

In some tests the wettable powders were combined with #2 sand and Golden Bear Oil #1356 in the following basic formula: 45.6 kg sand : 1.14 kg WP : 0.63 kg oil. The relatively small quantities used for these tests were mixed thoroughly first, followed by the wettable powder and a final thorough mixing.

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

[2]Chevron Chemical Company, San Francisco, California.

[3]The Cyclone Seeder Company, Inc., Urbana, Indiana.

[4]Abbott Laboratories, Chemical and Agricultural Products Division Oakwood Road, Long Grove, Illinois 60047.

[5]Biochem Products, Post Office Box 264, Montchanin, Delaware 19710.

In selecting the various dosage rates for any particular test series, consideration was given to the total number of AA units/mg, company recommendations when applicable, past experience with a particular formulation and the type of breeding source selected for the test. The total number of AA units/mg ranged from one to five fold or less for any dosage series and formulation.

Study Areas. Test sites were selected in cooperation with the Alameda, Marin-Sonoma, Diablo Valley, and Sutter-Yuba Mosquito Abatement Districts. Tests against *Aedes squamiger* were conducted in January 1982 in brackish marsh sources near the Oakland International Airport, Alameda County. During this period the salt concentration was recorded at 9700 ppm by Hach Kit analysis. The site was a typical *Ae. squamiger* source with entensive stands of pickleweed (*Salicornia* sp.) throughout. The depth of the water ranged from a few centimeters to over 60 cm in the 100 M^2 plots. Water temperatures varied from a low of 5° C to a high of 13° C. The age of the population ranged from 1st to 4th instar reflecting the sequential hatching of this univoltine species. Bactimos and Vectobac wettable powders were applied in water with a Solo backpack sprayer.

A series of catch basins were tested in Berkeley, Alameda County, from mid-July through August of 1982. Three control and nine test basins were sampled with a long handled dipper. Each basin was dipped five times in a 10 minute interval prior to and 48 hours post-treatment. The basins were then checked in the same manner on a weekly basis thereafter. A single donut shaped pellet weighing approximately 12 grams (5×10^6 AA units) was dropped into each of the nine test basins. Larval populations were represented by all instars of *Culiseta incidens* and *Culex pipiens*. In July from 60 to 90% of the larvae were *Cs. incidens*, however, by the end of the observation period in August all larvae were of this species. The numbers of larvae varied considerably during the course of the observations.

Studies were conducted in a single drainage canal in Marin County during May of 1982. Residual water remaining after the heavy winter runoff provided excellent breeding conditions for *Culex tarsalis* and *Cs. incidens*. Water depth was relatively constant at about 20 cm. Water temperatures in the middle of the canal ranged from 24° to 26° C during the midday. The canal was approximately five meters in width and was divided into 10 meter lengths (50 M^2 plots) each separated by about one meter. Algal mats covered the surface of several of the plots by as much as 90%. The population consisted of about 75% *Cx. tarsalis* and the remainder *Cs. incidens*. Wettable powders were applied with the Solo sprayer.

Irrigated pasture tests were conducted in Contra Costa County at sites historically recognized for breeding large populations of *Aedes* spp. During late July and early August the larval populations consisted of approximately 94% *Ae. nigromaculis* 6% *Ae. melanimon*. The pasture grass was dense and emerged above the water surfaces in most areas. Water depth ranged up to about 10 cm in depth in the test areas. A Bactimos WP/sand/oil mixture was applied with a Cyclone seeder over 1000 M^2 plots.

The final test series was conducted at Gray Lodge Wildlife Refuge in Butte County during late August, September, and early October 1982. Several fields flooded for waterfowl habitat during this period produced large populations of *Ae. melanimon*. *Ae. nigromaculis* was found only rarely among these larval populations. Several fields with relatively extensive populations were selected for testing. The density of larval populations was highly variable not only among the fields but also among the test plots in any one field. Although larval development varied among the fields due to the different flooding times, it was fairly synchronous within the fields. Some plots consisted of relatively open water while others had extensive emergent vegetation. Water depth was as much as 75 cm in some plots but generally ranged from a few to 30 cm. Selection of plots for testing the various formulations was based partly on the extent of the emergent vegetation. For example, granular material, sand mix or pellets were used in areas where vegetation was more extensive. Water temperatures were relatively mild and ranged from 18.8° to 30° C during the course of these studies. The formulations of Bti used were Vectobac WP, WP/sand, ES, granules and "slow release" pellets. The Bactimos formulations included WP/sand and granules. A Solo sprayer and whirlybird seeder were used to dispense the liquid and solid materials during these tests.

RESULTS AND DISCUSSION.–Table 1 shows the effect of Vectobac and Bactimos WP against populations of *Ae. squamiger* larvae. Observations 48 hours after treatment indicated that the Vectobac WP had reduced populations by 33%, 79%, and 92% at dosage rates equivalent to ¼, ½, and ¾ kg per hectare, respectively. At similar rates the Bactimos WP reduced populations by 77%, 92%, and 90%, respectively. The effectiveness of these relatively low treatment levels in typical *Ae. squamiger* habitat indicate that Bti could play a useful and selective role for the control of this species. Other formulations such as granular or sand mixtures may be more effective than the wettable powders where emergent vegetation is dense.

Table 1. Treatment of *Ae. squamiger* with Bti in brackish water, Alameda County, California. January 1982.[1]

Formulation kg/ha	$\bar{x}$ larvae/dip Pre-tx	SD	Post-48hrs.	SD	% Reduction
[2]Vectobac WP					
0.25	7.3	7.3	4.9	7.7	33.
0.50	9.2	9.9	1.9	3.6	79.
0.75	8.4	8.2	0.7	1.5	92.
[3]Bactimos WP					
0.25	7.4	9.9	1.7	2.8	77.
0.50	4.7	6.0	0.4	1.0	92.
0.75	10.3	10.1	1.0	1.3	90.
Controls	9.1	7.6	9.4	7.8	—

[1]Plot size = 100m^2. 3 replicates at each dosage and control, 12 dips each replicate.
[2]Wettable powder: 2000 AAunits/mgm product.
[3]Wettable powder: 3500 AAunits/mgm product.

In these tests with *Ae. squamiger*, a 48 hour observation period was used because many larvae, particularly the later instars, were still alive after 24 hours although many appeared sluggish and "sick." Apparently many of these "sick" larvae died, as population counts 48 hours post-treatment revealed a sharp reduction over the control plots and only a few sluggish appearing larvae were seen in most treated plots. Originally it was believed that the low water temperatures between 5° and 13° C were responsible for the prolonged die-off; however, constant temperature experiments at 8° C in the laboratory with wild-caught 3rd instar *Ae. squamiger* larvae in brackish water revealed a complete mortality after 24 hours at rates equivalent to 0.25 kg/ha. These results suggest that low temperatures were not primarily responsible and that other still unknown factors are involved.

Previous tests have demonstrated that Bti wettable powder is effective against *Cx. pipiens* and *Cs. incidens* in catch basins in the Bay Area. During the past season a slow release formulation (Briquet) by Bactimos was applied to test catch basins in an attempt to suppress populations for longer periods of time. Problems associated with the catch basins selected for treatment and control included marked changes in water volume, contamination with motor oil, detergents, leaf litter, and other trash and debris. These uncontrollable changes were at least partly responsible for the wide variations in mosquito larvae from one sampling period to the next, and for the complete disappearance due to drying and oil contamination in two control and five test basins. Results in Figure 1 from the remaining three tests and single control basin show early instar larvae to be relatively unaffected by the treatment. It is believed that possibly the early instars were not ingesting the Bti due to particle size or something else associated with the pellet. Late instar larvae in test basins remained at low levels for about five weeks; however, the levels of 3rd and 4th instar larvae in the one remaining control were also low, thus making it difficult to draw any conclusions. More extensive testing is necessary to prove efficacy of this material against catch-basin mosquitoes.

The results of treatment with Bactimos WP and FC and Vectobac WP against *Cx. tarsalis* and *Cs. incidens* in a drainage canal in Marin County are shown in Table 2. Results were generally very good to excellent in all test plots except in those where algal mats covered the water surface extensively. In plots where 90% of the surface was covered, the reduction of larvae was strongly affected. Presumably under these conditions much of the pathogen remained in the mats and thus was unavailable to the larvae residing below. However, where the emulsifiable solution was used at 2.4 kg/ha and algal mats covered about 80% of the surface, the results approached those of the open-water plots.

Irrigated pasture tests against *Ae. nigromaculis* in Contra Costa County are shown in Table 3. The sand/Bti/oil mixture proved very effective in reducing *Ae. nigromaculis* populations in dense pasture vegetation. The lower efficacy at the higher dosage plots was a few centimeters deeper than pre-treatment levels. It is believed that the Golden Bear oil used as a binder for the wettable powder had no direct effect on larval mortality since it amounted to only about 150 and 300 grams per hectare for the low and high dosage, respectively.

The final series of tests reported here were conducted at Gray Lodge Wildlife Refuge in Butte County. As part of the development of an integrated control strategy for the refuge, several different formulations were tested in order to evaluate the most effective ones for conditions on the refuge.

A variety of Vectobac formulations were the principal materials examined during these studies. The results of all investigations with Bti at Gray Lodge are shown in Table 4. Vectobac WP was applied with water and in a sand/oil mixture at rates that ranged from .25 to 1.2 kg/ha over 400 M^2 plots. The high concentration of wettable powder at .75 kg/ha was very effective in reducing later instars of *Ae. melanimon* while the results at the lower rate of .5 kg/ha against 2nd instar larvae were not as encouraging. When mixed with sand and oil, effective control was achieved at rates below ½ kg/ha. In two tests the amount of Bti was reduced while the amount of sand was increased or held constant, i.e., .38 kg wettable powder in 30 kg sand/ha and .25 kg wettable powder in 20 kg sand/ha. It was believed that effective control might be possible with lower rates of Bti, providing there was an adequate dispersal of the pathogen in the test area. The 0.38 kg/ha in 30 kg/sand proved relatively effective reducing larval populations by 91%. However, the lower rate of 0.25 kg/ha in 20 kg/sand was not particularly effective and the percent reduction among the plots was highly variable ranging from 60 to 80%.

Table 2. Treatment of *Cx. tarsalis* and *Cs. incidens* with Bti in an open drainage canal, Marin County, California. May 1982.

Formulation kg/ha	$\bar{x}$ larvae/dip Pre-tx	SD	Post-48hrs.	SD	% Reduction	% Algal Mats[2]
[3]Bactimos WP						
0.72	3.3	2.7	1.1	1.3	67.	90
0.36	6.5	3.7	0.4	0.7	93.	0
[4]Bactimos FC						
2.4	4.1	3.4	0.3	0.7	93.	80
1.2	11.4	8.5	0.8	0.9	93.	0
[5]Vectobac WP						
1.0	13.0	7.4	3.1	2.8	76.	10
0.5	12.8	11.9	6.0	5.8	53.	90
Controls						
Set 1	6.4	4.4	6.7	6.1	—	10
Set 2	10.4	5.8	12.6	10.6	—	90

[1]Plot size = $50m^2$. 3 replicates at each dosage and control set, 15 dips each replicate.
[2] % surface area covered.
[3]Wettable powder: 3500 AAunits/mgm product.
[4]Emulsifiable solution: 1000 AAunits/mgm product.
[5]Wettable powder: 2000 AAunits/mgm product.

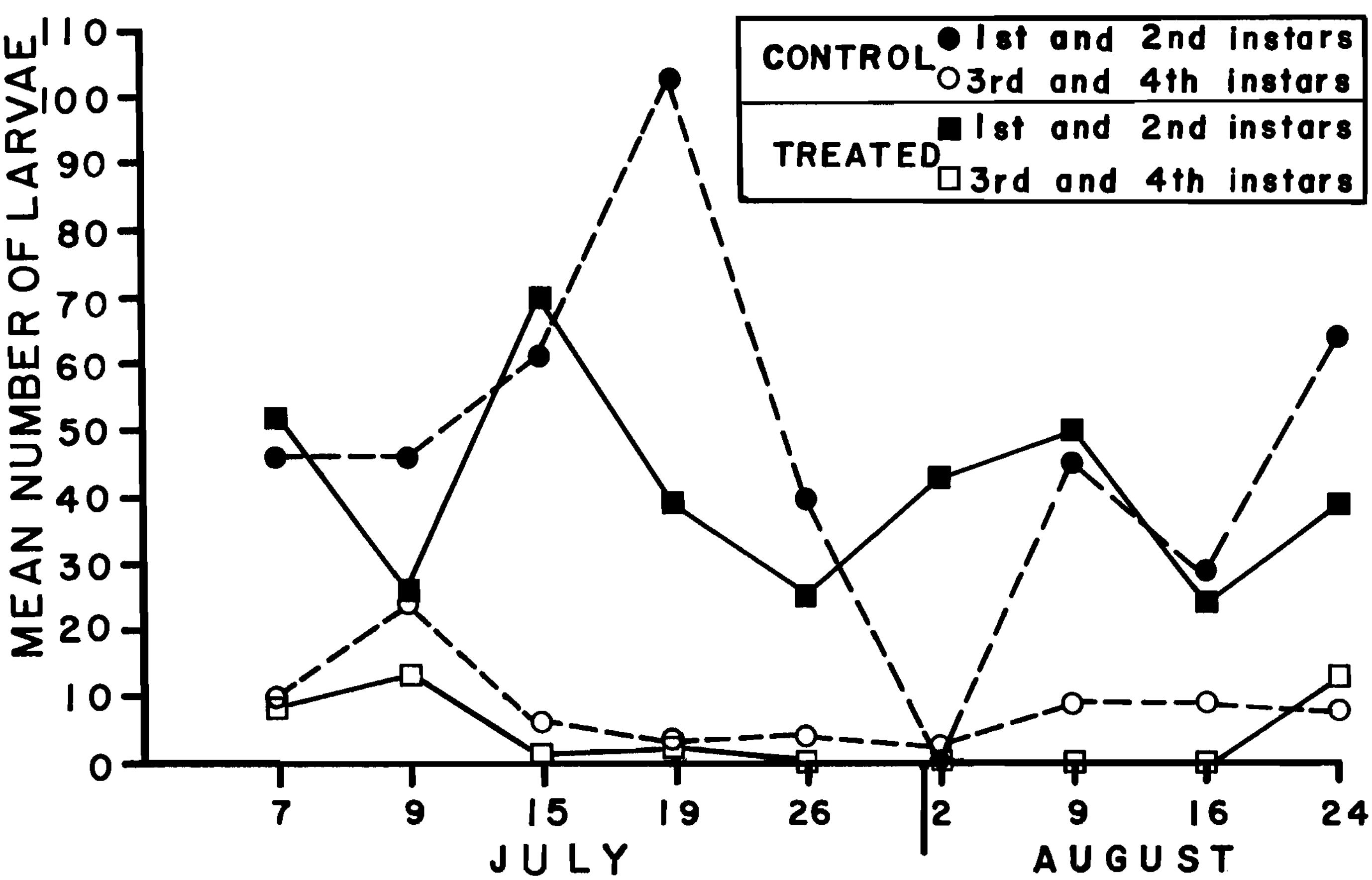

Figure 1. Mortality of *Culiseta incidens* in catch basins after treatment with Bactimos® slow release Briquet (400 AA units/mgm).

Table 3. Treatment of *Ae. nigromaculis* and *Ae. melanimon* with Bti in irrigated pastures, Contra Costa County, California. July 1982.[1]

Formulation		x̄ larvae/dip					
kg/ha	(sand kg/ha)	Pre-tx	SD	Post-24hrs	SD	%Reduction	Sample Size[1]
[2]Bactimos	wp/sand						
0.50	(20)	4.9	4.9	0.9	1.3	81	3 x 25
0.25	(10)	4.3	3.5	0.2	0.4	96	2 x 30
Controls		5.7	7.3	5.3	2.9	7	3 x 25

[1]Plot size = 1000m^2. Sample size: no. replicates X no. dips.

Ae. nigromaculis = 94%.
Ae. melanimon = 6%.

[2]Wettable powder: 3500 AAunits/mgm product mixed in sand & oil.

The Vectobac ES applied at rates of ½ to 1 kg/ha reduced populations from 87% to 99%. All plots in these experiments were composed of relatively late instar larvae in water less than 20 cm deep.

The Vectobac granules were effective at the high and at one of the paired lower rates. The cause for the differences between these treatments is not clear; however, water currents resulting from transfer of water from one field to another and differences in depth among the plots may have been responsible factors.

The slow-release formulation of Vectobac pellets was applied at rates of 8.3 kg/ha. This material effectively reduced large populations of 2nd instar larvae concentrated along the shore edge of plots in which the water was only 4 to 5 cm in depth (Table 4). This same rate failed to reduce populations of *Cx. tarsalis*, *Anopheles freeborni*, and *Aedes* spp. in water 30 to 50 cm in depth (results not shown here). It is believed that the effective toxin available from this "slow release" formulation was insufficient and/or unavailable to the larvae in deep water.

Bactimos granules applied at the recommended dosage of 4 kg/ha reduced 4th instar populations effectively. A lower dosage may be effective with this material. The Bactimos WP in sand and oil mix at .73 and .36 kg/ha reduced 4th instar populations by 93% and 84%, respectively (Table 4).

CONCLUSIONS.–There now appear to be Bti formulations that are either practical or approaching practicality for several major mosquito breeding habitats. Although more needs to be done in the area of formulation, the results of these studies indicate the efficacy of products now available and under test is very encouraging. Further, the wettable powder formulation mixed with sand and oil appears to be an effective method for dispersing Bti in thick emergent vegetation.

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Table 4. Treatment of *Aedes melanimon* with Bti in flooded fields, Gray Lodge Wildlife Refuge, Butte County, California.[1] September-October 1982.

Formulation kg/ha	(sand kg/ha)	larval instar	x larvae/dip Pre-tx	SD	Post-24hrs.	SD	% Reduction	Sample Size[1]
[2]Vectobac	WP							
0.75		3.4	50.7	50.9	2.1	3.4	96	3 x 20
0.50		2	22.7	14.5	4.2	6.0	81	2 x 20
[3]Vectobac	WP/sand							
1.20	(50)	4	12.6	8.7	0.5	0.8	96	5 x 20
0.60	(25)	4	2.5	2.0	0.2	0.6	92	4 x 20
0.50	(20)	2	32.6	34.5	0.6	0.9	98	3 x 20
0.38	(30)	2	10.7	6.8	1.0	1.8	91	3 x 20
0.25	(20)	2	14.8	10.2	4.0	3.2	73	3 x 20
[4]Vectobac	ES							
1.00		3.4	73.9	83.3	1.1	1.6	99	3 x 20
1.00		4	5.7	6.3	0.2	0.5	97	3 x 20
0.75		3.4	7.3	6.9	0.8	1.5	89	4 x 20
0.50		4	5.2	4.7	0.7	1.2	87	4 x 20
[5]Vectobac	Granules							
7.5		4	30.0	42.3	1.7	3.0	94	2 x 40
5.0		2	11.1	8.3	2.3	1.9	79	3 x 20
5.0		2	37.0	34.6	0.7	1.1	98	3 x 20
[6]Vectobac	Pellets							
8.3		2	60.8	101.5	0.6	1.1	99	3 x 20
[7]Bactimos	WP/sand							
0.73	(28)	4	20.4	16.2	1.4	1.5	93	5 x 20
0.36	(14)	4	4.0	3.2	0.7	1.3	84	3 x 20
[8]Bactimos	Granular C							
8.0		4	5.6	5.1	0.5	1.0	91	3 x 20
4.0		4	12.0	7.4	1.5	1.5	88	4 x 20

[1]Plot sizes = 400m^2. Sample size: no. replicates x no. dips.
[2]Wettable powder; 2000 AA units/mgm product.
[3]Wettable powder mixed with sand and oil.
[4]Emulsifiable solution: 1000 AA units/mgm product.
[5]300 AA units/mgm product
[6]250 AA units/mgm product.
[7]Wettable powder: 3500 AA units/mgm product.
[8]160 AA units/mgm product.

Controls: 3-5 reps. each dosage; not shown because no appreciable reduction in larvae noted.

EVALUATION OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 FORMULATIONS FOR CONTROLLING MOSQUITOES IN THE MENDOTA WILDLIFE AREA[1]

F. S. Mulligan III and C. H. Schaefer[2]

ABSTRACT

Liquid formulations of Bactimos™ and Teknar™ (*B.t.* H-14) were effective in the control of immature *Culex tarsalis* Coquillett at 0.5 kg/ha. An airplane application of Teknar AC to a Mendota Wildlife Area wetlands field provided operational control of *Cx. tarsalis* at 0.56 kg/ha. A 2% (wt/wt) corncob-grit, granule formulation of Bactimos was more effective than a Bactimos sand granule by ground application. Immature *Cx. tarsalis* at Mendota were reduced by 97% with an aircraft application of the corncob granules. Multiple applications of methyl parathion (0.1 kg AI/ha) produced major reductions in immature mayflies and dytiscid beetles, while applications of Vectobac™ (*B.t.* H-14) did not.

INTRODUCTION.–The protein toxin of *Bacillus thuringiensis* serotype H-14 (*B.t.* H-14) has proven to be effective in controlling immatures of various mosquito species (Garcia and DesRochers 1979). Innocuity of the toxin to associated, aquatic invertebrates, other than such closely related nematoceran families as Simuliidae and Chironomidae, has been reported by Colbo and Undeen (1980), Miura et al. (1980) and Purcell (1981). The toxin does not depress the population of immature coleopterans, thus they can be effective in controlling mosquitoes (Mulligan and Schaefer 1981). Such a biological toxin can be integrated with natural predation in pesticide sensitive habitats where maintenance of aquatic fauna is desired. State and federal wildlife refuges represent such habitats.

Located in western Fresno County, the Mendota Wildlife Area is a refuge administered by the California Department of Fish and Game. This wildlife area is one of the major mosquito producing sources in the Fresno Westside MAD (FWMAD). The principle species, at which the larvicide operations are directed, are *Aedes melanimon* Dyar and *Culex tarsalis* Coquillett. At present the FWMAD relies chiefly upon the board spectrum poisons, ethyl and methyl parathion. In areas where organophosphorous-resistance is low, parathion continues to be used in mosquito control because of its efficacy and low relative cost.

This paper reports the evaluation of new *B.t.* H-14 formulations for use in the wildlife habitat. Also the impacts of *B.t.* H-14 and parathion on a portion of the wildlife area's aquatic fauna are compared.

MATERIALS AND METHODS.–The following formulations of *B.t.* H-14 were evaluated in field tests: Abbott laboratories Vectobac™ wettable powder (WP) (2000 *Aedes aegypti* International Toxic Units per mg); Sandoz, Inc. Teknar™ aqueous concentrate (AC), (1500 AA units per mg); Biochem Products Bactimos™ WP (2500 AA units per mg) and Bactimos™ flowable concentrate (FC) (1000 AA units per mg). Also tested were two granular formulations. A corncob-grit granule formulated with Bactimos primary powder (2% wt/wt) was supplied by Control Systems (Minneapolis, MN). The other granule was a user-prepared sand formulation composed of 2.5% Bactimos WP and 14.3 ml of binding agent (supplied by Biochem Products) per kg of sand.

Formulation efficacy against natural mosquito populations was evaluated. Formulation, rate, area and species treated, date and application method are listed in Table 1. The immature mosquito populations were sampled by counting the numbers in each of 30-50 dips taken with a standard dipper along a transect across each field. An adjacent, untreated control area for each trial was sampled likewise. Relative differences in populations were attributed to the treatments.

Each field test was assigned a Field Test No. (FT#). Since it is impractical to include all details recorded for each test, e.g., temperature, wind, sky-cover, water depth, etc., this information will be on file and available to interested persons.

Multiple applications of 5EC methyl parathion (Occidental Chemical Company) at 0.1 kg A.I./ha and Vectobac WP at 1.1 kg/ha were made to separate 11 ha wetlands fields on either side of an untreated control field on the Mendota Wildlife Area. The material was sprayed from a fixed wing airplane at 9.4 liters aqueous suspension per ha. Criterion

[1]California Department of Fish and Game, located in western Fresno County, California.

[2]University of California, Mosquito Research Laboratory, 5544 Air Terminal Drive, Fresno, California 93727.

Table 1. Summary of field applications of *Bacillus thuringiensis* serotype H-14 formulations to various mosquito breeding sources during 1982.

FT# 82	date	area (ha)	species	instar	method of application	formulation[2]	rate kg/ha	percent reduction
09	5-4	0.01	*Cx. tarsalis*	2,3,4	whirling disc	Bio-sand G	0.29	99
10	5-6	0.05	*Cx. tarsalis*	2,3,4	hand sprayer	Bio-WP	0.10	93
		0.02				Bio-FC	0.50	97
		0.03				San-AC	0.50	100
11	6-9	0.02	*Aedes*[1]	3,4	hand sprayer	Bio-WP	0.25	93
				3,4	horn seeder	Bio-sand G	0.29	70
				2	horn seeder	Bio-sand G	0.29	83
12	8-11	0.02	*Cx. tarsalis*	2,3,4	whirling disc	Bio-sand G	0.29	63
						Bio-sand G	0.29	70
13	8-25	0.2	*Cx. tarsalis*	2,3,4	horn seeder	Bio-sand G	0.30	70
					horn seeder	Bio-sand G	0.45	16
					whirling disc	Bio-corncob G	0.20	86
14	10-11	10	*Cx. tarsalis*	2,3,4	airplane	Bio-corncob G	0.24	97
15	10-18	4	*Cx. tarsalis*	2,3,4	airplane	San-AC	0.56	98

[1] *Aedes* spp. refers to a mixed population of *Ae. melanimon* (92%) and *Ae. nigromaculis* (8%).
[2] Formulation designations: Bio-biochem Products; San-Sandoz, Inc.; WP-wettable powder; FC-flowable concentrate; AC-aqueous concentrate; corn-cob G-corn-cob grit granules; and G-sand granules.

for the necessity of an application was based on an action threshold of 1.0 immature (combined 3rd, 4th and pupal stages) per 10 dips (3 liters of sample water) (FWMAD).

The populations of aquatic fauna near the water surface, were sampled by dipper. Twice a week for each field, 5 samples, composed of 10 dips each, were concentrated on a 34 mesh/cm screen, placed in 95% alcohol and returned to the laboratory where the organisms were counted under a microscope.

RESULTS AND DISCUSSION.–Results of the formulation testing are shown in Table 1. Both of the liquid formulations, Teknar AC and Bactimos FC, performed well at 0.5 kg/ha (FT #10), the low end of their recommended dosage rates. Each liquid was easily handled and suspended well in water. By comparison the Bactimos WP, with a greater relative laboratory assay activity, was only slightly less effective at 5X lower rate. A low rate (0.56 kg/ha) of the Teknar AC applied by airplane provided operational control of *Cx. tarsalis* in a wetlands field of the Mendota Wildlife Area (FT#15).

A sand granule, which can be readily formulated from Bactimos WP by the user, was prepared and tested. After an initial high reduction of *Cx. tarsalis* in a small, confined habitat (FT#9), less than adequate control was obtained for the sand granule applications (consistently around 70% reductions). Neither the method of application, horn seeder or whirling disc granule spreader, nor the target species affected the results; although, 2nd instar larvae appeared more susceptible (FT#11). Also by increasing the percentage of WP on the same amount of sand carrier in FT#13, the efficacy was actually decreased. In the latter instance, more binder was added with the increased amount of WP. It is speculated that the added binder may have strengthened the binding matrix and allowed less release of WP upon impact with the water surface.

Further, activity of the sand granules declined with time in storage. With storage at 22 ± 2°C, the granules showed 9, 18, 35 and 38% loss of activity below that of newly formulated granules, 23, 30, 36 and 41 days after being formulated.

Corncob-grit granules of Bactimos at a lower rate out-performed the sand granules. An aerial application of corncob granules to a wetlands field in the Mendota Wildlife Area produced a 97% reduction in *Cx. tarsalis*. The increased efficacy of the air test over that obtained on the ground was in part due to the amount of actual ingredient applied, but primarily it was attributed to the more complete coverage provided by the increased volume of the carrier per treatment surface area.

The heavy protein toxin of *B.t.* H-14 does not diffuse well through water and spreaders seem inefficient in dispersing the toxin; thus more particles of the carrier per unit treatment area are needed to evenly and thoroughly distribute the toxin. Corncob-grit, by being less dense than sand, offered more particles and more binding surface area per weight than sand. The light weight of the corncob-grit made it more subject to wind drift, however it penetrated vegetative cover adequately.

During the course of the impact study of multiple applications of *B.t.* H-14 and methyl parathion, the following aquatic insect immatures were sampled in numbers sufficient for correlation: Ephemeroptera, *Callibaetis* sp.; Coleoptera, *Laccophilus mexicanus mexicanus* Aube, *L. m. atristernalis* Crotch, *Thermonectus basillaris* (Harris); Diptera, *Aedes melanimon* Dyar and *Culex tarsalis* Coquillett. All three of the coleopteran species were of the predacious water beetle family, Dytiscidae.

The initial flooding of the fields produced *Ae. melanimon*, a flood-water mosquito. This species predominated in the samples for the first week in each field and were thereafter replaced by *Cx. tarsalis* for the duration of the study period. A methyl parathion treatment on June 2, 1981 resulted in 100% control of *Ae. melanimon*. Additional parathion treatments on June 10 and June 24 reduced *Cx. tarsalis* populations by 88 and 100%, respectively. Applications of Vectobac WP were less effective, *Cx. tarsalis* were decreased by 85 and 91% after treatments on June 18 and 24. These values were based upon combined means for 2nd, 3rd and 4th instar larvae.

Figure 1 shows graphs of the sample means for mayflies, combined dytiscid beetles and mosquitoes (3rd, 4th and pupal stages), for each of the fields. Major reductions in the number of mayflies and dytiscid beetles as well as mosquitoes corresponded to each application of parathion, which resulted in cycles of depression and resurgence. In contrast the two groups reached steady levels which were comparable in both the untreated and *B.t* H-14 treated fields.

Mid-June depressions in the numbers of mayflies and dytiscid beetles of the *B.t.* H-14 field were slight in comparison to the parathion-induced reductions. There was no indication that these depressions resulted from the first *B.t.* H-14 treatment, based upon the lack of response to a second treatment. Indeed the untreated mayfly population showed a similar depression. Also the first 2 weeks in the *B.t.* H-14 field were marked by unstable water depth caused by the prolonged time required to flood the field, more than twice the flooding time of the other two fields.

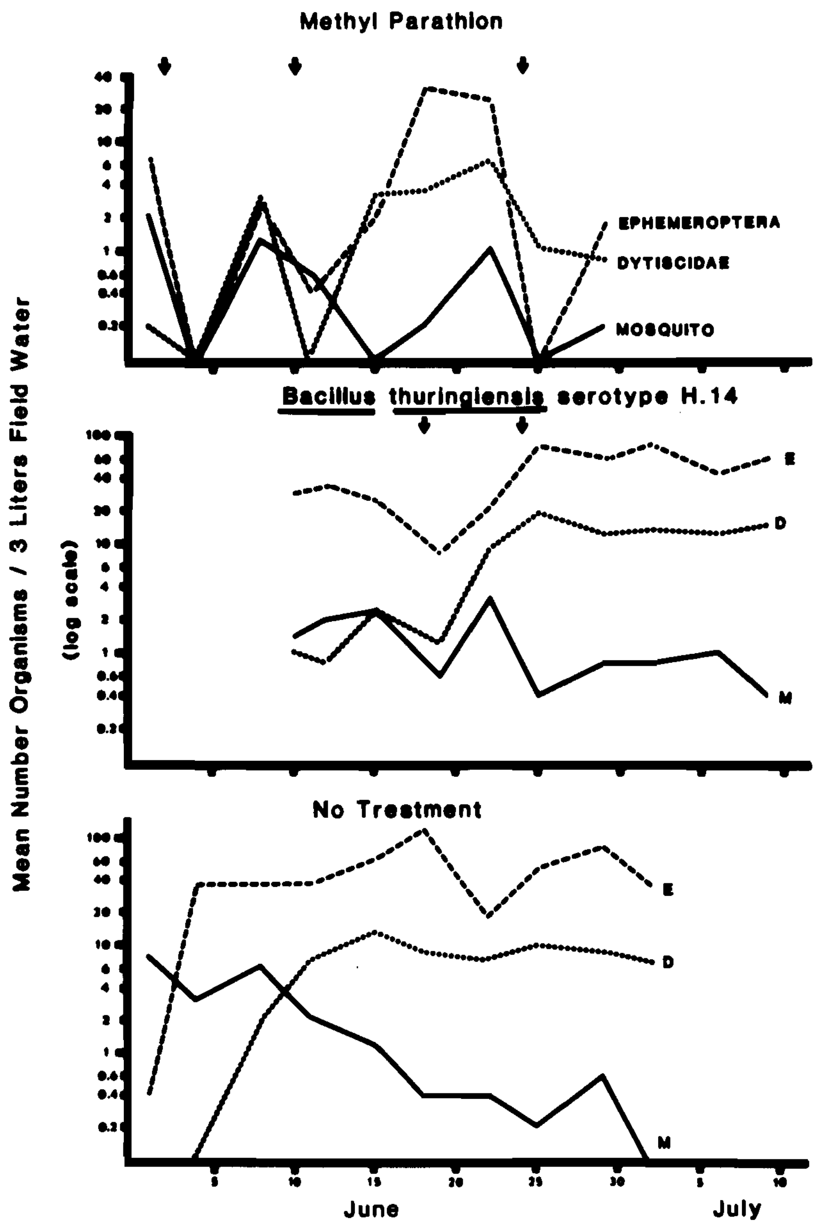

Figure 1. Comparative impacts on selected, immature, aquatic insect populations of multiple methyl parathion (0.1 kg Al/ha) and *B. thuringiensis* serotype H-14 (1.1 kg/ha) applications (arrows) to separate Mendota Wildlife Area wetlands fields, with an untreated control.

As the number of dytiscid beetle larvae in the untreated field increased, the number of older stage mosquito immatures decreased. With only natural predation and without control agent application, the number of mosquitoes in the untreated field dropped below the action threshold after 2 weeks and remained below for the duration of the study. This inverse correlation between the populations of these predators and their prey was also evident in a previous Mendota study (Mulligan and Schaefer 1981); when after a single *B.t.* H-14 application and in presence of high predator numbers, no mosquito immatures were recovered for 22 days. Predator numbers were significantly greater in the earlier study.

In this study, the integration of *B.t.* H-14 with predators did not decrease the number of necessary applications below that of parathion. (An early treatment against *Ae. melanimon* was needed but cancelled due to the prolonged period of flooding). While predacious dytiscid immatures will not suppress the number of mosquito immatures beneath an action threshold in every case, the use of a control agent compatible with predator populations is most desirable. Not only is the faunal richness of the habitat enhanced, but the possibility of benefits from natural predation is preserved.

ACKNOWLEDGMENT.–The assistance and cooperation of personnel of the Mendota Wildlife Area (CA Dept. of Fish and Game) and the Fresno Westside, Fresno and Kern Mosquito Abatement Districts is gratefully acknowledged. This work was supported, in part, by a special California State appropriation for mosquito control research.

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THE EFFICACY OF *BACILLUS SPHAERICUS* IN CONTROLLING MOSQUITOES BREEDING IN SEWER EFFLUENT[1]

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ABSTRACT

Two strains of *Bacillus sphaericus*, 2362 and 1593, produced by H. Dulmage were tested against a laboratory strain of *Culex pipiens* in secondary and primary sewer effluent. Both strains induced complete mortality although higher concentrations of the bacteria were needed in the primary effluent. Studies also showed this bacteria capable of continued growth in secondary sewer water which contained mosquito larvae at the time of inoculation.

INTRODUCTION.–*Bacillus sphaericus* is an aerobic spore-forming microorganism found in soil and aquatic systems. The first strain of *B. sphaericus* pathogenic for mosquito larvae was isolated in 1965 in California from 4th instar *Culiseta incidens* (Kellen et al. 1965). Since that time a number of strains pathogenic for mosquito larvae have been isolated around the world (WHO 1980). In general, the bacteria shows a wide range of activity against mosquito species, but is particularly active against the genus *Culex*.

B. sphaericus is known to recycle under certain conditions especially those where the water is rich in organic materials. Studies have been conducted in sewerage treatment facilities in Florida and have shown promising results in prolonged control, an apparent result of recycling of the bacteria in the sewer water. Hornby et al showed that *B. sphaericus* offered an economical method for control of *Cx. nigripalpis* in sewage units with 100% control as long as the spore concentration remained above 1×10^2 spore ml. Detectable control was observed as low as 1.7×10^1; 100% control was observed for over 60 days and was correlated with a spore count of between 6×10^4 to 2×10^3 (Hornby et al. 1979).

Studies were conducted in this laboratory to determine whether *B. sphaericus* was efficacious against *Cx. pipiens* in primary and secondary sewer effluent and the extent to which it would recycle under these conditions.

MATERIALS AND METHODS.–*Bacillus sphaericus* strains 2362 and 1592 produced by H. Dulmage[1] were supplied by E. Davidson[2]. The strains were provided in a dry powder formulation which contained approximately 10^7 spores/mg. In an initial experiment to determine the effectiveness of these strains in sewer water, a bioassay was conducted at four concentrations of the bacteria: 1×10^{-3}, 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} mg/ml. Tests were conducted in primary and secondary sewer effluent as well as in distilled water. Sewer water was collected from the Novato Sewer Plant (predominantly domestic sewage) in Marin County. A laboratory strain of *Cx. pipiens*, 3rd instar, was used as the test organism and experiments were conducted in 6 oz. glass jam jars containing a total volume of 100 ml. All tests were conducted indoors at a temperature of 21° ± 2° C. Abbott's method was used to correct for mortality in the controls.

Another experiment was designed to monitor the length of activity and degree of toxicity of *B. sphaericus* strain 2362 against *Cx. pipiens* in secondary sewer water. Jars containing 100 ml of sewer water and either 10 or 50 3rd-instar larvae were inoculated with three concentrations of bacteria: 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} mg/ml of the dried powder formulation. Mortality was recorded after 48 hours and 72 hours. After 12 days, 3rd instar *Cx. pipiens* were once again added to these jars (the dead larvae had not been removed) and mortality was recorded after 24 and 48 hours. All dead larvae were then removed from the jars and the test water for each concentration was diluted with distilled water 10, 100, and 1000-fold. Each dilution was replicated 3 times and 10 3rd-instar larvae were again used as the test organism. Mortality was recorded at 48 and 72 hours. The dead larvae that had been removed from the jars were washed several times in distilled water and added to jars containing 100 ml of distilled water. Ten *Cx. pipiens* larvae were then added each week for 6 weeks and mortality was recorded 24 hours after each addition of larvae.

RESULTS AND DISCUSSION.–*Bacillus sphaericus* strain 2362 appears to be the most active against *Cx. pipiens* in both primary and secondary sewer effluent (Figure 1). Figure1

[1]This research was supported in part by Special State Funds for Mosquito Research in California.

[2]H. Dulmage: USDA, Brownsville, Texas.

[3]E. Davidson: Zoology Department, Arizona State University, Tempe, Arizona.

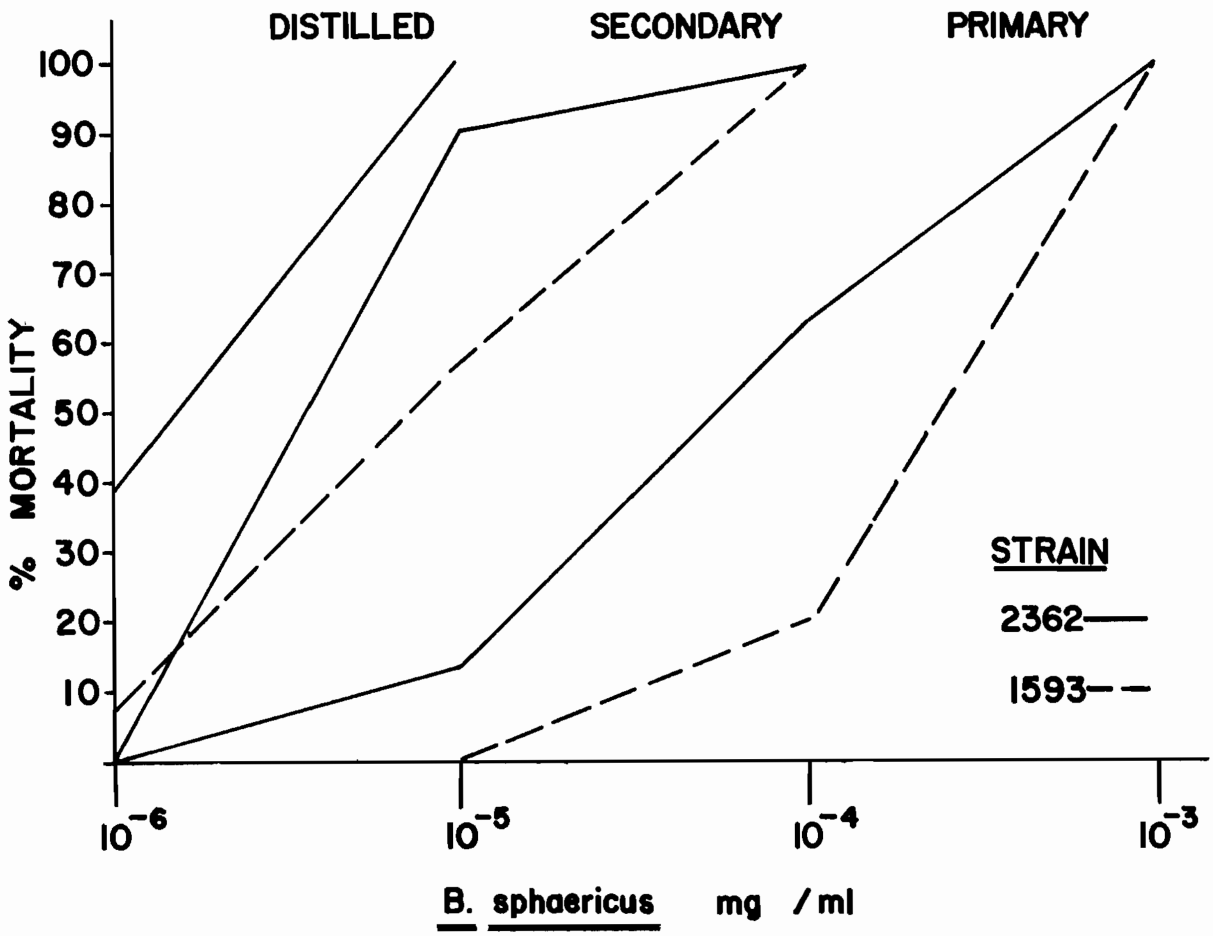

Figure 1. The effects of *Bacillus sphaericus* strains 2362 and 1593[1] against *Culex pipiens* in primary, secondary sewer effluent and distilled water. Water temperature 15±5° C.

also shows the difference in mortality rates when strain 2362 is tested against *Cx. pipiens* in distilled water. The mortality in the distilled water, although reported in this graph as post 72 hours, was actually completed between 24 and 48 hours. This was in contrast to the mortality in the sewer water which was not at all evident until 48 hours post inoculation. Thus, the observation period, which would generally end at 48 hours, was extended to 72 hours.

In the experiment designed to monitor the length of activity and degree of toxicity of *B. sphaericus*, mortality after 48 hours in the jars with 10 larvae was as expected from previously run bioassays. In the jars with 50 larvae the percent mortality was lower. For example, at 1 x 10^{-5} mg/ml the mortality was 23% as opposed to 70% in the jars with 10 larvae (Table 1). The jars were then set aside and eventually dead larvae were seen in all jars including those with the lowest concentrations. When *Cx. pipiens* was once again added after 12 days, 100% mortality was noted in jars that initially contained 1 x 10^{-4} and 1 x 10^{-5} mg/ml *B. sphaericus* and 90% mortality in jars with 1 x 10^{-6} mg/ml (Table 1). This mortality was recorded after only 24 hours. As there was no mortality in the controls, it was assumed that the *B. sphaericus* was not only responsible for the mortality

[1]Produced by H. Dulmage, USDA, Brownsville, Texas.

Table 1. Efficacy of *B. sphaericus* against *Cx. pipiens* (3rd instar) strain 2362 in secondary sewer effluent 72 hours and 13 days post inoculation.

No. larvae per jar	Concentration mg/ml	% Mortality Post-72 hours[1]	Post-13 days[2]
Sewer Water			
10	10^{-4}	100	-
	10^{-5}	70	100
	10^{-6}	0	93
	C	0	0
50	10^{-4}	100	-
	10^{-5}	23	100
	10^{-6}	0	93
	C	0	0
Distilled Water			
10	10^{-4}	100	-
	10^{-5}	100	-
	10^{-6}	37	100
	C(no food)	0	0
	C(food)	0	0

[1]Larvae added 0 hours.
[2]Larvae added post 12 days, mortality recorded post 13 days.
[3]½% solution Tetramin and liver powder added to these control (after 24 hours).

but also increasing in concentration in the test jars. The sewer water for each concentration was then diluted up to 1000 fold to determine the extent of this increase. Seventy-two hours after *Cx. pipiens* were added, extensive mortality was noted in most of the jars (Table 2). Interestingly, in jars originally inoculated with 1 x 10^{-6} mg/ml and diluted 1000-fold, mortality ranged from an average of 57% in the jars that initially contained 10 larvae to an average of 97% in the jars that initially contained 50 larvae. In the jars containing the dead larvae and distilled water, 100% mortality of *Cx. pipiens* was noted within 24 hours and each week thereafter for almost 6 weeks.

Table 2. Results of diluting sewer water held 13 days and containing three dosage levels of *B. sphaericus* strain 2362 each with 10 and 50 *Cx. pipiens* 3rd instar per test jar. Post 72 hours readings.

Original Concentration mg/ml	Dilution Factor	% Mortality 10L/JAR	50L/JAR
1 x 10^{-4}	10	100	100
	100	100	100
	1000	97	93
1 x 10^{-5}	10	97	100
	100	93	97
	1000	60	87
1 x 10^{-6}	10	87	100
	100	63	100
	1000	57	97

3 replicates/dilution. No mortality in controls.

CONCLUSIONS.–These results are extremely encouraging and support oberservations made by Hertlein et al. (1979) and others that show *B. sphaericus* capable of continued growth and activity against mosquitoes breeding in rich organic water for long periods of time. The results of the laboratory experiments presented here suggest that the bacteria are growing in the jars with the sewer water and larvae, probably using the cadavers for a nutrient source. There is also some indication that the initial population levels may be an important factor influencing the levels of bacterial growth.

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CONTEMPORARY APPRAISAL OF THE POPULATION DYNAMICS OF INTRODUCED CICHLID FISH IN SOUTH CALIFORNIA

E.F. Legner[1] and F.W. Pelsue Jr.[2]

ABSTRACT

Introduced cichlids, *Sarotherodon* and *Tilapia*, continue to flourish in south California, providing excellent biological control of chironomids, mosquitoes and aquatic weeds, as well as constituting a significant game fishery.

Populations of three African cichlids, *Tilapia zillii* (Gervais), *Sarotherodon (Tilapia) mossambica* (Peters), and *S. hornorum* Trewazas, that were introduced in the southwestern United States originally to improve the game fishery and subsequently as biological controls of aquatic weeds, mosquitoes and chironomid midges (Hallock & Ziebell 1970; Hauser et al. 1976, 1977; Legner & Medved 1973; Legner et al. 1973, 1975; St. Amant 1966), continue to flourish without management in irrigation channels and drains, golf course lakes, sewage treatment lagoons, flood control channels and the Salton Sea of south California. Although these fish can effectively reduce noxious aquatic weeds in the irrigation system (Legner 1979, Legner & Fisher 1980, Legner & Murray 1981), their manipulation has thus far proven too tedious for most irrigation districts (Legner 1979). Thus, these tropical species' effectiveness is largely confined to those water delivery channels and drains in which they can overwinter. Many of the drains in the lower Colorado Desert irrigation areas of southeastern California that have thermal inlets, sustain natural stands of these fish. However, among the channels, only the all-American and Coachella Main canals have the right conditions for overwintering. Presently, the population of *T. zillii* in the All-American Canal is large enough to evoke a significant reduction of the newly introduced aquatic weed, *Hydrilla verticillata* Royle, as well as other problem species, *Potamogeton pectinatus* L. and *Myriophyllum spicatum* var. *exalbescens* Jepson (Legner & Murray 1981).

In the Salton Sea, *T. zillii* is now one of the principal game fishes, with 1.8 k., 0.3 m-long fish commonly being caught by anglers the year round. A significant role of this herbivore/insectivore as a food source for increasing the densities of larger predatory game fish is also suspected.

The population of *S. mossambica- hornorum* complex, now firmly adapted to the Coyote Creek flood control channel in the Los Angeles basin (Legner & Pelsue 1980, Legner et al. 1980), annually attains densities exceeding 20 adult fish per square meter. Migratory populations of this fish have begun to regularly colonize other channels of the flood control system by gaining access from the littoral zone of the Pacific Ocean. Their biological control impact against midges of the genus *Chironomus* is especially pronounced, and sufficient to eliminate the need for insecticide applications. Behavioral changes in this fish population have progressed to a level where colonization of the swiftly-moving San Gabriel River with currents regularly exceeding 1 m/sec, has been possible.

Another population of *S. mossambica* has adapted to sewage treatment lagoons and golf course ponds in the Coachella Valley where chironomid midges are abated to non-annoyance levels (G. Stains, pers. commun.). Although the chironomid foraging ablity and tolerance of lower temperatures in *T. zillii* is superior to *S. mossambica* (Legner & Medved 1973), the former species has not been able to adapt to this habitat. The more fully expressed mouthbrooding habits of *S. mossambica* especially may account for this by enabling the survival of a greater number of fry in the presence of predatory bass, bluegills and catfish.

Expanded and intensified behavioral and ecological studies of these three cichlids in California are certainly justified given their obvious importance in practical mosquito, chironomid and aquatic weed suppression. However, there are presently no known scientific studies progressing.

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[2]Southeast Mosquito Abatement District, 9510 S. Garfield Avenue, South Gate, California 90280.

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PROGRESS REPORT ON MICROTURBELLARIANS AND OTHER FACTORS AFFECTING MOSQUITO ABUNDANCE IN RICE FIELDS

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The density of mosquitoes breeding in California rice fields has been shown to vary significantly from field to field, with a few fields often responsible for a large proportion of the mosquito population. It is necessary, therefore, to identify which are the most productive fields in order to effectively implement mosquito control programs.

Various factors that might contribute to differences in mosquito abundance were evaluated in this study. Possible differences between geographic areas, variation due to field age and water source, influence of rice height, presence of *Mesostoma lingua* (a microturbellarian flatworm predacious on mosquito larvae) and differences between laser planed and conventional fields were investigated.

Rice fields in the Sutter-Yuba Mosquito Abatement District were monitored every other week during the 1982 summer. Dip samples using one-pint mosquito dippers were taken by three people spaced 20 meters apart taking 3 dips in one spot at 5 meter intervals. This was repeated 10 times going out perpendicular to the levee and then 10 times returning to the levee. Mosquito and flatworm numbers were recorded the day of collection upon return to the laboratory. Water temperature, rice height and water depth were recorded for fields beginning July 13.

As suggested by Collins and Washino (1979), large flatworm populations should exert considerable pressure on the *Culex* larval population. Local mosquito abatement personnel noted that areas in North Yuba County met the requirements for high flatworm numbers. They wondered how this might fit in with unusually high light trap catches in that area in previous years. Therefore, we investigated the differences in flatworm abundance and larval mosquito populations between the area north of Marysville in North Yuba County called "District 10" and the area in South Sutter County between East Nicolaus and Rio Oso extending up to Highway 65 and South Beale Road. The flatworm numbers were slightly higher in the East Nicolaus area, but the extreme variability prohibits making a correlation between flatworm numbers and larval populations. For *Culex tarsalis* (Table 1) there is no significant difference in larval abundance in the two areas except for the period beginning 8/16 when the South Sutter area had more larvae. The South Sutter area also has more *Anopheles freeborni* (Table 2) during the periods beginning 6/21 and 8/16.

It has been hypothesized by Collins and Washino (1979) that fields during their first year in rice have a higher density of *Cx. tarsalis* larvae than those fields planted in rice for more than one year. They also suggested that fields irrigated with well water will have higher *Cx. tarsalis* densities than fields irrigated with ditch water. The results of this year's sampling (Table 3) showed no significant difference between either field age or water source. The results for *An. freeborni* (Table 4) were also compared. Combining the old and new ditch water fields and comparing larval abundance to that in well water fields, a significant difference is seen for the periods beginning 7/5, 7/19, 8/2, and 8/16. Well water field had higher larval densities in these sampling periods, while the opposite was true during the 8/30 and 9/13 sampling periods. Grouping the *Anopheles* data in terms of field age, there was no significant difference in larval abundance.

The microturbellarian flatworm *Mesostoma lingua* has been shown to prey on mosquito larvae quite readily in the laboratory and in predator exclusion sentinel cages in the rice fields. Therefore, we wanted to evaluate some factors of its distribution related to larval distribution. We found no consistent pattern (Figure 1) of distribution according to field age or water source. *Culex* larvae did have greater survivorship in sentinel cages early in the season. This coincides with the numbers of flatworms increasing later in the season as the seasonal peak for *Cx. tarsalis* is mid-July and the peak for *M. lingua* is mid-August. A definite predatory cause and effect cannot be established from these data.

The switch from tall to short stature varieties of rice has raised the question of how this might affect larval abundance.

Table 1. Comparisons of North Yuba County and South Sutter County collections of *Culex tarsalis* larvae[1].

Collection date	North Yuba	South Sutter
6/21-7/4	8.5 ± 14.4 (4)	18.5 ± 40.1 (13)
7/5-7/18	37.8 ± 54.9 (19)	32.4 ± 73.6 (22)
7/19-8/1	21.4 ± 65.7 (18)	16.3 ± 50.2 (23)
8/2-8/15	5.1 ± 9.0 (18)	19.3 ± 46.6 (22)
8/16-8/29	2.1 ± 4.1 (18)	10.8 ± 18.6 (22)
8/30–9/12	0.6 ± 1.2 (17)	--
9/13	0.2 ± 0.5 (4)	0.2 ± 0.4 (11)

[1]Figures include the mean number per 180 dips followed by the standard deviation over the number of fields in parentheses.

Table 2. Comparisons of North Yuba County and South Sutter County collections of *Anopheles freeborni* larvae in rice fields[1].

Collection date	North Yuba	South Sutter
6/21-7/4	0.0 ± 0.0 (4)	1.7 ± 2.5 (13)
7/5-7/18	1.9 ± 2.2 (19)	3.6 ± 5.8 (22)
7/19-8/1	8.7 ± 14.8 (18)	10.4 ± 18.1 (23)
8/2-8/15	45.9 ± 66.0 (18)	58.4 ± 84.7 (22)
8/16-8/29	37.6 ± 32.4 (18)	87.1 ± 71.2 (22)
8/30-9/12	47.9 ± 34.4 (17)	---
9/13	46.5 ± 43.2 (4)	42.1 ± 40.1 (11)

[1]Figures include the mean number per 180 dips followed by the standard deviation over the number of fields in parentheses.

Table 3. Comparisons of water source and mean number of *Culex tarsalis* larvae collected per 180 dips in rice fields[1].

Collection date	New Ditch	Old Ditch	New Well	Old Well
6/21-7/4	35.3 ± 36.3 (3)	0.4 ± 0.8 (7)	31.4 ± 57.7 (5)	4.5 ± 4.5 (2)
7/5-7/18	21.9 ± 36.0 (10)	22.6 ± 53.8 (14)	36.4 ± 44.0 (11)	82.3 ± 128.3 (6)
7/19-8/1	8.4 ± 11.6 (10)	16.8 ± 62.3 (15)	36.8 ± 92.3 (9)	13.3±13.5 (7)
8/2-8/15	2.2 ± 3.7 (9)	5.3 ± 8.0 (14)	16.73±27.3 (11)	39.8±82.7 (6)
8/16-8/29	1.1 ± 2.0 (9)	4.4 ± 9.8 (14)	12.6 ± 17.2 (10)	11.0±24.3 (7)
8/30-9/12	0.7 ± 1.1 (7)	0.20 ± 0.4 (5)	20. ± 2.0 (2)	0 ± 0 (3)
9/13	0.5 ± 0.7 (2)	0.25 ± 0.5 (4)	0 ± 0 (6)	0.33 ± 0.58 (3)

[1]Figures include the mean number of larvae ± standard deviation over the number of fields in parentheses.

Table 4. Comparisons of water source and mean number of *Anopheles freeborni* collected per 180 dips in rice fields[1].

Collection date	New Ditch	Old Ditch	New Well	Old Well
6/21-7/4	0.0 ± 0 (3)	0.1 ± 0.4 (7)	0.6 ± 0.5 (5)	0 ± 0 (2)
7/5-7/18	0.1 ± 0.3 (10)	0.9 ± 1.1 (14)	2.4 ± 4.4 (11)	8.0 ± 8.22 (6)
7/19-8/1	3.3 ± 2.7 (10)	2.7 ± 2.6 (15)	7.1 ± 9.5 (9)	37.0±24.8 (7)
8/2-8/15	16.5 ± 9.9 (9)	20.7 ± 16.2 (14)	69.6±101.3 (11)	151.2 ± 80.1 (6)
8/16-8/29	23.9 ± 16.7 (9)	52.9 ± 52.8 (14)	89.1 ± 71.8 (10)	106.9±68.6 (7)
8/30-9/12	63.4 ± 28.2 (7)	45.4 ± 48.3 (5)	32.0 ± 28.3 (2)	26.3±16.7 (3)
9/13	81.0 ± 25.5 (2)	42.5 ± 59.1 (4)	37.8 ± 35.8 (6)	26.0±12.3 (3)

[1]Figures include the mean number of larvae ± standard deviation over the number of fields in parentheses.

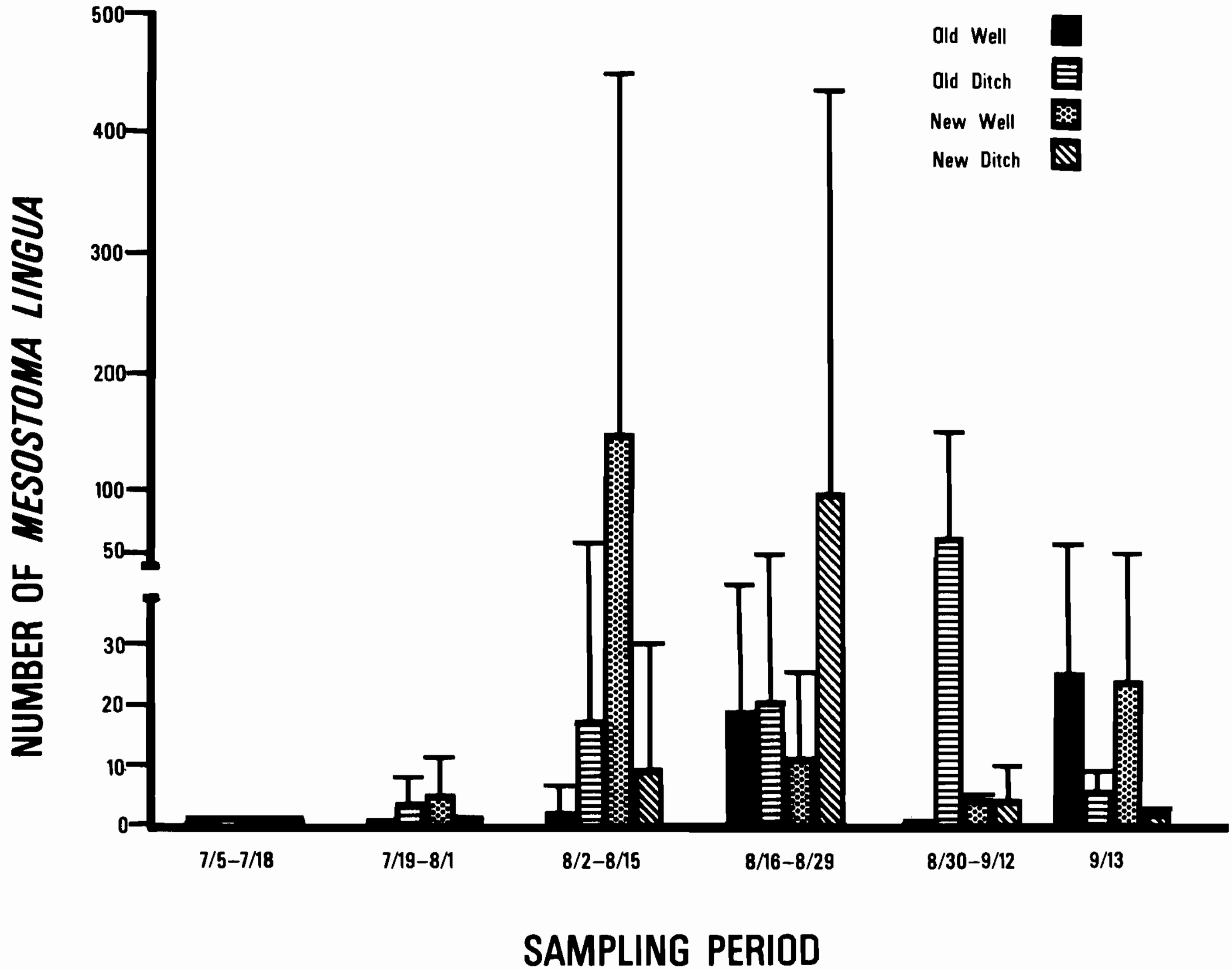

Figure 1. Number of *Mesostoma lingua* collected in dip samples (180 dips with standard mosquito dipper).

Looking at *Cx. tarsalis*, we found a slight negative correlation for the period beginning 7/19 and 8/2, with increased mosquito numbers with shorter rice. Other sampling periods showed no correlation between rice height and numbers of *Culex* larvae. This may be an artifact of the developmental time of the larvae being related to the planting time of the rice, although the data was grouped into 2 week sampling periods to minimize this effect.

Fields were evaluated for the possible effect of laser planing on mosquito breeding. Changes in field preparation result in changes in check size, levee contours, water depth, water flow and character of vegetation along the levees. There was no significant difference between laser planed and conventional fields for the *An. freeborni* or the *M. lingua*. However, there was a difference for the *Cx. tarsalis* with laser planed fields producing more larvae ($\overline{x}$ = 28.24 ± 59.9 larvae/180 dips, n = 71) than conventional fields ($\overline{x}$ = 9.04 ± 30.9 larvae/180 dips, n = 140).

When evaluating the influence of the previously mentioned factors, it is important to bear in mind the complexity of the interactions. Also, definitive statements regarding influence of individual factors cannot be made from one season's sampling.

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EVALUATION OF *GAMBUSIA AFFINIS, LEPOMIS CYANELLUS* AND THEIR INTERACTIVE EFFECT ON MOSQUITO CONTROL

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ABSTRACT

A study was initiated in 1982 to compare the effects of the mosquitofish, *Gambusia affinis*, and the green sunfish, *Lepomis cyanellus*, on mosquitoes and other organisms in experimental rice plots. Four treatments were compared in 24 (83 m^2) plots: 1) control - no fish, 2) *G. affinis* - 10 female and 10 male adults, 3) *L. cyanellus* - 20 adults (size range: 6.5-10.5 cm), and 4) both fish - 20 adults of each species. Fish were stocked between 15 June and 18 June.

Analyses of variance were performed comparing the numbers of dip-collected mosquitoes and various predators in the different treatments. The analyses incorporated seven sampling dates from 27 July to 7 September. *Culex tarsalis* and *Anopheles freeborni* were as abundant in the fish plots as in the control plots. *A. freeborni* populations were higher in the *L. cyanellus* plots than in the *G. affinis* plots ($p < 0.05$). Notonectids were more abundant in the control plots than in the fish treatment plots ($p < 0.01$), suggesting that these fish may directly reduce the population of this family by predation. Differences in the abundances of Belostomatidae, Anisoptera, Zygoptera, *Mesostoma* spp., and *Tropisternus* spp. (all potential predators of mosquitoes) between treatments were not demonstrated.

SURVIVAL AND PREDATOR EFFICIENCY OF *GAMBUSIA AFFINIS* FOR CONTROL OF MOSQUITOES IN UNDERGROUND DRAINS

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ABSTRACT

Mosquitofish, *Gambusia affinis* (Baird and Girard), survived in an impounded water area within an underground storm drain system in Fresno, California through a 14 week period during the summer of 1982. The number and relative condition (K-factor) of the stocked fish slowly declined with the depletion of the food supply, as determined by fish gut content analysis, at the tenth week after introduction. However, the mosquitofish were effective in reducing the number of adult *Culex quinquefasciatus* produced in the drain system. Reductions of 75, 89 and 94% below those of an untreated control area were obtained after the first, second and third month, respectively. While female mosquitofish (gravid before introduction) produced offspring, no mating of fish within the drain was found.

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PREY SELECTION BY *GAMBUSIA AFFINIS* IN CALIFORNIA RICE FIELDS:

EFFECT OF VEGETATION AND PREY SPECIES

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ABSTRACT

Prey preferences of *Gambusia* were tested both in a synthetic Plexiglas® ricefield model in the laboratory and in screened enclosures in rice fields. Three zones of vegetation within the rice field were selected (open water, submerged vegetation, and mature rice stand) with corresponding simulation in the laboratory by the use of appropriate plastic aquarium plants. Fish were presented with a selection of live prey items consisting of equal portions of up to three of the four possible species (fourth instar mosquito larvae of *Culex tarsalis*, adult cladocerans of *Daphnia pulex*, adult amphipods of *Hyalella azteca*, and second instar corixid bugs of *Cenocorixa* sp.). Fish were given 10 minutes to consume prey and then preserved in alcohol. Gut analysis of the stomach contents determined the number of each prey species consumed. Preliminary statistical analysis consisting of student's t-tests showed *Cx. tarsalis* to be the preferred species, *D. pulex* and *H. azteca* to be intermediate, and *C.* sp. to be the least consumed. Presence of vegetation increased overall prey consumption and differentially affected prey species selection. *Cx. tarsalis* left exposed by submerged vegetation were preferentially consumed over *H. azteca* which took refuge in the bottom vegetation.

CONTINUOUS PRODUCTION OF *GAMBUSIA AFFINIS*

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ABSTRACT

Methodology, as well as production figures, for a continuous production culture system for *Gambusia affinis* was investigated. Preliminary work indicated that fish grown at 25°C grew as fast as those grown at 30°C and survival was better at 25°C. Fry grown at a density of .43 fry/l grew faster than fry at a density of .85 fry/l although total length gain per system was greater at .85 fry/l.

In continuous production trials fry were raised to 5 months of age. During this time gravid females in the population were induced to spawn at 3,4 and 5 months. Number of males mature, females gravid, females that dropped fry and number of fry dropped were recorded. Fry produced per female that dropped was calculated. Results showed a marked increase in all categories at 5 months.

INTRODUCTION.–California has experienced a chronic shortage of mosquitofish, *Gambusia affinis*. Traditionally fish are seined and collected from local reservoirs of waterways such as sloughs. Consequently, there has been a progression of methods used to produce large numbers of these fish. Outdoor methods to date include overwintering ponds (Coykendall, 1977), raceways for artificial production (Reynolds 1977), and small shallow ponds for spawning fish (Johnson 1976). More recently, geothermal ponds have been utilized (Cheyne 1981). However, all three of these methods are subject to environmental stress such as drought, contamination and predation and there is a limited availability of geothermal sites. A fourth, more ideal alternative would be to produce fish in an intensive culture system which has both temperature and water quality control. Drazba and Gall (1980) reported on the feasibility of intensive culture of mosquitofish under confined space. This last alternative was selected as the method of choice and experiments were conducted to investigate the feasibility of continuous production of fish year-round at Contra Costa Mosquito Abatement District.

Continuous production in this report is defined as the year-round production of fry, which can only be accomplished if a constant supply of gravid females is available. In order to achieve this, each experiment had three phases: (1) holding populations of adults, (2) spawning chambers, and (3) growth of fry. The limitations of these phases would be relative to the carrying capacity of the system which is defined as "the animal load that a system can hold" (Spotte 1979). Initial work by Drazba (1980), indicated optimal fry growth around 1 fry/ liter, which was the basis for the following growth and production studies.

Basic growth parameters such as temperature and fish density for optimum production have been suggested by Cech (1981) and Drazba (1980) respectively. As both were deduced by small scale experiments, we chose to re-test these parameters in our production system. Based on preliminary results from our density and temperature work, we designed the following approach to achieving a continuous production system. Initial fry would be obtained by bringing gravid females into the lab and inducing them to spawn by isolating them and elevating the temperature. Fry would be separated into four groups and grown-out. At maturity these fry would be thinned out to mate. Gravid females would then be isolated and induced to spawn at predetermined intervals. Fry produced would be subsequently grown-out in the lab, space permitting, or removed to stock ponds. Some fry would be retained to replace aging broodstock as needed.

MATERIALS AND METHODS.–The District converted a small storage building into an aquaculture laboratory for production of fish. Within the building there are two independent, recirculating aquatic systems as previously reported (Beesley 1981). Each system is comprised of four tanks (7′ x 3′ x 18″) connected to a sump (22″ x 22″ x 30″), a 55-gallon cylindrical carboy biofilter and a 10-gallon water heater which is in turn controlled by a thermoregulator. Water is pumped from the sump to the biofilter, gravity fed to the tanks and returns to the sump by gravity overflow. The biofilter is composed of 4 layers and types of rock, a 6″ bottom layer of California Gold Rock, a 6″ layer of dolomite, a 6″ layer of pea gravel and a 6″ top layer of aquarium gravel. Both systems are fully independent of each other.

In all trials the initial fry were obtained by bringing gravid females into the lab, placing them in 4″ by 4″ strawberry baskets lined with 1/8″ or 8x8 mesh window screen and elevating the temperature to 30°C. This induced 90% of the females to drop fry within 7 days (unpublished data). Fish were fed twice daily with Tetramin® flake (ad libitum) and supplemented with 4 g of *Artemia nauplii* weekly. Photoperiod was maintained at 16:8 light-dark by fluorescent lights on an automatic timer. All measurements were made in total length.

The two densities tested were .85 fry/l and .43 fry/l; trials were conducted at 25°C for 31 days. Two replicates of each density were tested in each system. The two temperatures tested were 25°C and 30°C; trials were conducted for 8 weeks at .24 fry/l. Three replicates of each temperature chosen were tested in each system.

Two consecutive continuous production tests were conducted for five months at 25°C in one system. Each trial consisted of 1800 fry obtained from a mass spawn of wild caught gravid females. Four tanks were initially stocked with 450 fry each (1 fry/liter) for fry grow-out. Populations were thinned out at eight weeks to 120 fish/tank (.3 fish/l) for the duration of the test. Sexually mature males and females were counted and spawning tests were run at 3, 4 and 5 months. Spawning tests were conducted by transferring the gravid females to the other system at 30°C and isolating females in individual chambers for two weeks. Numbers of females that spawned were noted and fry were counted. Females were then returned to the original tank and system at 25°C. This procedure was repeated at four and five months, although mature males were not recorded at four months.

RESULTS AND DISCUSSION.—Growth rate was significantly greater (F.05) at .43 fry/l than .85 fry/l (Table 1), while survival exceeded 90% at both densities. Drazba and Gall (1980) reported the highest rate of growth for fry occurred at a density of .8 fry/l, which probably reflects differences in system designs including rate of flow, biofilter size, and raceways or tanks. Regardless of the differences in optimal density, both studies indicate that maximal biomass gain/system was achieved at the higher density. In other words, although the growth/fry was slower at .8 fry/1, total length gain for all fish was greater: 210 vs. 114. Length gain is calculated by multiplying the total number of fish times average gain/day/fish. Consequently, from a mass-rearing standpoint the number of fish being reared would be more important than the density/liter provided mortality is not high.

As seen in Table 2, there was no significant difference in growth rate (F. 05) at 25°C vs. 30°C while there was significant difference (F.01) in survival (F.01). Mortality was predominant at the higher temperature and was observed to occur primarily in young males between 4-6 weeks of age in both trials.

The results of continuous production trials are seen in Table 3 and all gravid females were isolated for spawning. There was a constant increase in gravid females and mature

Table 1. Average growth in *Gambusia* fry, measured in total length (mm) at two densities (25 C; 31 days).

Fry per liter	Total number fry	Initial length	Final length	Length gain	Gain/day/fish	Total length gain	Survival %
.85	1400	10.1	14.7	4.6	.15	210	92
.43	600	10.1	16.0	5.9+	.19	114	96

+ indicates a significant difference at the 5% confidence level.

Table 2. Average growth in *Gambusia* fry, measured in total length (mm) at two temperatures (.24 fry/l; 8 weeks).

Temperature C	Total number fry	Initial length	Final length	Total length gain	Gain/day/fish	Total/gain/day	Survival %
25	300	7.0	25.9	18.9	.34	102	97
30	300	7.0	25.1	18.1	.32	97	70++

++ indicates a significant difference at the 1% confidence level.

Table 3. Continuous production of *Gambusia affinis* (25° C; .3 fish/l).

Trial	Age (months)	Number fish	Number mature males	Number gravid females	Number females dropped	Number fry dropped	Fry/female
1	3	480	17	44	9	54	6.0
	4	452	–	30	6	60	10.0
	5	443	122	137	110	836	7.6
2	3	480	14	56	7	64	9.1
	4	464	–	46	22	125	5.7
	5	458	108	69	52	634	12.2

Table 4. Maturation and reproduction of *Gambusia affinis* in a continuous production system, an average of two trials.

Age (months)	% gravid females	% mature males	% gravid females spawned	Number fry/female spawned	Number fry produced
3	10	3	16	7.38	59
4	8	—	37	6.61	92
5	23	26	70	9.07	735

males with time. There was no apparent difference between trials in numbers of mature males over time but there was a substantial difference in number of gravid females at the 5 month mark, 137 in trial 1 vs. 69 in trial 2. Nevertheless, in both trials the percentage of gravid females that dropped fry was more directly associated with time. This continuum was also seen in the increase in total number of fry dropped. A more complete picture is provided in Table 4 which is a summation of both trials. Increased egg development by females was evident by comparing the percentage of sexually mature males and females to the percent which spawned. Although the number of fry/female dropped slightly between months four and five, the total number of fry produced continued with time, with a marked increase the fifth month (1470 fry). This could be associated with the increase in number of mature males at five months (26%), but this could also be due to the increase in gonadotrophic activity as exhibited by the marked increase in gravid females (23%).

The fecundities attained (Table 4) were lower than those realized by Busack (Gall, personal communication), for *Gambusia* of the same age in isolated aquaria, 22.8 fry/female at 2.5 months of age and much less than wild females induced to spawn in this system, 39.3 fry/female of unknown age (unpublished data). The higher fecundities were probably due to the larger size of the females used by Busack, and the older age of fish used in our unpublished data. Although fry were observed once from fish two months old our data does not support the contention that early maturity and reproduction at 60 days (Busack 1981) is to be expected in a closed recirculating system used for continuous production.

CONCLUSIONS.—Based upon the results, intensive culture of *Gambusia affinis* is feasible. Success would be predicated on a continuous supply of gravid females and production of adequate numbers of fry. The number of fry per female may not be as critical to overall success as the total number of fry produced as seen in Table 4, and the total length gain for the system used appears to be more important than actual gain/day/fish as seen in Table 1.

Within the limits of a small scale system there are two possibilities for manipulating mass production. One would be to have a reliable source of gravid females, induce them to spawn, and transplant fry for appropriate uses. Brood stock would have to be replaced when fry production declined. During the colder months, brood stock would best be replaced by females which had matured under artificial conditions, as those brought in from the field are not likely to spawn within a desired period of time. The other method would be continuous production. In this method the supply of gravid females is produced by raising fry to maturity. Thereafter, excess fry obtained from this brood stock can be removed to alternate sites for appropriate use whether field stocking or over the counter distribution to the public. There are two principle differences between these two methods, in the former the brood stock is maintained outside the lab and all the fry are transplanted, while in the latter the brood stock is maintained within the system and a small percentage of fry are periodically retained and raised to maturity to produce the next brood stock. The first method is limited by availability of gravid females in the colder months. With continuous production a year-round supply of fish is more realistic and production would be a function of the size and efficiency of the system.

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BROADENED VIEW OF *MUSCIDIFURAX* PARASITES ASSOCIATED WITH ENDOPHILOUS SYNANTHROPIC FLIES AND SIBLING SPECIES IN THE *SPALANGIA ENDIUS* COMPLEX

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ABSTRACT

Strain differences of fecundity, cold hardiness, mating behavior, response to RH, and habitat foraging exist within some species of the *Muscidifurax* clade. The heretofore considered *Spalangia endius* Walker of Australia is probably a sibling species completely reproductively isolated from the *S. endius* of New Zealand and North America.

The genus *Muscidifurax* comprises a group of closely related species (superspecies) of parasitic Hymenoptera which attack pupae of Muscidae in accumulated animal wastes. Only five species have been described (*M. raptor* Girault & Sanders, *M. zaraptor* Kogan & Legner, *M. raptoroides* Kogan & Legner, *M. raptorellus* Kogan & Legner, and *M. uniraptor* Kogan & Legner) (Kogan & Legner 1970), but additional species probably exist (Legner 1969, Kogan & Legner 1970).

All five described *Muscidifurax* species occur in geographic isolation in the Nearctic and Neotropics, except two species which are sympatric in the western Nearctic. The suspected ancestor of the clade is widely distributed in Europe, Africa, North America and probably occurs in Australia, New Zealand, Hawaii and the Philippines, etc. (Legner 1972; Legner & Olton 1968; Legner et al. 1967, 1976). It has not been found in the Neotropics, and there are no known clinal patterns. The genus has not been reported from Asia nor equatorial regions (Legner & Olton 1968, Legner & Greathead 1967, Legner et al. 1967).

Distinct morphological characteristics separate the five species (Kogan & Legner 1970, Legner et al. 1976), which also possess distinctive courtship patterns (van den Assem & Povel 1973). Pronounced behavioral differences among the species include partial and full gregariousness, solitariness, thelytoky, arrhenotoky, gigantism, variable reproductive potentials and capacities to penetrate the host pupal habitat, etc. (Kogan & Legner 1970; Legner 1969, 1967, 1977; Legner et al. 1976).

The genus *Muscidifurax* usually occurs sympatrically with another genus of parasitic wasps, the *Spalangia*, and more rarely with the genera *Figites*, *Trichopria*, *Pachycrepoideus* and *Sphegigaster* (Legner & Greathead 1969, Legner & Olton 1968, Legner et al. 1967). However, *Muscidifurax* species are primarily peripheral foragers in the accumulated excrement habitat, while the *Spalangia* habitually range to greater depths in search of host pupae (Legner 1977). Other parasitic species associated with Muscidae either attack different host developmental stages (eggs and larvae), or appear in competition for pupae with the intrinsically superior *Muscidifurax*, which could account for their comparative scarcity (Legner 1977).

Muscidifurax species are best adapted to cooler weather periods prevalent in late spring and autumn at temperate latitudes (Ables & Shepard 1976, Legner 1977).

Because *Muscidifurax* are prevalent in or near accumulated animal excrement where they parasitize host flies that also breed selectively in this habitat, they fit the endophilous eusynanthropic category (Legner et al. 1974), placing their very existence outside of the Ethiopian Region wholly dependent on human habitation. In sub-Saharan Africa, wild bovines produce such suitable natural accumulated excrement habitats as reported by Legner & Greathead (1969) in East Africa. This apparent dependence on the barnyard existence outside of Africa suggests that the genus is exotic to the Western Hemisphere and its persistence depends on continued human agrarian settlement (Kogan & Legner 1970, van den Assem & Povel 1973).

The identified sibling species, all confined to the Western Hemisphere, could have evolved within the recent time period of European settlement, or during the past 400 years (Kogan & Legner 1970, van den Assem & Povel 1973). There is only one record of a *Muscidifurax* species being deliberately imported in the Western Hemisphere, to Hawaii from South Africa (Silvestri 1914), but 10 years after the genus had already been described from Illinois (Kogan & Legner 1970).

Therefore, the genus *Muscidifurax* very probably invaded from the Eastern Hemisphere (Europe or Africa). However, if it did exist in America prior to European colonization, it must have been greatly restricted in population density compared to its present widespread distribution in accumulated excrement. It is rarely collected outside the eusynanthropic fly habitat, such as in wild animal dung, birds nests, etc. This is substantial evidence for its pre-Columbian restricted population size, if it occurred in America at all. Whatever the case may be, the advent of Europeans with their livestock greatly increased host habitat and population size, and in turn guaranteed much larger numbers of parasite individuals. The resultant greater capability for interbreeding may have provided more opportunity for gene recombination. Local temporary explosions of *Muscidifurax* on the European-created high fly host densities may have allowed for repeated temporary survival of a greater number of genotypes before severe competition eliminated them. These normally unexpressed genotypes could then have had repeated opportunity for mating with others in the explosive population sites, creating greater opportunities for new gene combinations to occur, and possibly new species to evolve. In this manner, the situation may have been akin to that of the colonial checker-spot butterfly, *Euphydryas aurinia* (Rott.) in England (Ford & Ford 1930), and the common house sparrow, *Passer domesticus* (L.) in North America (Johnston & Selander 1964). Also, refer to Remington (1968) for additional possibilities.

Marked strain differences have been recorded within *M. zaraptor*, including fecundity, cold-hardiness, heat tolerence and habitat foraging capability (Legner 1977, Legner & Badgley 1982). For biological control, *M. zaraptor* appears as the most suitable candidate to introduce for increased muscid mortality. However, the addition of other species of this genus to the muscid ecosystem is not as apt to increase natural control. For example, the gregarious *M. raptorellus* compensates for its apparent high fecundity on a single host by reduced searching capacity (Legner 1967). On the other hand, *M. uniraptor*, a thelytokous species, is more narrowly adapted to extremes in temperature and RH than the other species, and its continuous production of ♀♀ progeny is not certain. Cultures either produce predominantly sterile ♂♂ progeny after several days of oviposition (Legner, unpublished data), or show reduced reproductive potential when continuous hosts are present (Legner & Gerling 1967).

A case of sibling species is now apparent within *Spalangia endius* Walker. Cultured isolates from Perth and Mareeba, Australia are completely reproductively isolated from New Zealand and American isolates of what, heretofore, has been considered the same species (Legner, unpublished data). However, behaviorally, the New Zealand strain of *S. endius* is more distinct from American strains than are the isolates from Australia and America, which are behaviorally more similar to each other than are either to the New Zealand strain (Legner & Badgley 1982; Legner, unpublished data). For biological control, the New Zealand strain with its higher fecundity and tolerance of low RH and temperatures, is a better candidate to increase natural control of muscid flies than the Australian sibling. However, whether these superior traits can persist after hybridization with resident American strains in the field remains to be verified.

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ECOLOGICAL IMPACT OF MOSQUITO CONTROL RECIRCULATION DITCHES ON SAN FRANCISCO BAY MARSHLANDS: STUDY CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

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ABSTRACT

From 1977 to 1983, a series of field studies have examined the ecological impact of mosquito control recirculation ditches on selected aspects of San Francisco Bay salt marshes, including water table height, groundwater and soil surface salinity, pickleweed *(Salicornia virginica)* production, plant diversity, aquatic invertebrate diversity and biomass, fish diversity and density, salt marsh song sparrow *(Melospiza melodia samuelis)* density, and terrestrial arthropod diversity, biomass, and population density; results of these studies are summarized. Three recommendations based on ecological and environmental considerations are suggested: accurately identify salt marsh ponds that produce mosquitoes; avoid ditching in marshes with porous soils; minimize ditch depth. Pond inundation height, pond area, and percentage cover of emergent vegetation may be used to predict which ponds will produce mosquitoes and should be ditched. Ditching in porous soils may alter marsh plant composition and require high maintenance costs. Shallow ditches result in less spoil and less pond drainage than deep ditches, and are equally effective in mosquito control. Salt marsh management requires effective, economical, environmentally compatible mosquito control, which a properly designed ditching program can offer.

INTRODUCTION.–The use of recirculation ditches for mosquito control in tidal salt marshes has evolved through several stages over the past century. Designs have included gridded networks of ditches to drain the entire marsh (Smith 1904), shallow, hand-dug ditches to connect ponds to tidal channels (Reiley 1951), and the diverse procedures of Open Water Marsh Management programs including dendritic ditching, pond deepening, and the addition of pond radials (Ferrigno and Jobbins 1968). Unfortunately, the recommendations for ditch construction offered by Ferrigno and Jobbins are specific to salt marshes on the Atlantic coast, since Pacific coast marshes differ in geomorphology and plant composition (Chapman 1974). The only design criteria for ditch construction in Pacific coast salt marshes were provided by Herms and Gray (1940), but economic factors, ditching equipment, and knowledge of tidal marsh dynamics have changed dramatically in the past four decades and have rendered these recommendations somewhat obsolete.

At present, ditches in Pacific coast marshlands connect ponds to natural tidal channels. They are dug using a hydraulically operated plow pulled by a Spryte all-terrain vehicle; the ditches are steep-sided, and 45 cm wide and 60 cm deep. After completion of a ditch, the Spryte operator drives the vehicle back over the spoil line to press the spoil down and eliminate the possibility of inundating tidal water being trapped behind the spoil.

In 1977, we undertook a project to evaluate the ecological impact of such ditching on San Francisco Bay salt marshes (Resh and Balling 1979, Resh et al. 1980). This research was then followed by a study to determine methods for optimizing ditching efficiency (Balling and Resh 1983a). This paper presents a summary of the results of both studies and offers specific recommendations for the use of ditching in the management of Pacific coast salt marshes.

STUDY IMPLICATIONS AND MANAGEMENT RECOMMENDATIONS.–The results of our impact studies in selected San Francisco Bay marshes (Figure 1) suggest that ditches have little of what would traditionally be considered as adverse ecological effects on marsh plants, terrestrial arthropod communities, fish, or birds (Table 1). However, a comparison of ditched with unditched potholes indicates that ditching does adversely affect aquatic invertebrate diversity (Table 1).

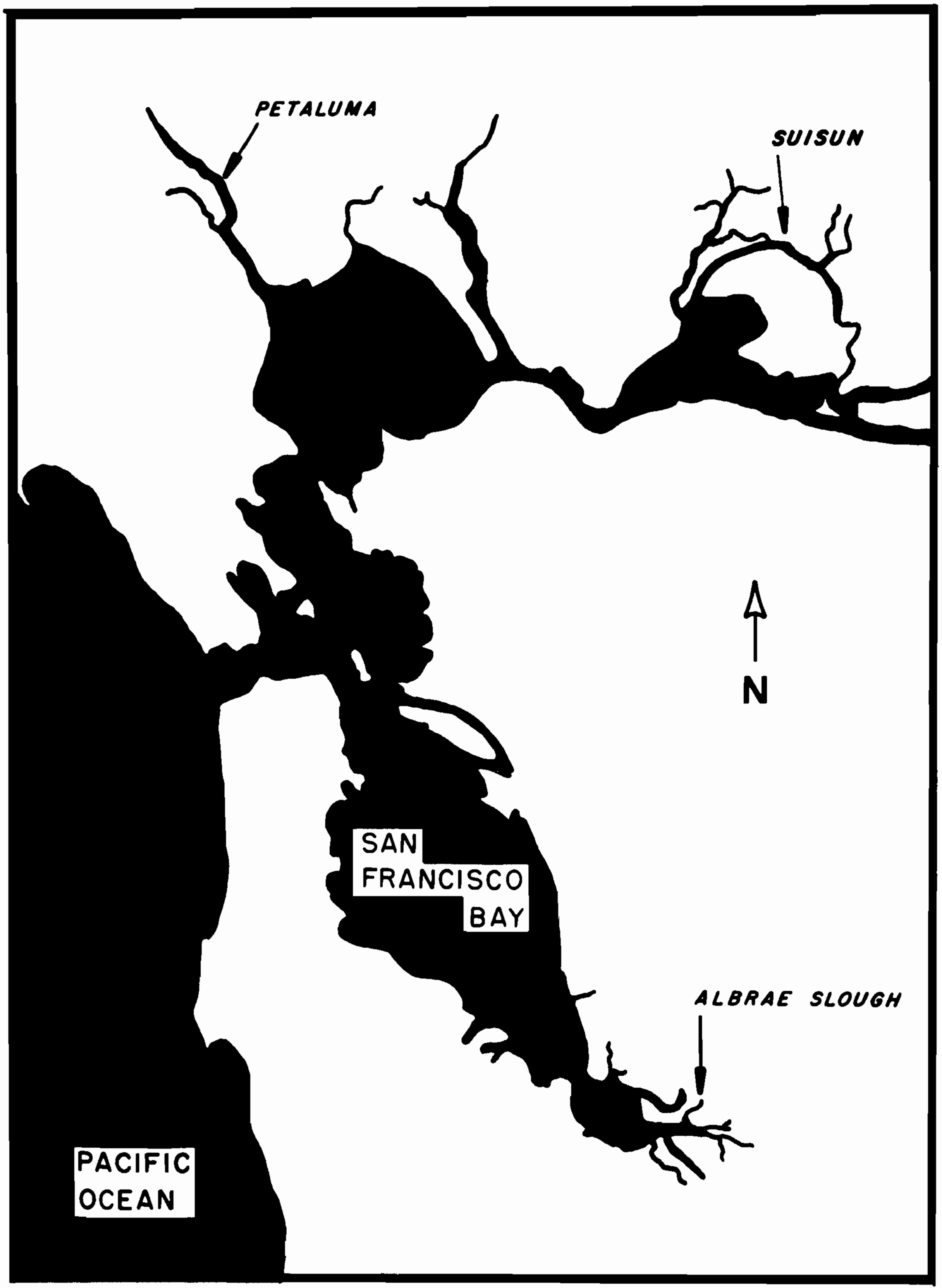

Figure 1. Location of the salt marsh study sites referred to in Table 1.

These results suggest that unnecessary ditching should be avoided in San Francisco Bay salt marshes.

In order to reduce the ecological impact of mosquito control recirculation ditches, and increase the effectiveness and efficiency of their use, we have three recommendations that should be considered in the design of future ditching programs:

1) Ponds that produce mosquitoes should be identified, and only these ponds should be ditched.
2) Ditching should be avoided in marshes with porous soils.
3) Ditch depth should be minimized.

Identification of which ponds produce mosquitoes. By accurately distinguishing the ponds that produce mosquitoes and should be ditched from those that do not produce mosquitoes and should not be ditched, the amount of unnecessary ditching can be minimized. There are both ecological and economic reasons for not ditching ponds that will not be mosquito production sites. First, significant hydrological, and consequently biological, changes occur within ditched ponds, as indicated by the decrease in invertebrate diversity (Table 1). Second, it is impractical to consider monitoring every pond in a marsh to determine which produce mosquitoes (e.g. there are over 15,000 ponds in Petaluma Marsh), and it would be both ecologically and economically imprudent to ditch all ponds in a marsh.

In order to develop accurate and economically expedient criteria for the identification of mosquito-producing ponds, we studied the mosquito populations of 44 ponds in eight marshes

Table 1. Summary of studies designed to evaluate the effects of mosquito control recirculation ditches on selected physical and biological features of San Francisco Bay marshlands.

Feature	Study Site	Localized Effect	Reference
water table height	Petaluma Marsh	decreases	Balling and Resh 1983b
	Suisun Marsh	no change	Balling and Resh 1983b
groundwater salinity	Petaluma Marsh	decreases	Balling and Resh 1983b
	Suisun Marsh	decreases	Balling and Resh 1983b
soil surface salinity	Petaluma Marsh	decreases	Balling and Resh 1983b
pickleweed (*Salicornia virginica*) production	Petaluma Marsh	increases	Balling and Resh 1983b
plant diversity	Suisun Marsh	increases	Balling and Resh 1983b
terrestrial arthropod diversity			
wet season	Petaluma Marsh	decreases	Balling and Resh 1982
dry season	Petaluma Marsh	increases	Balling and Resh 1982
year round	Suisun Marsh	no change	Balling and Resh 1982
terrestrial arthropod biomass	Petaluma Marsh	no change	Balling and Resh 1982
terrestrial arthropod population densities	Petaluma Marsh	no change (in >50% spp.)	Barnby and Resh 1980, Barnby and Resh 1983
aquatic invertebrate diversity	Petaluma Marsh	decreases	Resh and Balling 1983
aquatic invertebrate biomass	Petaluma Marsh	no change	Resh and Balling 1983
fish diversity	Albrae Marsh	increases	Balling et al. 1979, Balling et al. 1980
fish density	Albrae Marsh	increases	Balling et al. 1979, Balling et al. 1980
salt marsh song sparrow (*Melospiza melodia samuelis*) density	Petaluma Marsh	increases	Resh and Balling 1983; Collins and Resh, in press

surrounding the San Francisco Bay to determine which environmental factors may be used as predictors of larval occurrence and abundance (Balling and Resh 1983a). From this analysis, it became apparent that the most important predictor of both occurrence and abundance of mosquito larvae was pond inundation height. Ponds that are frequently inundated, e.g. those at or below an elevation that is halfway between mean high water (MHW) and mean higher high water (MHHW), will not produce mosquitoes, and thus should not be ditched. Above this height (which is the mosquito production threshold in San Francisco Bay salt marshes), pond area and percentage cover of emergent vegetation also become important predictors. Ponds above the mosquito production threshold will generally produce mosquitoes if they are less than 100 m^2 in size; ponds above the threshold and greater than 100 m^2 will produce mosquitoes if they have greater than 30% cover of emergent vegetation, as often occurs when the pond's banks grade into the surrounding vegetation. Using pond inundation height, pond area, and percentage cover of emergent vegetation, we have developed decision rules that permit accurate identification of potential mosquito-producing ponds (Balling and Resh 1983a).

Avoid ditching in porous soils. In Atlantic coast salt marshes, ditching in porous soils has often resulted in a substantial lowering of the marsh water table, which has promoted the invasion of woody, upland shrubs (Bourn and Cottam 1950, Miller and Egler 1950). Soils of San Francisco Bay marshes are composed primarily of silts and clays, and as a result, the water table drainage in these marshes only occurs within a 2 to 3 m distance perpendicular to the ditch. Although this drainage locally diminishes the physical harshness of the salt marsh (e.g. less waterlogging of the soils, lower soil surface and groundwater salinities), it is apparently insufficient to promote the invasion of woody, high marsh or upland shrubs along the ditch banks (Balling and Resh 1983b).

Although absent along the shallow ditches, shrubs such as *Baccharis douglasii* and *Grindelia humilis* are commonly found along the larger and deeper sloughs of San Francisco Bay marshes. This invasion by upland and high marsh shrubs indicates that, given sufficient drainage, some species are capable of colonizing the lower elevations of Pacific coast salt marshes. The addition of ditches in porous soils (e.g. sandy soils in coastal marshes) could therefore drain the water table sufficiently to promote such an invasion, and consequently alter the plant community structure of the marsh.

A second reason to avoid ditching in sandy soils is that since sand is not cohesive, the ditch banks cannot be supported. The resultant bank slumping greatly increases maintenance costs, and may well promote additional mosquito production within the blocked sections of ditches.

Minimize ditch depth. Ditches need not be deep to be effective. As noted in our discussion on identifying which ponds produce mosquitoes, mosquito breeding does not occur below the mosquito production threshold (i.e. the elevation halfway between MHW and MHHW, Balling and Resh 1983a). The addition of a ditch effectively lowers the height of a pond to the height of the ditch bottom; therefore, it is only necessary for a ditch to lower pond height to below that of the mosquito production threshold for mosquitoes to be eliminated. Even in the highest marshes of the San Francisco Bay, a ditch only 40 cm deep will serve this purpose.

Shallow ditches offer two advantages in marsh management; they result in (1) less spoil and (2) less pond drainage. Linear placement of large amounts of spoil parallel to the ditch can cause the formation of small dikes, behind which tidal water can be trapped and mosquito production occur (Miller and Egler 1950). The greater the volume of spoil produced, the greater is the risk that dikes may form. In contrast to linear placement and flattening, if the spoil is piled in mounds to reduce the problem of dike formation, the typically planar, unbroken topography of the coastal marsh is disrupted. In order to avoid the possibility of creating artificial dikes or making excessive changes in marsh appearance, ditches dug in the San Francisco Bay Area have usually followed existing swales (which are often tidal channels that have filled with sediments), thereby making use of the natural drainage patterns of the marshes.

Shallower ditches also increase the number of ponds in which water remains during low tide. These areas can then serve as refuge for resident fish populations, such as the mosquitofish *(Gambusia affinis)*, the three-spine stickleback *(Gasterosteus aculeatus)*, and the rainwater killifish *(Lucania parva)* (Balling et al. 1979, 1980).

Ultimately, ditches will seek their own depth according to the size of the area they drain. That is, ditches will fill with sediments until water movement during ebb tide is sufficient to remove newly deposited sediment (Krone 1982). As a result, ditches should progressively fill from the pond-ditch interface (where the least movement occurs) toward the natural channel (where the most movement occurs). The rate at which a functioning ditch will fill is directly related to tidal sediment load, and indirectly related to drainage area. Maintenance should be necessary only for ditches that are blocked by vegetation or debris; this appears to happen infrequently in San Francisco Bay marshlands. For example, over 300 linear meters of ditches were dug in 1969 in a section of Petaluma Marsh; as of 1982, less than 5% of these ditches were blocked and in need of maintenance.

The effectiveness of shallow ditches in eliminating mosquitoes is apparently related to the frequency of tidal inundation. Past reports have suggested that ditches control mosquitoes by the tidal flushing of larvae out of the ditched ponds (Smith 1904), or by exposing larvae to increased predation by fish that have entered the habitat as a result of the improved access afforded by ditches (Ferrigno and Jobbins 1968, Balling et al. 1979, 1980).

To examine this problem further, we blocked five ditched ponds in Petaluma Marsh during neap tides, when ditched ponds are least frequently inundated. As a result of the manipulation, these ponds were no longer flushed by tidal action and were no longer accessible to immigrating fish populations. Following the next set of inundating tides, mosquito abundances in these ponds were compared to five similar, but unditched, ponds. Although four of the natural ponds contained mosquito larvae, none were found in the blocked, ditched ponds. The results of this test suggest that it was neither the fish nor the tidal flushing that eliminated mosquito larvae, but rather that no larvae were ever present in the habitat. This could be a result of the inhibition of either oviposition or egg hatching. Ferrigno and Jobbins (1968) have alluded to the need for a natural pond to be properly conditioned before it will produce mosquitoes; such conditioning may include making suitable oviposition sites available for *Aedes* females, or allowing sufficient drying time for the eggs prior to flooding (Horsfall 1963). Ditches, at least in San Francisco Bay salt marshes, apparently disrupt one or both of these requirements through an increase in inundation frequency, which is a consequence of the ditch lowering the inundation height of the pond.

CONCLUSION.–Decisions in salt marsh management are complicated by the numerous and often conflicting demands made by different regulatory agencies and interest groups on these habitats. Oftentimes mosquito control practices are in conflict with these demands. The development of specific, empirically determined guidelines for maximizing mosquito control, yet minimizing environmental disturbance, can simplify management decisions. The three recommendations that have evolved from the studies summarized above can assist regulatory agencies in providing both economical mosquito control and reduced ecological impact in the control of salt marsh mosquitoes in San Francisco Bay marshlands.

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COMPARISON OF SAMPLE PATTERNS FOR *CULEX TARSALIS* IN RICE FIELDS

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ABSTRACT

Most immature *Culex tarsalis* were found in the exit water end of the rice fields studied. Two sample patterns presented proved less time consuming than evenly spaced on transect patterns and showed equal or lesser sampling variability. Coupled with a sequential sampling plan either of the two patterns would allow for a rapid determination of treatable population levels.

INTRODUCTION.–Sampling for *Culex tarsalis* Coquillett in rice fields presents problems for those concerned with control of this mosquito. Fields where these mosquitoes are found usually are large in size and when population densities are low it is often difficult to sample since *Cx. tarsalis* exhibits a marked clumping in distribution (Mackey and Hoy, 1978; Stewart et al., 1983). Efficient methods are needed in order to assess populations in large areas under rice cultivation. Sequential sampling allows for increased efficiency in time usage when determining population levels above or below certain set limits. This technique has been applied to sampling of rice field mosquitoes previously (Wada et al., 1971; Mackey and Hoy, 1978; Stewart et al., 1983) and allows for a rapid determination of treatable population levels. Sequential sampling will help in optimizing effort if the sampler knows where in the field to take samples. The purpose of the work presented here was to establish critical sampling zones and provide for an efficient method in application of a sequential plan.

MATERIALS AND METHODS.–Four rice fields in western Fresno County were sampled intensively over the years 1980 through 1982. These fields were an average of 10.1 hectares in size and have been in rice for ten or more years with only infrequent fallow periods. Sampling was done using the standard white enamel dipper (473 ml). Four different sample patterns were used over the study in determining population levels of *Cx. tarsalis*.

The first sampling method, used in 1980, was establishment of 100 fixed sampling sites on each of four fields. The sampling sites were evenly spaced over the fields and sampling took place weekly over the rice growing season.

In 1981 one field out of this group was sampled biweekly using a transect pattern. Two transects were used and ran the length of the field from the water inlet portion to the water exit end. Using two transects a total of 52 samples were taken on each sample date. There was an even spacing of dipper samples with two samples taken per paddy over the length of the field on each transect.

Samples taken in 1982 on two fields involved the use of three different methods. The first was a transect pattern similar to that in 1981 but with the addition of samples so that 100 samples were taken per field. The other two patterns involved sampling from selective sites within the field instead of trying to cover the entire area. One of these patterns was a series of three semicircular sampling transits in three different areas of the field. The second was a zigzag pattern starting at the water exit end of the field and diagonally traversing the paddies moving toward the central section of the field. Figure 1 shows these two patterns on a rice field. Using either the zigzag or semicircular patterns, 60 samples were taken per field per sample date. These two patterns were used in order to evaluate what we considered time saving methods.

In 1982 a large field in Kings County (approx. 90 ha) was sampled using three transects across the field. Figure 4 shows the sampling transects in relation to paddy configuration and water flow in the field. Fifty to 100 samples were taken evenly along each transect line on 23 sample dates during 1982.

The contents of each dipper sample taken were counted for each immature stage present, recorded and returned. A sample of collected larvae was preserved in alcohol for identification in the laboratory. The amount of time necessary to complete sampling for each of the patterns was recorded.

Data was analyzed to provide mean values per field and variance associated with the mean. Ninety five percent confidence limits and the coefficient of variance (CV) were calculated for each mean value. An analysis was made of the location of capture of mosquitoes in the field relative to water flows for each method.

RESULTS AND DISCUSSION.–Figure 2 shows the distribution of *Cx. tarsalis* on a rice field in Fresno County from samples taken over three years. Over the three year period using any of the four sample patterns described, sixty percent or more of the immature mosquitoes were found in water exit one third of the field. Comparison of data collected in 1982 showed that transect sampling and zigzag and semicircular pat-

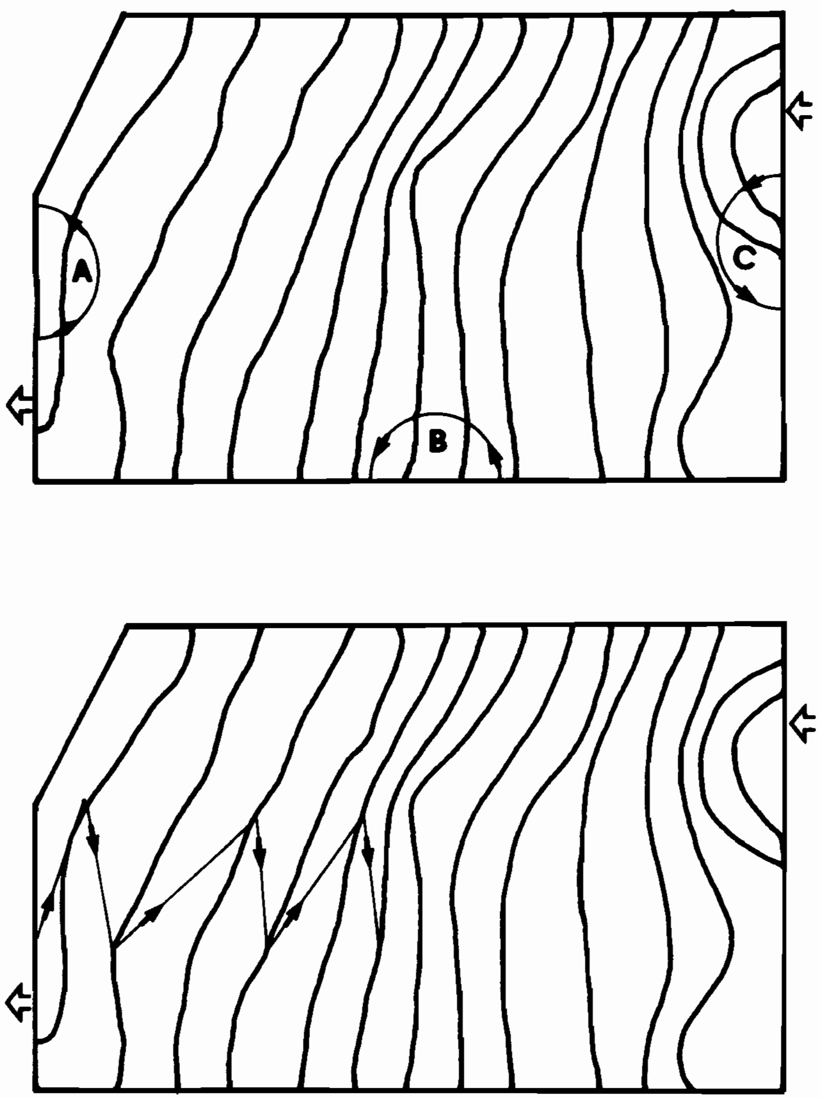

Figure 1. Above: Semicircular sample pattern. Below: Zigzag sample pattern. Open arrows indicate water flows into and out of the field.

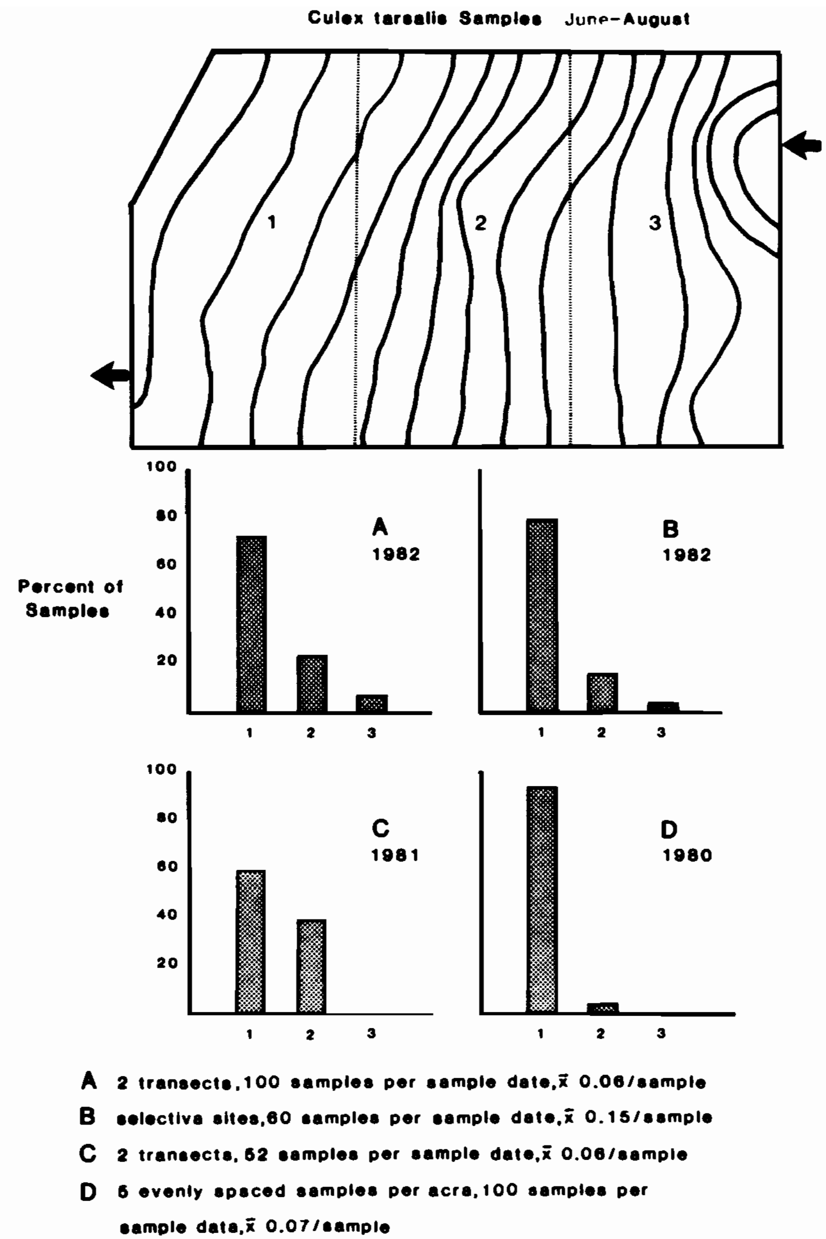

Figure 2. Distribution of *Cx. tarsalis* on a rice field in western Fresno County Graphs show percent of mosquitoes sampled from different zones within the field over the season.

terns indicated almost the same proportions of immatures in the different areas of the field. In this field, for the three years, ninety percent of the mosquitoes were found in the lower two thirds of the field.

Figure 3 shows three fields in Fresno County and the distribution of mosquitoes during the 1980 sampling season. Twenty percent or less of the mosquitoes were found in the one third of the field where water entered.

Shown in Figure 4 is the distribution of immatures on a rice field in Kings County. For the entire summer season of 1982 twenty percent of the mosquitoes were found nearest the water inlets to the field. This field produced large numbers of *Cx. tarsalis* and yet the water outlet and middle portions of the field supported the largest proportion of the population.

Our data show that on fields which generally do not produce large numbers of *Cx. tarsalis* (western Fresno County) most mosquitoes can be expected on the water exit half of the field. Even with a large mosquito producing field like that in Kings County the numbers can be expected to be greatest in the water exit portion of the field. Mackey and Hoy (1978) also indicated that the lower elevations in the rice fields they sampled seem to produce the most *Cx. tarsalis* immatures. With this in mind it would seem appropriate for sampling intensity to be concentrated in areas of rice fields where the largest part of the populations could be found. This could be used to simplify the use of a sequential sampling plan in allowing the sampler to take samples from areas most likely representing the mosquito populations.

Table 1. Comparison of confidence limits (95% CL) and coefficient of variation (CV) for samples taken during 1982 using different sample patterns. Means below 0.06 per sample were omitted.

mean/sample	Semicircular and Zigzag Patterns 95% CL	CV
0.13	± 0.12	3.62
0.23	± 0.16	2.83
0.30	± 0.19	2.47
0.33	± 0.18	2.15
0.36	± 0.17	2.00
0.42	± 0.26	2.40
0.46	± 0.36	3.33
	Transect Pattern	
0.06	± 0.07	4.00
0.08	± 0.07	3.38
0.22	± 0.21	3.36
0.40	± 0.38	3.43

A comparison of confidence limits and coefficients of variation (CV) for different sample patterns is shown in Table 1. Mean values of less than 0.06 per sample were omitted because the variances were at or near mean values and confidence limits would thus be large. Values below 0.06 would also indicate populations of mosquitoes at low enough levels so

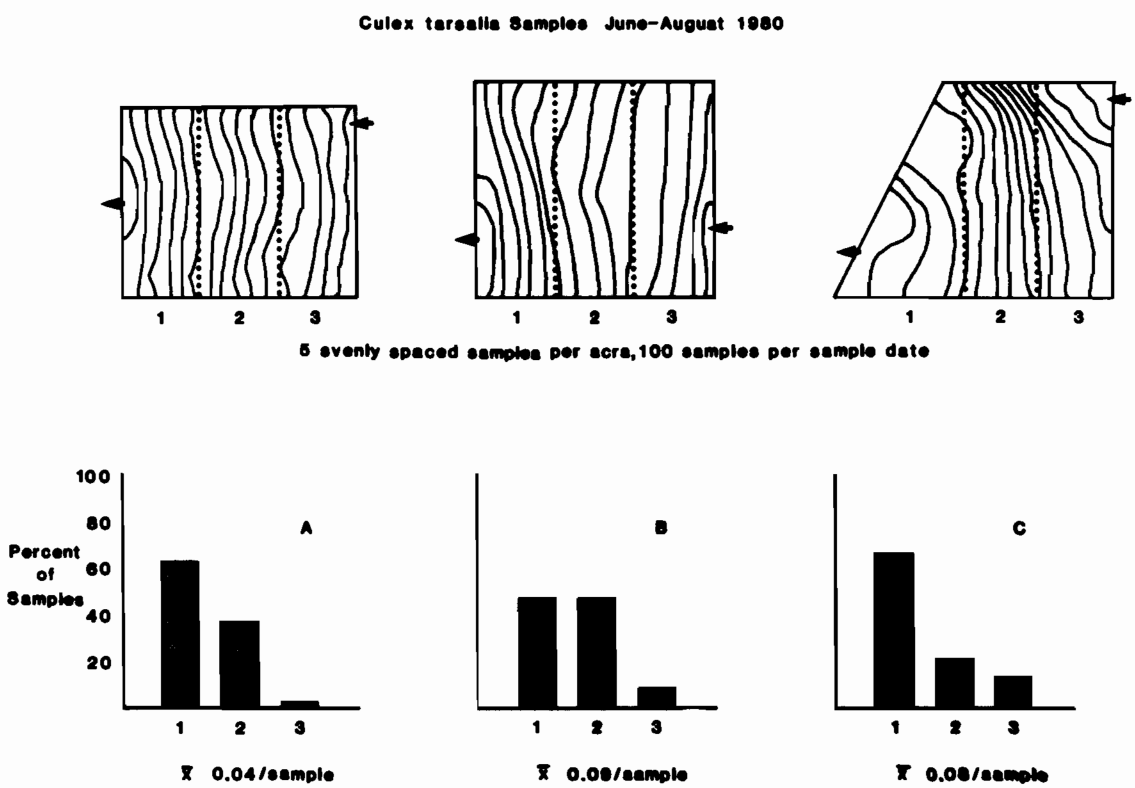

Figure 3. Distribution of *Cx. tarsalis* on three rice fields in western Fresno County. Graphs show percent of mosquitoes sampled from different zones within the field over the season. Arrows on the diagrams indicate water flows.

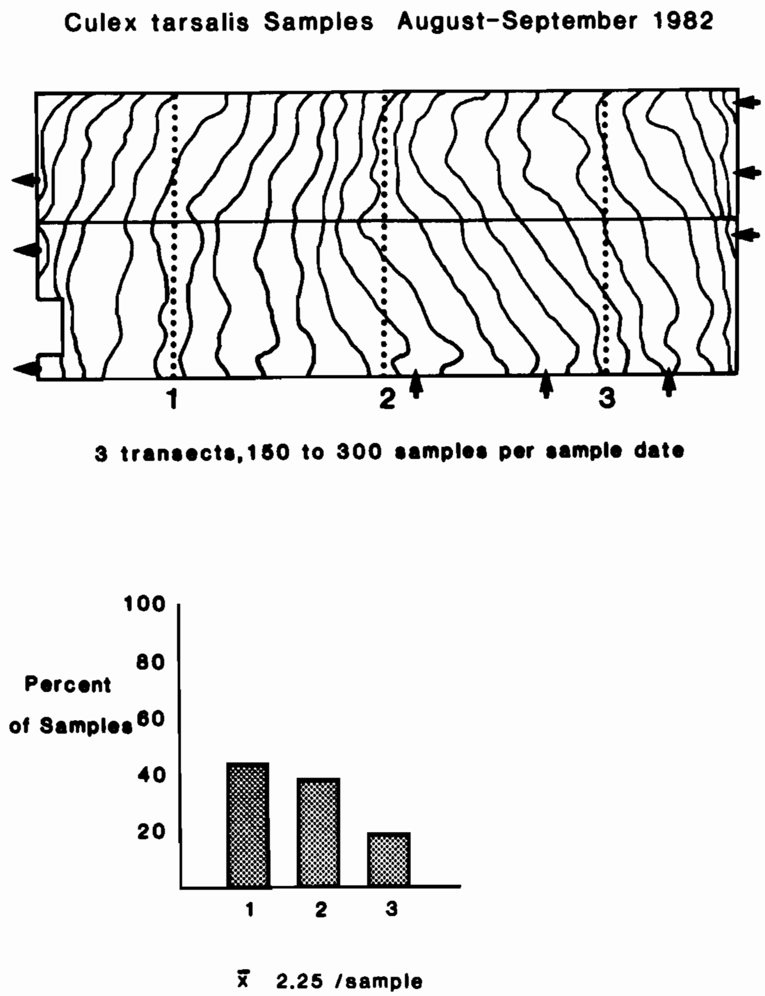

Figure 4. Distribution of *Cx. tarsalis* on a rice field in Kings County. The graph represents the percent of mosquitoes samples along designated transects in the field. Arrows on the diagram show water flows.

as not to be of concern for most control programs. The CVs shown for the semicircular and zigzag patterns are close to or lower than those for transect sampling. This indicates that these patterns have equivalent or lower variation than transect sampling and in terms of sampling error are not inferior.

Using the transect method we found that it took about one hour for one person to cover a 10 ha field completing 60 samples. For semicircular or zigzag sampling it took close to 30 minutes for 60 samples. The time considered here included return of the sampler to his vehicle. Samples taken in 1980 with evenly spaced samples over the entire field were clearly the most time consuming averaging over two and one half hours per person per field.

CONCLUSIONS.–We have found that most of the immature *Cx. tarsalis* can be expected in the lower or water exit portion of rice fields. By sampling in the lower and mid portions of the fields 80% or more the field's population would be subject to being sampled. The water entrance portion of the field could be considered as having the least likelihood of any portion of the field for producing mosquitoes.

The use of a sequential sampling plan like those presented by Mackey and Hoy (1978) or Stewart et al. (1983) could make assessment of treatable populations of *Cx. tarsalis* much more time efficient. A minimum sample size of 30 (Mackey and Hoy 1978) or 60 (Stewart et al., 1983) when no immatures are found would mean one half hour or less of sampling time per field. If population levels were high or in the range of concern for treatment (0.10 per dip for the local mosquito district) sequential sampling could allow for even less time spent assessing the population.

From our study we concluded that we could eliminate the water entrance end of the field from sampling. In the use of a semicircular pattern this would mean taking 30 samples from the exit end first and then another 30 from the mid portion.

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We thank Koda Farms Inc. and the Fresno Westside MAD personnel for their cooperation.

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SPATIAL DISTRIBUTION OF *CULEX TARSALIS* LARVAE WITHIN RICE PADDIES

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ABSTRACT

Data obtained from field studies indicate that *Culex tarsalis* breeding in rice fields were contagiously distributed within paddies and population densities were greatly enhanced by exposing foliage of submerged vegetation by lowering irrigation water depth.

INTRODUCTION.–It is well known among mosquito control personnel that the densities of immature mosquito populations in rice fields differ by a great magnitude from one field to another; most of the fields produce few or none, but some fields produce a considerable number of mosquitoes. Many speculations are made to explain this phenomenon, for example: (1) ovipositional preference of female mosquitoes (Kato, 1955), (2) high content of nitrogenous compounds (Schaefer et al. 1982) and (3) abundant larval food material (Nakamura et al. 1971) in irrigation water produce more mosquitoes. On the other hand, high population densities of natural enemies (Case and Washino, 1979) and algal toxin (Gerhardt, 1956) in the water prohibit mosquito production.

For the past several years, we have been studying rice field mosquitoes in the San Joaquin Valley. We have found that more immature stages of *Culex tarsalis* Coquillett, a dominant species of rice fields, were collected from lower ends (water exit side) of rice fields than higher ends, and within a paddy, some areas produced more mosquitoes than the others. In order to find out the cause of this patchy distribution of *Cx. tarsalis* in paddies the following study was undertaken during the 1982 rice growing season.

MATERIALS AND METHODS.–The most north end of Koda's rice field no. 5 located approximately 6 miles southwest of Dos Palos, CA in Fresno County was used for this study. The paddy measured ca. 1.5 acres and produced high numbers of immature *Cx. tarsalis* (2.14 larvae + pupae/dip). The paddy was arbitrarily divided into 80 sections in a grid pattern, a single dip sample was taken from each section and kept separately in alcohol vials. In the laboratory all samples were examined under dissecting microscopes for species and stages. To elucidate the relationship between mosquito production and habitats, all dip sites were classified into the following 6 categories:

1. Dense rice plant stand and deep water - dense growth of submerged vegetation present but foliage were not exposed to air.
2. Dense rice plant stand and shallow water - foliage of submerged vegetation exposed to air.
3. Sparse rice plant stand and deep water - no submerged vegetation exposed.
4. Sparse rice plant stand and shallow water - foliage of submerged vegetation exposed.
5. No rice plant and deep water - no submerged vegetation exposed.
6. No rice plant and shallow water - foliage of submerged vegetation exposed.

RESULTS AND DISCUSSION.–Table 1 shows the dipping results. Most mosquitoes were collected from rice growing areas where foliage of submerged vegetation were exposed to air. Figure 1 illustrates the relationship between densities of mosquito collection and the six categories of physical feature of the paddy. The frequency distribution of immature collections is shown in Figure 2. These data clearly indicate a contagious distribution; that is, more mosquitoes were collected from the areas where the foliage of submerged vegetation were exposed to air than the areas where the foliage of submerged vegetation were not exposed. Frequency distribution of collection are skewed to the left and the variance

(10.7) exceeded the mean (2.14).

The study area is a well established permanent rice field, therefore populations of many indigenous natural enemies of mosquitoes--damselfly, dragon fly nymphs, beetle larvae etc.--are well established. In addition to these indigenous predators, usually the Fresno Westside MAD plants ca. 500 to 700 mosquitofish per acre. These indigenous and augmented natural enemies suppressed mosquito population below the economical threshold level.

As the spring of 1982 was unusually cold and wet, rice sowing in these fields were delayed several weeks into early May. Consequently, to accelerate rice growth, water temperatures in the fields were raised by reducing the water depth. This practice resulted in a tremendous mosquito production. It was apparent that physical barriers created by dense exposed foliage of the submerged vegetation had a significant effect on mosquito production by providing predator-free areas for oviposition and larval development. Mosquito larvae had evolved predator avoidance and escape mechanisms by hiding within exposed foliage of submerged vegetation. This finding may explain in part why more immature stages of mosquito collections were made from isolated areas of (1) dense rice plant growth, (2) tall water grass or rice plant lodging, (3) foot prints or (4) animal nests in rice paddies.

In summary, results of field studies clearly indicate that *Cx. tarsalis*, breeding in rice fields are contagiously distributed within paddies and population densities were greatly enhanced by exposing foliage of submerged vegetation through lowered water levels. In use of the sequential plan for estimating

Table 1. Effect of various physical conditions within paddies on mosquito production.[a]

Rice Plant Stand (B)	Submerged vegetation (A) submerged	exposed	Mean (B)
Dense	2.71 (7)	3.98 (45)	3.34
Sparse	2.27 (15)	3.62 (8)	2.94
No Plant	0 (1)	.25 (4)	.12
Mean (A)	1.66	2.62	2.14

[a] Numbers in the table denote number of immature mosquitoes/dip; numbers in parenthesis indicate sample size.

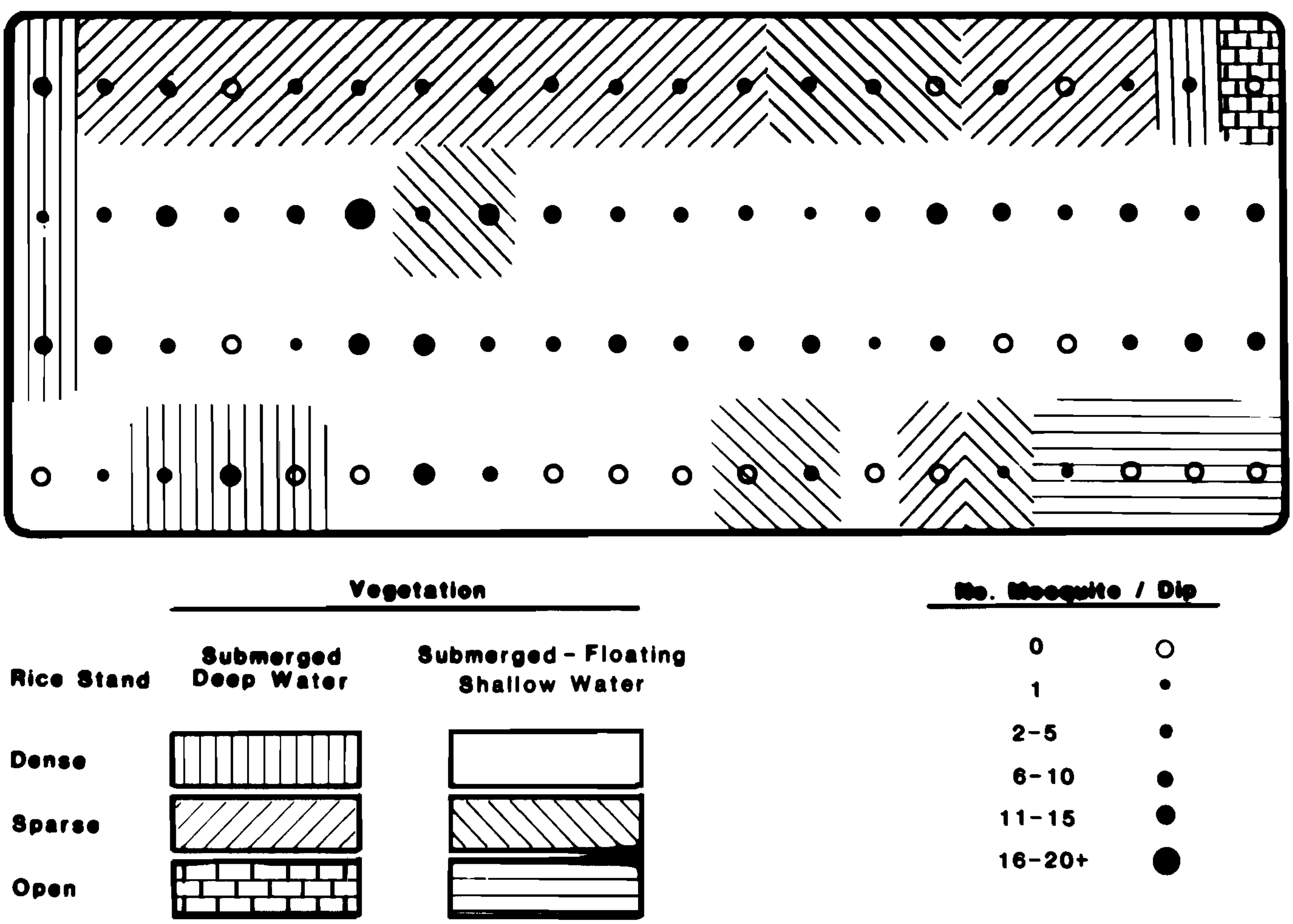

Figure 1. Schematic diagram showing the relationship between habitats and immature mosquito collection.

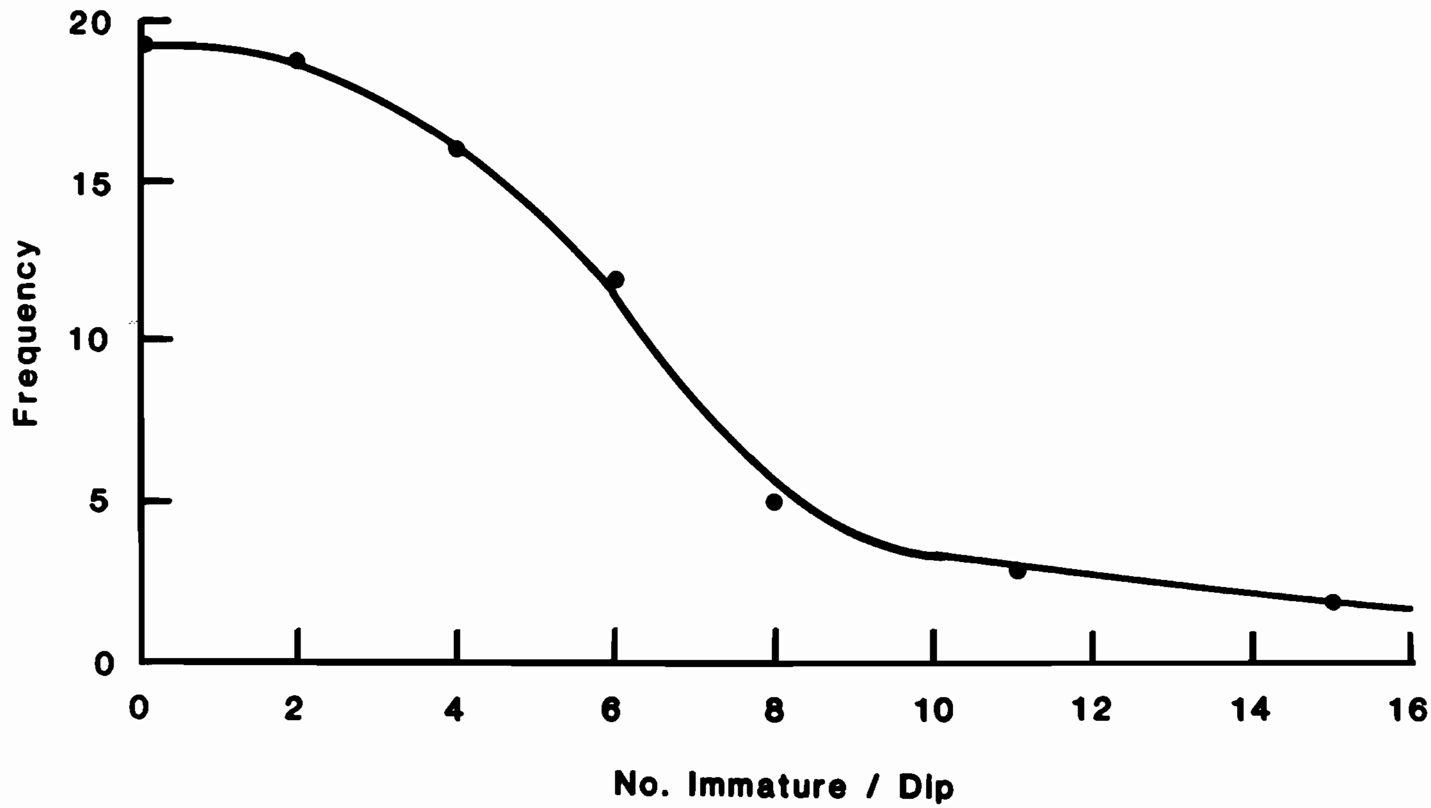

Figure 2. Frequency distribution of immature stages of *Culex tarsalis* in a rice paddy.

population densities of *Cx. tarsalis*, sampling sites should be selected with care to include these hot spots of mosquito breeding areas.

ACKNOWLEDGMENT.–This study was supported, in part, by a special California State appropriation for mosquito control research and by USDA cooperative agreement No. CR-806771-01. We thank the Koda Farm and the personnel of the Fresno Westside Mosquito Abatement District for cooperation.

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THE RELATIONSHIP OF MOSQUITO PRODUCTION TO DAIRY WASTE WATER MANAGEMENT: A REGRESSION ANALYSIS

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ABSTRACT

A stepwise multiple regression was used to determine correlation between variables which described dairy waste water management practices and associated mosquito production. The survey included dairies within the Delta Vector Control District (Tulare County, CA). Mosquito production from waste water storage ponds was found to be less when the following management conditions were employed: (1) A full pond level was maintained by mixing and/or refilling with fresh water after irrigation pumping or by a reduced storage capacity in relation to waste water input. (2) Weeds were eliminated in and around the pond. (3) Wind-induced, surface wave action was promoted by a square shaped pond construction and by elimination of freeboard. (4) Agitation of the ponded water was provided by irrigation pumping or by recycling of waste water for flush-down. (5) Solid waste was prevented from entering the pond through the use of a mechanical separator or a small, rectangular solids separating pond.

INTRODUCTION.–In the Central Valley of California, used water derived from dairy operations is usually discharged into waste water storage ponds. Generally the rich organic waste is only temporarily stored until it is utilized in irrigation of agricultural crops, to which it adds fertilizing value. However this process of collecting and temporarily storing manure-laden water often results in severe mosquito problems. For the Delta Vector Control District (VCD) of Tulare County, the cost of controlling *Culex quinquefasciatus* Say emergence and dispersal from waste water storage ponds is one of the biggest expenditures of district funds.

During the past 10 years, small, single-family dairies have been largely replaced by big, commercialized operations. As a result of increased herd size and water consumption, the size of waste storage systems has likewise increased. Within this group of new, large dairy facilities, there is a wide range of waste water management schemes. These different management types exhibit the whole gamut of mosquito breeding problems, from very low to high.

To evaluate the relationship between mosquito production and waste water management, a study was conducted during the summer of 1982 in cooperation with the Delta VCD. The problem was addressed by proposing to correlate actual mosquito production to various variables which could describe the management practices employed by dairies within the district for the handling of waste water.

METHODS.–To gather pertinent data, a survey questionnaire was developed which addressed all aspects of dairy operation in relation to the management of waste water. Quantification of these management characteristics was accomplished by visiting each dairy within the district and by interviewing each owner/operator. Cooperation from the dairymen was excellent, as questionnaires were completed for 50 of the 52 possible dairies.

Management and construction characteristics for each of 84 ponds were gathered and quantified and condensed from the questionnaires into 16 independent variables. These included:

1. LENGTH of pond,
2. WIDTH of pond,
3. DEPTH of pond,
4. SHAPE (length ÷ width) of pond,
5. STORAGE capacity of pond,
6. RECYCLE (recycling waste water for flush-cleaning alleyways),
7. MIXFILL (mixing fresh water in the pond and/or refilling pond with fresh water),
8. PUMPFEQ (pumping frequency for irrigation),
9. floating SOLIDS coverage on pond,
10. WEEDS coverage on pond,
11. SEPATOR (presence of mechanical solids separator),
12. number of milking cows in HERD,
13. IRRIG (acreage of land irrigated with waste water),
14. HERDIRR (herd size in relation to irrigated acreage),
15. STORIRR (storage capacity in relation to irrigated acreage) and
16. STOHERD (storage capacity in relation to herd size).

Weekly data gathered by the Delta VCD in its surveillance and larvicide operations were used to calculate the mosquito productivity for each pond. This information obtained from June through August represented the dependent variable.

The data were analyzed by a CDC 720 computer with the SPSS stepwise multiple regression routine (Nie et al. 1975 and Hull and Nie 1981).

Limitations. There were two major limitations upon this study. One concerned scope, the other concerned method. The scope of the study was limited to management analyses of dairies for the handling of waste storage ponds which affected mosquito production. Therefore, information used for analysis consisted primarily of indirect factors. Direct factors, e.g. water temperature and wave action, were not included. Due to the limited time frame of the study, it was not possible to measure all variables at the desired level with the questionnaire and field survey method. Also the measurement of certain variables were possibly more subjective than others. These inherent limitations added to the residual error factor of the regression analysis.

RESULTS. The purpose of the solid waste separating pond was to collect floating solids in a small pond and to prevent these solids from entering the larger storage pond. Because of the difference in function of the two pond types, a separate analysis was made for each. Regression statistics of the significant variables for each are listed in Table 1.

Solids separating ponds. Less mosquito production occurred with the following management conditions (in order of significance):

1. More storage pond capacity in relation to irrigated acreage (Note this variable did not directly relate to separating pond).
2. Less floating solids coverage on pond surface.
3. Use of a mechanical solids separator.
4. Larger milking herd size.
5. More depth.
6. Less frequent pumping.
7. Less weed coverage.
8. Less length.
9. More mixing and/or refilling with fresh water.
10. Less separating pond capacity in relation to herd size.
11. More rectangular than square shape.

Waste water storage ponds. Less mosquito production occurred with the following management conditions (in order of significance):

1. More mixing and/or refilling with fresh water.

Table 1. Stepwise multiple regression statistics of dairy waste water managment variables which were significantly correlated to mosquito productivity (in order of significance).

Rank	Variable	Multiple Correlation Coefficient	Determinant (R^2)	Simple Correlation Coefficient	Overall F
		SOLIDS SEPARATING PONDS			
1	STOIRR	.43551	.18967	-.43551	3.74509
2	SOLID	.59814	.35777	.42087	4.17809
3	SEPATOR	.66260	.43903	-.24934	3.65232
4	HERD	.73711	.54333	-.02022	3.86672
5	DEPTH	.81142	.65840	-.22572	4.62567
6	PUMPFEQ	.84314	.71089	.20242	4.50801
7	WEED	.86455	.74744	.29267	4.22776
8	LENGTH	.88940	.79102	.14144	4.25838
9	MIXFILL	.90866	.82565	-.24986	4.20953
10	STOHERD	.92697	.85927	.10336	4.27414
11	SHAPE	.92714	.85961	-.27609	3.33975
		STORAGE PONDS			
1	MIXFILL	.38586	.14889	-.38586	9.97135
2	WEED	.46308	.21445	.30862	7.64366
3	HERDIRR	.57238	.32762	-.19855	8.93288
4	DEPTH	.62054	.38507	.18541	8.45382
5	HERD	.67387	.45410	-.22755	8.81740
6	STOHERD	.69689	.48566	.04645	8.18324
7	PUMPFEQ	.70853	.50202	-.23644	7.34470
8	LENGTH	.71435	.51030	.03543	6.51285
9	SHAPE	.72231	.52174	.09642	5.93937
10	RECYL	.72677	.52819	-.20528	5.37368
11	SOLID	.72856	.53079	.23254	4.83355
12	SEPATOR	.72900	.53144	-.12966	4.34781
13	WIDTH	.72938	.53199	-.19466	3.93477

2. Less weed coverage.
3. More milking stock in relation to irrigated acreage.
4. Less depth.
5. Larger herd size.
6. Less storage pond capacity in relation to herd size.
7. More frequent pumping.
8. Less length.
9. More square than rectangular shape.
10. More recycling of waste water for flush-down of alleyways.
11. Less floating solids coverage on pond surface.
12. Use of a mechanical separator.
13. More width.

DISCUSSION.–To better visualize the relationship of the significant variables in producing a "mosquito free" separating pond, a flow chart is presented in Figure 1. The relationship of some of the variables, particularly the most significant - STORIRR, is obscure. The cause may be a matter of the different methods employed to produce the desired function.

On one hand a separating pond may be constructed in a narrow rectangular shape with a small capacity per herd. Friable solids can then accumulate on the surface and completely cover the water. An increased depth in conjunction with reduced capacity will facilitate this process. A siphon arrangement draws the "solid free" waste water to the storage pond. This method is very efficient in reducing mosquito production in connected storage ponds.

Another approach is to prevent solids from entering the separating pond. Mechanical solids separators are effective in this regard. An added benefit of less solids is reduced weed growth, especially on the pond surface. Surface weed growth can be a problem in the former type where floating solids are encouraged.

Finally solids can be diluted by mixing and/or refilling the pond with fresh water. In addition agitation from this process may help break up and resuspend solids. Also turnover time of waste water is shortened by increasing the out-flow to the storage pond. In this latter approach, however, the problem may simply be shifted to the storage pond.

All of these approaches can effectively reduce mosquito production in separating ponds, but each has its own drawback. In the case of the first approach, that of friable solids formation, the pond must be periodically cleared of the accumulated solids. Again, use of the third approach may only delay the problem until the storage pond, where it becomes more difficult to control. The most effective alternative is the mechanical solids separator, but the models in use within the Delta District at present are prone to mechanical malfunction. Clearly a more mechanically sound and energy efficient design is needed.

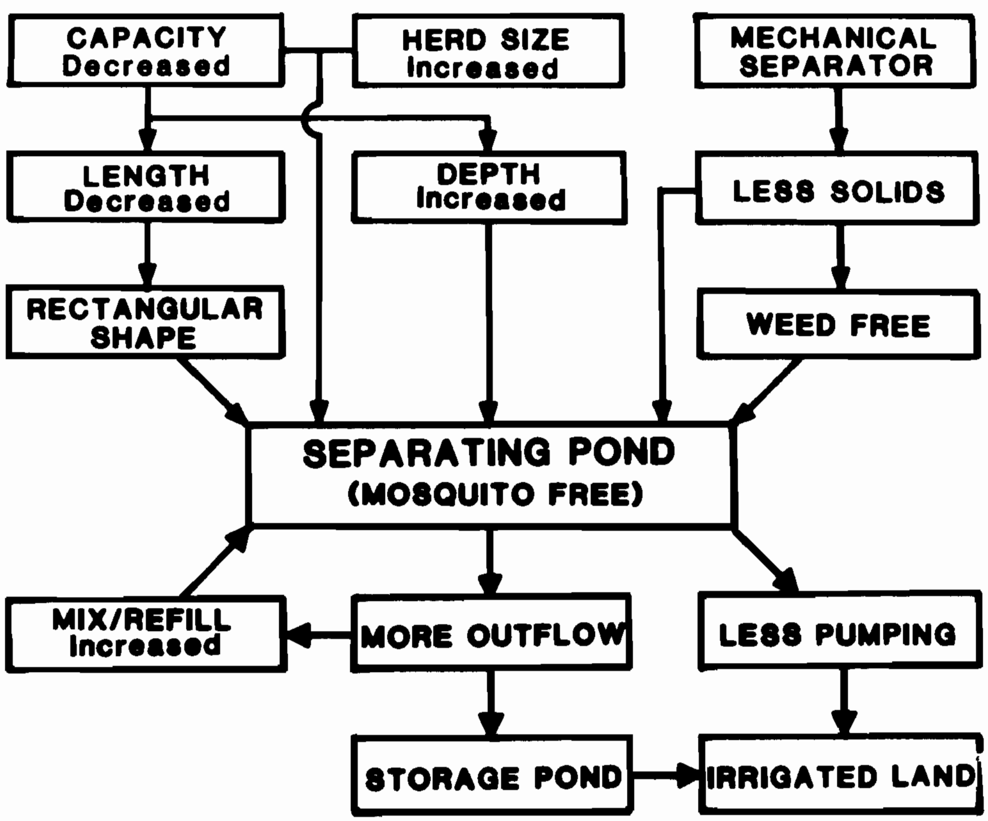

Figure 1. Flow chart showing the relationship of construction and management variables which result in a solids separating pond with diminished mosquito productivity.

A flow chart of significant variables for waste water storage ponds is presented in Figure 2. Construction features are important in the development of a mosquito free pond. A more square rather than narrow, rectangular shape decreases mosquito production. It is speculated that the enhanced access of wind to the water surface promotes wave action which results in the reduced mosquito breeding. A wind shield can result from excessive freeboard (the amount of unfilled depth), if a pond is constructed overly deep and is not filled to capacity.

Very shallow water can promote weed growth across the pond bottom and can provide mosquito breeding harborage. With a smaller storage capacity per herd size a fuller pond is achieved. If the storage capacity is too large or if the irrigation pumping demand is too great to maintain deeper water, then mixing and/or refilling the pond with fresh water is needed to eliminate freeboard.

The addition of fresh water to a waste water storage pond also reduces suspended solids and decreases formation of floating solids, which can harbor mosquito breeding. A mechanical solids separator can eliminate floating solids from a storage pond, as can a solids separating pond. Agitation of the waste water produced by mixing/refilling further breaks up and resuspends solids. Pumping for irrigation and recycling of waste water for flush-down of alleyways also provides agitation.

It is important that dairy waste water be used for irrigation of cropland; not only to gain the obvious nutrient value and to recondition sodic soils, but also to reduce mosquito production. Waste water storage ponds are for temporarily holding water destined for irrigation. More frequent pumping, especially when fresh water is mixed or refilled into the pond, is most beneficial.

Weed growth provides harborage for mosquito breeding. Factors which promote weed growth, e.g. shallow water and floating solids, need to be eliminated. Also a rigorous regime of weed control must be implemented. To allow for herbicide and possible spot larvicide operations, unhindered access around the periphery of the ponds is needed.

With the advent of large dairies and incumbent large ponds, it is no longer feasible to rely upon chemical larvicide operations. Therefore the answer lies in managing the ponds to eliminate mosquito production.

Recommendations: - The following recommendations can help to reduce or eliminate the production of mosquitoes from dairy waste water storage ponds: (1) maintain a full pond level through mixing and/or refilling pond with fresh water after pumping for irrigation or by a reduced storage capacity in relation to herd size, (2) eliminate weeds in and around pond, (3) promote wind-induced wave action by construction of a square shape and eliminate freeboard by reducing constructed depth, (4) promote agitation of the ponded water by pumping for irrigation or by recycling waste water for flush-down of alleyways and (5) prevent floating solids from entering pond through use of a mechanical separator or with a

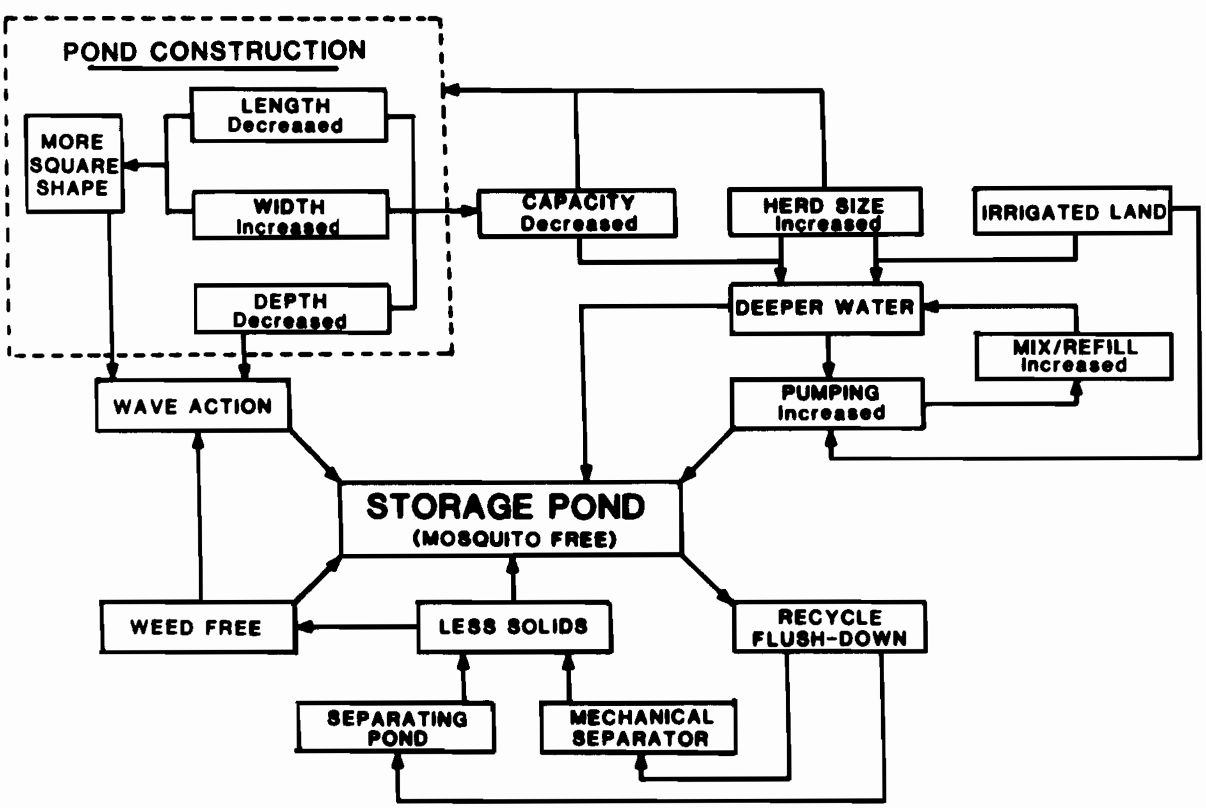

Figure 2. Flow chart showing the relationship of constuction and management variables which result in a storage pond with diminished mosquito productivity.

small, rectangular solids separating pond.

ACKNOWLEDGMENT.–The assistance and cooperation of Tom Schultz, Tulare County Dairy Farm Advisor and John C. Combs, Dr. W. Donald Murray and James Philpot of the Delta Vector Control District is gratefully acknowledged. This work was supported, in part, by a special California State appropriation for mosquito control research.

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THE COYOTE HILLS MARSH MODEL, CONCEPTUAL FRAMEWORK AND DIRECTIONS OF RESEARCH

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The Coyote Hills freshwater model is a computer-operated, simulation model being developed to integrate the two seemingly opposite management objectives of mosquito control and wildlife management of a freshwater marsh. The marsh is managed by naturalists of the East Bay Regional Park District to introduce students and citizens to the rich variety of flora and fauna associated with the marsh. Unfortunately, the marsh also produces a rich variety of mosquitoes.

In past years the Alameda County Mosquito Abatement District has accomplished mosquito control primarily by planting mosquitofish. Although relatively high populations of mosquitoes of the species *Aedes squamiger*, *Culex tarsalis*, *Cx. erythrothorax*, *Anopheles* spp. and *Culiseta inornata* have, at times, been monitored at the site, pesticides have only been applied on rare occasions to control the winter salt marsh mosquitoes, *Aedes squamiger*. These mosquitoes became necessary to control because they were strong fliers invading distant residential areas. The other mosquitoes, for the most part, have been unnecessary to control because the park is located some distance from residential areas. This may change, however, since plans have been made to create residential housing adjacent to the park, thus portending increased public demand for mosquito control on the marsh.

The Coyote Hills freshwater model, in its broadest sense, is a beginning effort to bring together the two factions of wildlife and mosquito management, and to increase their understanding of the dynamics of the marsh. Hopefully, the increased understanding will lead to development of a marsh management system that will allow an ecologically rich freshwater marsh to exist adjacent to, and in harmony with, a residential development. In its most specific sense, the model may eventually be used to assist in making specific decisions concerning the course of action to be taken to control mosquitoes in a manner compatible with the wildlife management objectives of the marsh.

The first version of the Coyote Hills freshwater model was developed by a team of six scientists led by Dr. James Schooley of California State University at Hayward. It was presented by Dr. Schooley at a workshop on Systems Modelling sponsored by the Northern Chapter of the Society of Vector Ecologists. A thorough description of this first version was presented by Dr. Schooley at last years' C.M.V.C.A. conference (Schooley et al. 1982).

CONCEPTUAL FRAMEWORK.–The essence of the model is the relationship between the population of *Anopheles* spp. and its predators, primarily mosquitofish, *Gambusia affinis*. The effectiveness of predation by *Gambusia* is affected by the biomass of pondweed, *Potamogeton pectinatus*. As pondweed increases in biomass during the growing season it provides an increasingly effective refuge for the *Anopheles* larvae. The initial level of *Anopheles* larvae, and mosquitofish are required inputs to the model and must be accurately determined by field sampling. The biomass of pondweed is a function of the depth the water will be held in the marsh throughout the growing season and is determined by an equation found in the literature.

The mathematical core of the model is a difference equation which determines the level of Anopheline larvae. The equation includes the components of predation by *Gambusia* and the interference of predation by pondweed. During the operation of the model, the difference equation calculates the number of Anopheline larvae to add or subtract from the population in a given period of time, in this case one day. The computer calculates the level of Anopheline larvae for each day. Difference equations are particularly suited to operation

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by a computer because the computer can run many iterations in just a few seconds.

DIRECTIONS OF RESEARCH.–The model in many ways acts as blueprint by vividly illustrating the research needs. The first steps in operating the model requires the user to provide input. The accuracy of the input will undoubtedly have an affect upon the validity of the results. For this reason, some of the initial research efforts have been aimed at developing effective methods to assess the populations of *Anopheles* spp., mosquitofish and pondweed, and developing a method to measure average depth of the marsh. Obviously one cannot expect the model to provide valid results until effective sampling techniques have been developed to measure these parameters.

During the development of the mathematics of the model, it became evident that the literature would not provide all of the necessary information. Numerous assumptions had to be made based upon the best estimates of the team. Assumptions were made about growth rates of *Anopheles* spp., the predation rates of *Gambusia*, and the phenology of pondweed at Coyote Hills, just to name a few. It became quite clear that many of these assumptions would need to undergo laboratory and/or field testing.

The first version of the model may well be simplistic and leave out important invertebrate and vertebrate predators of *Anopheles* spp. Indeed, the model may leave out any number of important physical or biological components. For this reason, a general assessment of the biological and physical components of the marsh has also been deemed important.

On June 18, 1982 a number of interested scientists met to review the model and identify the research needs. The resultant research work load was divided into general categories and the researchers chose areas within which they could accomplish their research. In this way it was hoped that researchers would be able to pursue a productive avenue of research without duplicating the efforts of other researchers working on the model. The following two papers are reports by principal researchers on their research projects.

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A COMPUTERIZED DATA BASE AND SIMULATION SYSTEM TO SUPPORT DECISIONS IN THE ALAMEDA COUNTY MOSQUITO ABATEMENT DISTRICT

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The ACMAD has taken the opportunity to reanalyze the computer system with the recent aquistion of a new computer and hard disk system. This has enabled the systems team to expand the computer system to handle many more functions with greater efficiency. The heart of the new system is a Radio Shack TRS 80 Model II computer with an 8 megabyte hard disk storage unit.

Much of the system analysis necessary to build the new system had been accomplished in 1980 when the District installed its first micro-computer. At that time, the following objectives were set for the system (Roberts 1980):

1) To efficiently process data that will measure the effectiveness of the mosquito control program.
2) To efficiently generate required reports.
3) To develop and utilize models to predict levels of mosquitoes and thereby assist in making treatment decisions.
4) To quantify the work performed by the District.
5) To define "high priority" mosquito sources, through cost evaluation and set appropriate work schedules for the physical control program.
6) To measure the insecticide pressure on any given species and avoid resistance problems.
7) To determine the costs of specific program elements and enhance program budgeting.
8) To check current inspection and treatment schedules with those of the past and modify the schedule as required.

These objectives had been met by the old system, but they could not be integrated easily due to the lack of storage space on the floppy disks. Another limitation of disk storage was the inability to build and routinely update a complete data base of mosquito source information. There was simply not enough on-line storage and access for all the mosquito sources in the District. Without adequate on-line storage capacity it was virtually impossible to build and update a data base capable of creating inspections and treatment schedules for all the important mosquito species and to provide the necessary input to system models. Also, users of the old system had to deal with a computer that was not always friendly, sometimes cryptic, and at other times unavailable due to heavy use.

By following a system development process as outlined by Page and Hooper (1979), the system team was able to build a new system better able to meet the District's objectives. System development included the following steps:

1) **System analysis** - general background, organization, structure, document and procedure review, deficiencies, recommendations.
2) **Statement of Objective** - overall purpose, specific objectives, required output, required data, necessary controls, new policies or procedures.
3) **System Design** - scope and boundaries, specific requirements, conceptual design, resource requirements, benefits.
4) **System Specifications** - system description, system flowchart, computer requirements, data management summary, implementation, schedule.
5) **Programming** - narrative description, user instructions, sample input, sample output, test data program listing.
6) **Implementation** - hardware requirements, personnel orientation, training, testing, file conversion, parallel operation.
7) **Evaluation** - documentation review, cost analysis, user acceptance, internal control, deficiencies, recommendations.

The ACMAD systems team consists of the manager and three mosquito control technicians. In initial planning sessions, job assignments were issued in three areas: programming, documentation, and logic. One technician was assigned responsibility in each area and the others assisted. The manager led the group in planning a program schedule. The first task to be accomplished was the upgrading of the old data base which included a list of all known mosquito sources in the District, with information as to ownership, location, source type, size, methods of treatment, hours required to inspect and treat. The information was copied by computer from existing disks, and additional information was gathered from the supervisors and technicians and entered by keyboard. The new hard disk storage unit allowed the District to store all this information in one place, making it possible to add a secondary group of files which are updated daily by the daily records. The secondary files accumulate operational and biological information on each source which can be accessed within a few seconds. The biological data being added to existing source information includes the following:

1) Mosquito species present at source, the species prioritized according to health and pest considerations.
2) Endangered species present at source, such as the red-bellied harvest mouse and the clapper rail.
3) Non-target organisms such as fish, birds, mammals, and invertebrates.
4) Larval predators in sources including fish and invertebrates.
5) Major vegetation types.

The District's computer is now utilized to store all operational data, transmit data from one facility to another, update the before mentioned data base, generate inspection and treatment schedules for *Aedes squamiger*, predict population levels of *Culex pipiens*, generate monthly reports, provides information on inventoried mosquito sources, and is used along with a Daisy wheel printer for word processing. The following projects are now in progress to further upgrade the computer system:

1) Daily inputs are being expanded to include weather, tidal, and insecticide resistance data.
2) Programs are being developed to generate inspections and treatment schedules for all high priority mosquito species.
3) A simulation model is being developed of a freshwater marsh to assist in treatment decisions. A general model is being developed to assist in predicting whether important species of mosquitoes are approaching established thresholds.

The District is in the process of consolidating facilities with the construction of a new office and shop in Hayward. The consolidation will eliminate the need to transfer data by phone. Also, in the plans for the new office are additional terminals to be placed where needed in the building.

The system team found that the initial programming and planning went much quicker than anticipated but an unanticipated amount of time was spent debugging programs. Integration of the programs and procedures with the existing system occurred quickly and smoothly.

Although a considerable amount of time was spent with field personnel going over source information, the time was well spent. The effort has created a data base of mosquito sources, each of which can be modified, created, subdivided, eliminated, or checked in a matter of seconds. The daily cards of employees serve to update source data continually. The District's new data base and simulation system, without doubt, has provided the District's decision makers with a greater amount of readily accessible, current information to assist in their decisions.

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THE COYOTE HILLS MARSH MODEL: CALIBRATION OF INTERACTIONS AMONG FLOATING VEGETATION, WATERFOWL, INVERTEBRATE PREDATORS, ALTERNATE PREY, AND *ANOPHELES* MOSQUITOES

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ABSTRACT

Initial field studies to calibrate computer-based simulation of *Anopheles* spp.-habitat interactions indicated that distribution of larvae closely corresponds to the distribution of floating mats of sago pondweed (*Potamogeton pectinatus*); larvae are seldom found in the open water of the pond center or along the margins. Pondweed mats may increase larval survival by impeding fish predation and by supporting alternative prey, e.g. chironomid larvae whose densities are directly related to pondweed standing crop ($p < 0.001$). Waterfowl may increase predation by creating pathways, which allow fish to penetrate the mats. Floating pondweed also supports invertebrate predators that can reduce mosquito survival, e.g. predaceous damselflies whose densities are also directly related to pondweed standing crop ($p < 0.001$). Interactions among predators, alternative prey, waterfowl, and *Anopheles* spp. are currently being quantified in relation to pondweed biomass to account for changes in density of mosquito larvae.

INTRODUCTION.–The Alameda County Mosquito Abatement District (ACMAD) has selected computer-based systems analysis to integrate existing methods for controlling populations of Anopheline mosquitoes at the Coyote Hills Marsh, Coyote Hills Regional Park, Alameda County, California. The practical and theoretical aspects of the Coyote Hills Marsh Model have been described by Schooley et al. (1982), Roberts (1982), and Roberts et al. (1983). This paper presents the experimental design and initial results of on-going field studies that will be used to calibrate the simulation of interactions among the target species (*Anopheles* spp.), populations of other aquatic invertebrates, waterfowl, and floating vegetation. Thus far these studies have addressed three fundamental questions: (1) What is the habitat for *Anopheles* spp. at Coyote Hills? (2) What important biological factors interact with *Anopheles* spp. and affect their density? (3) What are the temporal and spatial dimensions of this *Anopheles* spp. population? That is, what sample size, sampling frequency and location of sample plots will best characterize the dynamics of this population.

Site Description. Until the last decade of the nineteenth century, the area now called the Coyote Hills Marsh comprised a small part of the upland margin of a nearly continuous band of salt marshes that bordered much of the San Francisco Bay (Fig. 1A). At the beginning of this century efforts to dike and "reclaim" adjacent marshland for agriculture or other commerical uses reduced tidal inflow and drainage around Coyote Hills. The entire area persisted as poorly drained bottomland (Fig. 1B) with saline soils until the late 1940s, when a private hunting club opened part of the area to freshwater influence. Since then, the marsh has become part of the Coyote Hills Regional Park and has been subjected to a series of transformations using stream diversions and flood gates to distribute freshwater to various portions of the original salt marsh; in one large portion (6 ha) of our study area, heavy machinery was used to shape basins and channels. Thus by 1977, a man-made freshwater marsh replaced the previous natural saline and brackish water marshland (Fig. 1C).

However, remnants of the original salt marsh exist at Coyote Hills. As a result, the local area supports both salt-tolerant and freshwater vegetation (Fig. 2). Pickleweed (*Salicornia virginica*) and salt grass (*Distichlis spicata*) typically dominate the areas that have saline soil, whereas the margins of the man-made freshwater marsh support cattails (*Typha* sp.) and bulrushes (*Scirpus* sp.). The water surfaces of the interior portions of the freshwater marsh are not covered by vegetation during the winter months, but from late spring through autumn most of these same areas support dense mats of sago pondweed (*Potamogeton pectinatus*) on the water surface.

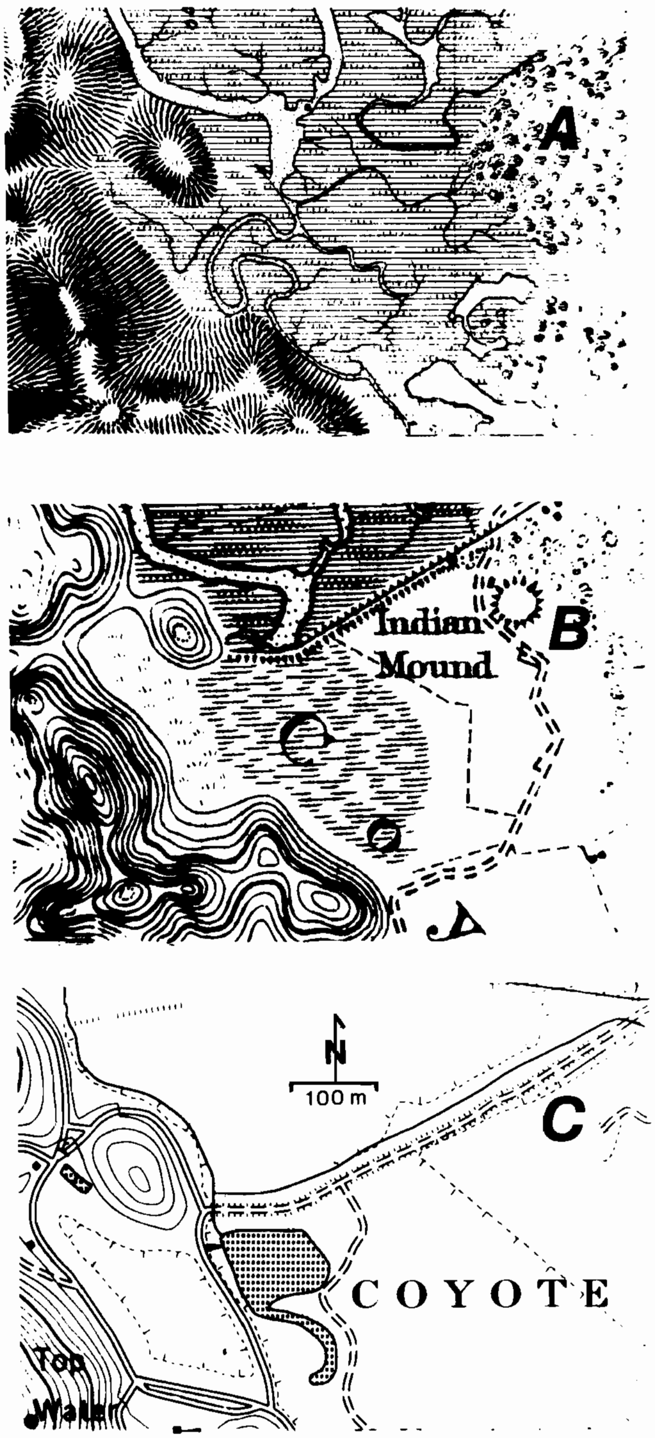

Figure 1. Changes in landform and landuse at Coyote Hills Marsh as indicated from details of the following maps: (A) Southern Part of San Francisco Bay California, Survey of the Coast of the United States, U. S. Coast and Geodetic Survey, 1882; (B) San Francisco Bay Southern Part, U. S. Coast and Geodetic Survey, 1911; (C) Newark, California topographic quadrangle, U. S. Geologic Survey, 1959 (photorevised 1980).

Habitat Description. Baseline information about the species composition and life histories of mosquitoes at the Coyote Hills Marsh has been collected by ACMAD. These data indicate that the marsh supports *Culex tarsalis*, *Culiseta inornata*, and *Anopheles occidentalis* and *Anopheles freeborni*. The baseline data also indicate that *An. freeborni* replaces *An. occidentalis* during mid-summer, and thereafter is characterized by a stable age structure until recruitment discontinues in late autumn. This information has been used to establish the timeframe for monitoring *Anopheles* spp. in the Coyote Hills Marsh.

In order to determine the spatial distribution of *Anopheles* spp. at Coyote Hills, we sampled larvae along transect lines that included representative portions of the various plant communities (Fig. 2). These transect data indicate that different species of mosquitoes correspond to different plant communities, and that *Anopheles* spp. are essentially restricted to the interior areas of the freshwater marsh where pondweed also occurs (Table 1). Therefore, the distribution of pondweed may be used to generally delineate the spatial boundaries of the *Anopheles* spp. habitat.

Table 1. Percentage of total individuals for each species of mosquito collected within each plant community at the Coyote Hills Marsh on 8 July 1982.

taxon	pickleweed	cattail & bulrush	pondweed
Culex tarsalis	100	1	1
Culiseta inornata	0	98	0
Anopheles spp.	0	1	99

Interacting Biological Factors. The Coyote Hills Model presently recognizes two organisms as factors that interact with *Anopheles* spp.: the mosquitofish, *Gambusia affinis*, which preys on *Anopheles* spp., and the pondweed, which functions as refuge from *Gambusia*. Later in this report we will discuss pondweed-mosquito interactions. Other research groups are currently examining *Gambusia* ecology at the Coyote Hills Marsh (Schooley et al. 1982, Schooley 1983).

Although the model recognizes that vegetation and fish affect the density of *Anopheles* spp., our initial field studies have indicated that survival of these larvae is also influenced by other aquatic invertebrates and waterfowl. Based upon fish stomach analysis and quantitative examination of the invertebrate community at Coyote Hills, we have identified two insect taxa, damselfly nymphs (including *Enallagma carunculatum*, *E. civile*, and *Ischnura cervula*) and epiphytic chironomid midge larvae (including *Paralauterborniella subcincta*, *Dicrotendipes nervosus*, *Cricotopus* spp., *Coryneura* spp., and Tanytarsini spp.) as organisms that coexist with the target species and affect its density. Both damselflies and midges are abundant in the marsh and function as alternative prey for *Gambusia* and other resident fish (e.g. stickleback, *Gasterosteus aculeatus*, and Sacramento blackfish, *Orthodon micro-*

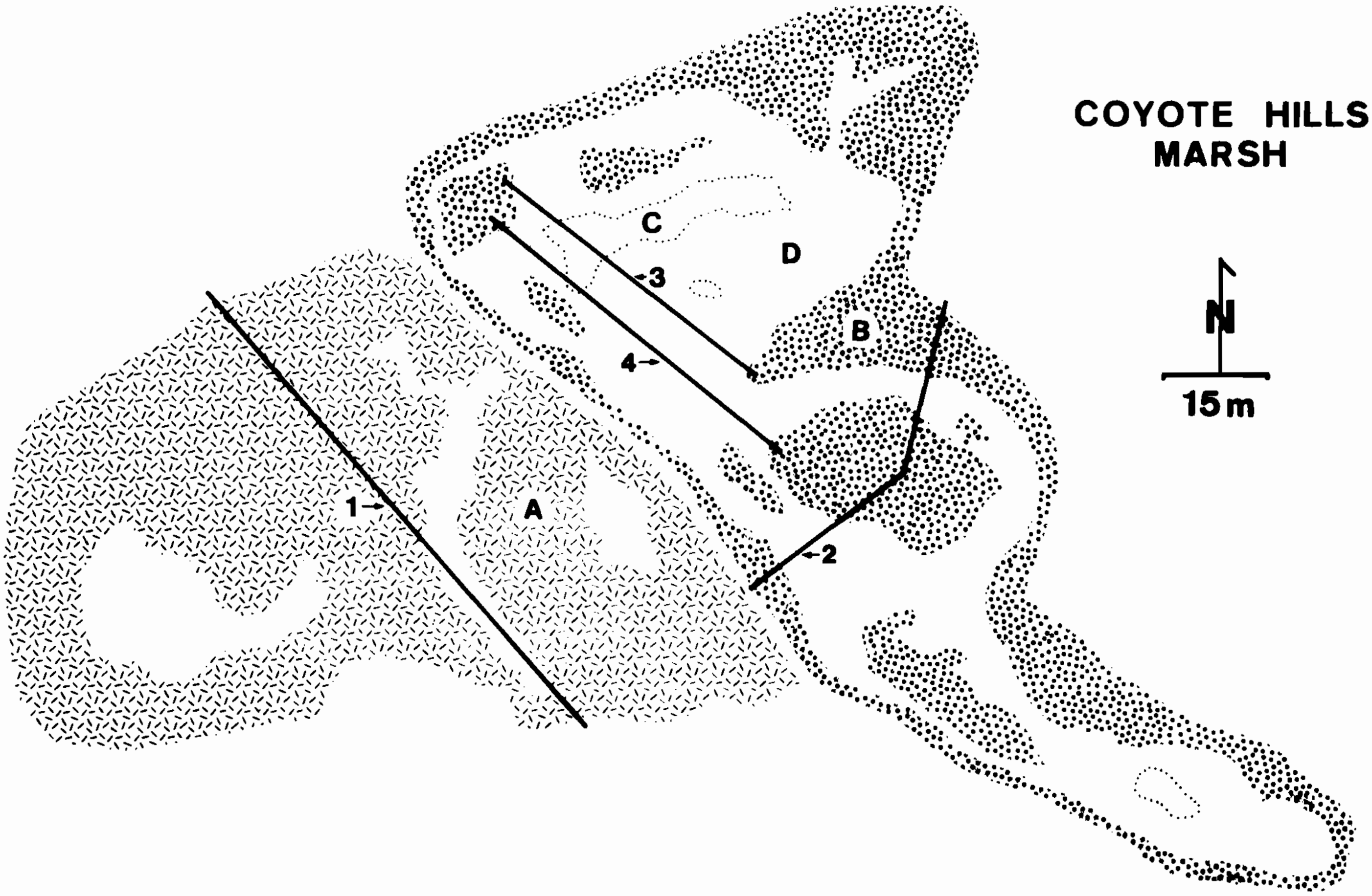

Figure 2. Vegetation cover and transect locations. Stipple-types A and B represent the distributions of the pickleweed and cattail-bulrush plant communities, respectively. Areas contained within dotted lines (e.g. area C) represent permanent open water. Other areas (e.g. area D) that surround permanent open water represent the ephemeral pondweed cover. A thin border of open water (not shown on this map) exists between the pondweed and cattails. Transects 1 and 2 were used to determine boundaries for the *Anopheles* spp. habitat. Transects 3 and 4 were used to test for relationships between density of *Anopheles* spp. larvae and either water depth or distance from open water.

lepidotus) that are predators of *Anopheles* spp. Furthermore, damselflies can be voracious predators of mosquito larvae.

Waterfowl, especially American coots (*Fulica americana*), indirectly affect the density of mosquito larvae by creating pathways through the pondweed mats at the water surface. These pathways appear to allow *Gambusia* and other predatory fish to penetrate the interior reaches of the pondweed canopy, and thus reduce its effectiveness as refuge for mosquitoes.

Determination of Scale. The freshwater basins and channels that support both *Anopheles* spp. and pondweed vary in crossectional profile from being steep-sided to having sides that steepen gradually (Fig. 3). Since the pondweed canopy is re-established each spring from seeds or rhyzomes that are embedded in the marsh bottom, water depth can affect the timing of pondweed emergence. Depth might also influence temperature, nutrient turnover, and consequently, surface micro-fauna and flora. Therefore, we used data from transect lines that crossed basin contours (Fig. 2) to determine if density of mosquito larvae is related to water depth. These data indicate that larvae were uncommon in shallow water; otherwise density and depth were apparently unrelated (Fig. 4).

Since these shallow areas represent a zone between the cattails and pondweed that does not support floating or emergent vegetation, we hypothesized that other areas of open water might also lack *Anopheles* larvae. Samples taken along transects that spanned open water and floating vegetation indicated that the density of *Anopheles* spp. larvae decreased abruptly at both the outer and inner margins of the pondweed cover (Fig. 5). In all likelihood, such margins tend to be less effective as refuges from fish predation. However, the floating mats of vegetation also vary in thickness, due to differences in both plant stem density at the marsh bottom and the presence of waterfowl pathways at the water surface. This heterogeneity of plant cover might result in variable access for fish, which in turn might cause the density of mosquito larvae to vary spatially, even within interior areas of the pondweed cover (e.g. Fig. 5; distances 6-15 m).

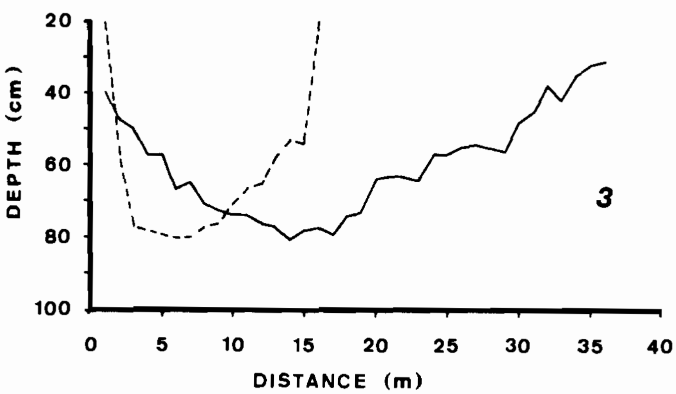

Figure 3. Depth profiles for freshwater basins. Dashed and solid lines represent steep-sided and gradually deepening basins, respectively. Both basins are man-made.

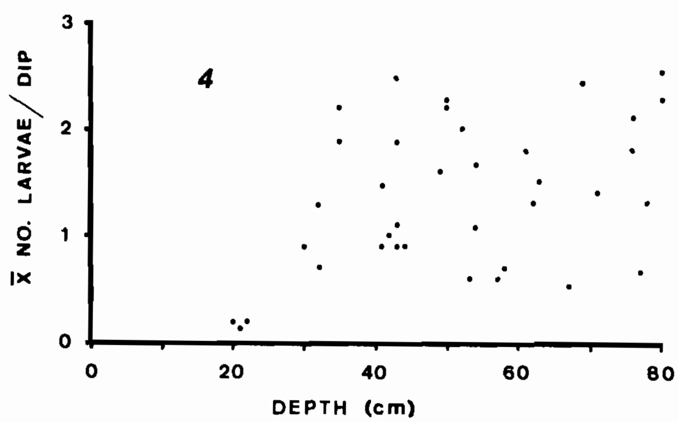

Figure 4. Relationship between water depth and density of *Anopheles* spp. larvae. The 20 cm depth corresponds to shallow open water along the outer edge of the pondweed cover.

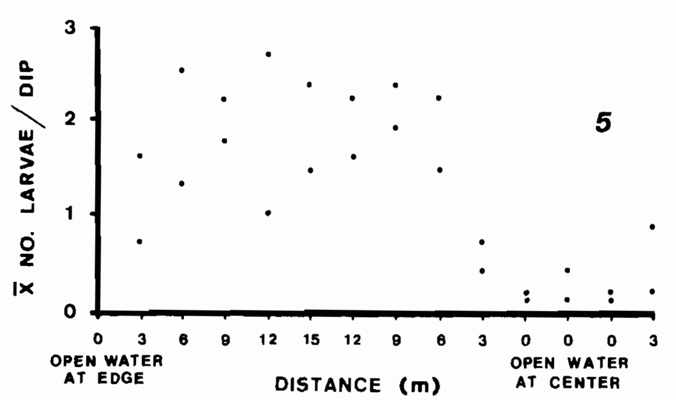

Figure 5. Relationship between distance from open water and density of *Anopheles* spp. larvae. The 15 m distance corresponds to the most interior position in the pondweed cover, relative to either the open water at the margins of the pondweed or that in the middle of the freshwater marsh (see Fig. 2).

The transect data that were used to examine the effects of depth and distance from open water clearly demonstrate that the density of *Anopheles* spp. can vary significantly over short (i.e. < 1 m) distances. Furthermore, when samples from shaded and sunlit locations were compared, significant differences were found for the density of some invertebrates, including *Anopheles* spp. (Table 2). Field observations also indicated that *Anopheles* spp. larvae repeatedly move away from encroaching shadows. These findings suggest that the scale used to collect the transect data (i.e. 3 m intervals) is too large for measuring the biological interactions that involve *Anopheles* spp.

Table 2. Comparisons of invertebrate densities in shaded and sunlit locations at the Coyote Hills Marsh. Densities ($\bar{x} \pm$ sd) of damselfly nymphs and Cladocera are per 0.062 m^2 of surface pondweed cover and included individuals in the underlying water column. *Anopheles* spp. densities are $\bar{x}$ per three standard dips.

taxon	sunlit	shaded	p - value
Damsefly	32 ± 16.6	3 ± 2.4	0.005
Cladocera	33 ± 14.0	142 ± 81.0	0.014
Anopheles spp.	6 ± 3.8	1 ± 1.0	0.023

Given this requirement for smaller scale and the obvious importance of the pondweed cover, we have begun to examine the interactions that occur relative to the biomass of either a single plant (i.e. one shoot from a rhyzome or the growth from a single seed) or all plants contained within a small pull-up or drop-type sampler. Using individual plants as sample plots, we found a significant positive relationship between plant biomass and total counts of epiphytic midges (Fig. 6). Likewise, data from pull-up samplers indicate that plant biomass and density of damselfly nymphs were positively correlated (Fig. 7).

Based upon these results, we have substituted the line-transect sampling approach for a stratified-random design that will restrict field efforts to a central portion of the pondweed canopy. According to this design, either individual plants or small quadrats will constitute sample plots, depending upon the biological factor being measured.

Future Research. Following our determination of habitat boundaries and the appropriate scale for field studies, we began to examine in detail the interacting biological factors that affect the density of *Anopheles* spp. at Coyote Hills Marsh. Both field and laboratory studies are underway to describe the bionomics of the chironomid and damselfly assemblages and the phenology of the pondweed. Field experiments will examine emergence success for *Anopheles* spp. under different conditions of plant cover, and predator and alternative prey densities. Such studies will enhance the use of the Coyote Hills Marsh Model by reducing the number

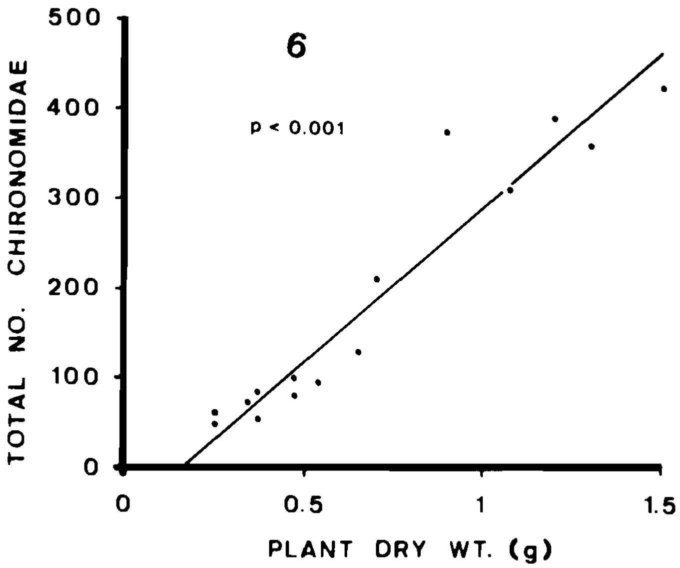

Figure 6. Relationship between total numbers of epiphytic Chironomidae and biomass of individual pondweed plants. P-value refers to significance of the Pearson product-moment correlation coefficient.

and kinds of measurements required as input and by improving simulation of field conditions.

ACKNOWLEDGMENTS.—These studies are the result of cooperative efforts among personnel of the University of California at Berkeley, the California State University at Hayward, the East Bay Regional Park District, and the Alameda County Mosquito Abatement District. We wish to thank G. Grodhaus and R. Garrison for identifying the Zygoptera. Support for these studies was provided by University of California Mosquito Research Funds and the Alameda County Mosquito Abatement District.

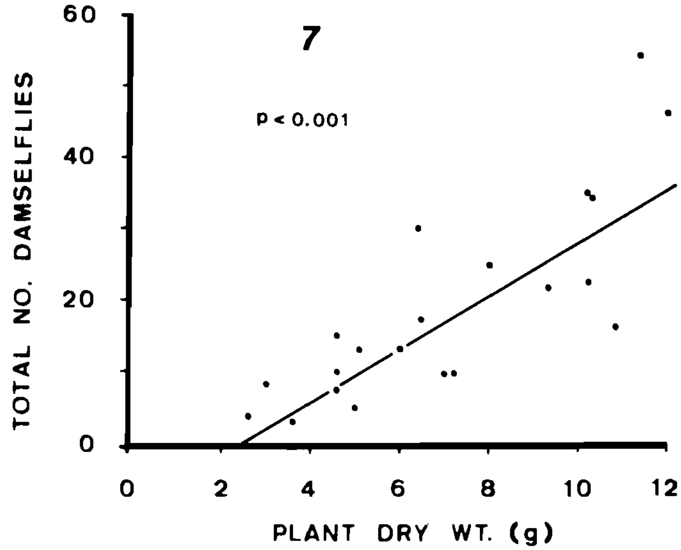

Figure 7. Relationship between total number of damselfly nymphs and biomass of all plants contained within 0.062 m^2 of pondweed cover and the underlying water column. P-value refers to significance of the Pearson product-moment correlation coefficient.

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THE COYOTE HILLS MARSH MODEL: CALIBRATION OF THE INTERACTIONS AMONG FISH AND FLOATING VEGETATION

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ABSTRACT

Studies on the distribution and abundance of fish were done in the freshwater marsh of the Coyote Hills Regional Park, Fremont, California. The purpose of the studies was to calibrate an ecosystem simulation model designed for vector control managers. Preliminary results suggest that the modeling assumptions of fish densities were too low and that *Gambusia* distribution is correlated to the distributions of other fish in the marsh. Modeling assumptions concerning the relationship of fish distributions to pondweed appear reasonable.

INTRODUCTION.–The first phase in ecosystem modeling is design, often using empirical data or theoretical predictions from other systems. The Coyote Hills Marsh Model (Schooley et al. 1982) is no exception. In short, the model deals with the behavior of aquatic predators and their prey in the freshwater marsh in Coyote Hills Regional Park, Fremont, California. The utility of such a simulation for vector control is discussed in Roberts (1982) and Schooley (1983). Summaries of marsh morphology and vegetation are given in Schooley et al. (1982) and Collins et al. (1983).

The only site specific data available on the marsh vegetation and aquatic fauna during the initial design phase of the model consist of miscellaneous field notes taken during routine mosquito dipping by the staff of the Alameda County Mosquito Abatement District (G.E. Conner, personal communication). Therefore, seasonality, population densities, pondweed phenology, predator-prey relationships and microhabitat selection were all derived from sources other than the marsh being modeled. The most common source was data from rice fields in Central California.

The second phase in modeling is calibration. During this phase the major biotic and abiotic compartments of the model are compared to empirical data to verify functional significance and test the reasonableness of assumptions concerning behavior and interrelationships. This is not the same as validation, where actual model behavior is compared to system behavior over a period of time. Calibration is just that, calibrating the internal assumptions and relationships in the model to be close to the general pattern of the marsh, not to specific conditions.

The calibration studies include the phenology of pondweed and invertebrate interactions (Collins et al. 1983); and fish interactions and feeding behavior (Schooley 1983). The following is a discussion of the results of the calibration studies of fish interactions. The four areas calibrated were: average densities of fish, especially the mosquitofish (*Gambusia affinis*), the correlation between fish density and pondweed density, assumed to be negative for mosquitofish; correlation between the distance to open water and fish density, assumed to be negative for mosquitofish because of reduced foraging efficiency in areas of heavily occluded pond surface; and independence of fish distributions.

MATERIALS AND METHODS.–After preliminary sampling in July 1982, four regular sampling efforts were made at three week intervals from August through October 1982. The samples were taken from the northern portion of the main freshwater marsh using a portable drop-box. The portable drop-box was the only practical quantitative sampler for waters dense with floating vegetation. The box consisted of a wooden frame of one cubic meter lined with plastic on four sides. The box was carried overhead into the marsh and tossed approximately three meters, sinking immediately. Subsequent tosses were made 15 to 25 m from the previous, in undisturbed water. Six tosses were made in the first two sampling efforts and five in the last two for a total of 22 samples. Fish were dip-netted from the box and preserved on ice for later weighing and measuring. For each sample, the water depth, air temperature, water temperature (surface and bottom) were recorded. Then, pondweed (*Potamogeton*) was gathered from a 0.25 m^2 quadrant next to each drop. Finally, distance to the nearest shore, next-nearest shore, boardwalks or observation platforms and open water were recorded. Open water is defined as any area greater than one square meter which does not have pondweed at the surface. Distances were measured and used to eliminate covariance of fish species due to any tendency to shoal, seek cover, or select microhabitats with less occluded water surfaces. Distances less than 40 m were measured to the nearest 0.1 m; distances greater than 40 m were estimated to the nearest 0.5 m. Statistical analysis was performed at the computing center of California State

University, Hayward, using the Statistical Package for the Social Sciences (SPSS).

RESULTS.–Six fish species were collected in the drop-boxes (Table 1). Fish distributions were clumped and highly variable as indicated by the large standard deviation. The white catfish (*Ictalurus catus*) and the Sacramento squawfish (*Ptychocheilus grandis*) found in qualitative samples taken from a deeper, open-water arm of the marsh were not taken in any drop-box.

The relationships of fish density with pondweed biomass, distance to open water, and density of other fish species were examined using partial correlation analysis (Steel and Torrie 1960). A partial correlation coefficient measures the correlation between two variables when other specified variables are kept constant. Statistically variables are held constant by assuming linear relationships among the variables. This should then have the same effect as controlling these variables experimentally.

The partial correlation coefficients for fish density with pondweed are shown in Table 2. Carp was the only fish significantly correlated with pondweed biomass, as pondweed increased so did carp density. Only one of the carp caught was older than one year. Partial correlations between fish densities and distance to open water are shown in Table 3. Again carp was the only fish with a significant correlation. This relationship indicates that as the distance from open water increases the density of carp decreases. The coefficients for Sacramento blackfish and three-spined stickleback indicate a pattern of increasing density with increasing distance from open water; however, it was not statistically significant. The correlations between fish species are shown in Table 4. The positive correlations of mosquitofish with Sacramento blackfish and three-spined stickleback with sculpin were both significant. Interestingly Sacramento blackfish correlated negatively with both sculpin and three-spined stickleback, while mosquitofish correlated negatively to the former and positively to the latter. None of these however was statistically significant.

DISCUSSION.–In the marsh model the density of mosquitofish is not a fixed value, but a simulation option. Densities used in simulation examples ranged from 0.1 to 0.5 ind/m^2 while the actual average was approximately two orders of magnitude higher at 13 ind/m^2. The densities of three-spined stickleback and juvenile Sacramento blackfish were assumed to be insignificant. Thus these species were not included in the

Table 1. Fish species density plus or minus one standard deviation. (N=22)

Species	Density (No./m^2)
Gambusia affinis (Mosquitofish)	12.8 ± 16.9
Gasterosteus aculeatus (Three-spined stickleback)	16.8 ± 21.0
Orthodon microlepidotus (Sacramento blackfish)	8.4 ± 14.0
Cottus sp. (Sculpin)	2.7 ± 4.2
Cyrinus carpio (Carp)	1.1 ± 1.4
Lepomis microlophus (Redear sunfish)	0.4 ± 0.7
Total	42.2

Table 2. Partial correlation coefficient of fish species density with pondweed biomass after controlling for water temperatures, depth, distances to near shore, next-nearest shore, boardwalks and open water (N=13).

Species	Correlation coefficient
G. affinis	-0.11
G. aculeatus	-0.04
O. microlepidotus	-0.10
Cottus sp.	0.16
C. carpio	0.63 *
L. microlophus	0.05

*$p < 0.05$

Table 3. Partial correlation coefficients of fish species with distance to open water after controlling for water temperatures, depth, distances to nearshore, next-nearest shore, boardwalks, and pondweed biomass (N=13).

Species	Correlation Coefficient
G. affinis	-0.15
G. aculeatus	-0.26
O. microlepidotus	0.43
Cottus sp.	0.43
C. carpio	-0.53 *
L. microlophus	0.07

*$p < 0.05$

Table 4. Partial correlation coefficients between fish species after controlling for surface water temperature, depth, distances to nearshore, next-nearest shore, boardwalks, and pondweed biomass (N=13).

Species	Species				
	G. aculeatus	*O. microlepidotus*	*Cottus* sp.	*C. carpio*	*L. microlophus*
G. affinis	0.16	0.65 *	-0.44	0.19	0.26
G. aculeatus		-0.36	0.55 *	0.17	0.01
O. microlepidotus			-0.51	0.09	0.13
Cottus sp.				0.14	-0.25
C. carpio					-0.44

*$p < 0.05$

model. Since actual densities proved to be higher than anticipated these species will be included in feeding and pondweed distribution studies in 1983. This has important modeling implications as both of these fish are potential predators on mosquito larvae (Moyle 1976).

The relationships between mosquitofish density and the density and distribution of pondweed were consistent with modeling assumptions. As modeled, pondweed interferes with the success of feeding on mosquito larvae, rather than restricting their entrance. This assumption will be tested during the 1983 field season. The three-spined stickleback showed a distribution pattern similar to the mosquitofish relative to pondweed density and distribution. One hypothesis based on Tables 2 and 3 is that the mosquitofish may occur less frequently in dense pondweed areas, but is more likely to penetrate farther into dense pondweed stands than sticklebacks.

The final modeling assumption was that mosquitofish distributions were independent of the distributions of the other fish species. This assumption was not met. Reasons for this could be a substantial niche overlap or a predator-prey relationship between two fish. None of the correlations with potential predators (sculpin, carp and sunfish) are relevant to the model either on the basis of the direction of the correlation or the density of the potential predator. Future diet studies are planned to assess the modeling implications of the correlations of mosquitofish with Sacramento blackfish and three-spined stickleback. If they are determined to be competitors the model will be altered accordingly.

Future work will include completion of a full field season of calibration studies, analysis of fish secondary production and field testing of the effect of variable pondweed density and predator combinations on mosquito larvae survival.

ACKNOWLEDGMENTS.–This research was funded by the Alameda County Mosquito Abatement District and done in cooperation with the East Bay Regional Park District and the University of California, Berkeley. I also thank Ms. Nannette Franceschini who kindly typed an earlier draft of the manuscript.

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QUALITY CONTROL IN LABORATORY PRODUCTION OF *CULEX TARSALIS*[1]

S. Monica Asman[2,3] and Nancy F. Knop[3]

ABSTRACT

Field releases of radio-sterilized males indicated that laboratory-reared material was uncompetitive in the field due to assortative mating behavior. Flight-mill tests also demonstrated that a laboratory colony was less than 50% as competent in flight as was the field population. Laboratory selection may have occurred because females failed to oviposit.

Preliminary data on a 2-year study to produce laboratory *Culex tarsalis* with similar mating behavior to field populations suggest that a composite diet consisting of specific nutrients is essential to optimize all life functions. In oviposition-site preference experiments, data indicated that dark cups, and dark cups with an egg-raft associated pheromone, are preferred. Fatty acids used as substitutes for the natural pheromone also show promise in preliminary experiments. Electrophoretic analysis of enzymes indicate the material reared under a variable laboratory environment rather than a constant one retains more heterozygosity in gene loci patterns. In addition to electrophoretic monitoring, flight-mill studies and competition testing all of which must still be done should give more conclusive data on relationships between artificial rearing components and life-function parameters.

[1]These studies were supported by funds from the California State Appropriations for Mosquito Control Research in the University of California and by special funds for mosquito control research appropriated annually by the California legislature.
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SWARMING AND MATING BEHAVIOR OF *CULEX TARSALIS*: COMPARSION OF FIELD AND LABORATORY-ADAPTED POPULATIONS[1]

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ABSTRACT

Virgin adults that emerged from field-collected or laboratory- reared pupae were released into a quonset cage and observed during several dusks and dawns. Females were collected periodically to determine when mating occurred. Observation of mating pairs falling out of the swarms and dissection of females indicated that insemination occurs during the swarming intervals at dusk and dawn. In the quonset cage, field-collected males had smaller and fewer swarms, and fewer field-collected females were inseminated, suggesting that space is used differently by laboratory-adapted and field-collected *Culex tarsalis*. Laboratory-adapted and field-collected mosquitoes observed in the quonset field cage and a population observed in the field responded similarly to light changes at dusk and dawn.

[1]These studies were supported by special funds for mosquito control researsh appropriated annually by the California Legislature and from the National Grant AI-3028 Institute of Allergy and Infectious Diseases.
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LIFETIME MATING PATTERNS OF LABORATORY-ADAPTED *CULEX TARSALIS* MALES[1]

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ABSTRACT

The lifetime mating patterns of *Culex tarsalis* Coquillett males were determined under insectary conditions. Thirty males were offered new harems of virgin females daily, from emergence to death, while individually confined in 4 liter carton cages. The number of males alive, males mating, and females inseminated per day was used to calculate male life tables, applying statistical methods developed for female mosquitoes.

Cohort survivorship was estimated to be 98% per day and life expectancy was 29 days at emergence. The mortality rate was greatest between days 15 and 21 of adult life, when 33% of the cohort died. The number of males mating per day was greatest between days 2 and 9, decreasing to zero by day 37. Mating activity was renewed between days 46 and 55. The maximum number of females mated per male per night was 4.

Mean lifetime reproductive effort was 12.2 females inseminated per male per generation (range = 0 to 29). Four males accounted for 105 (29%) of the total 360 inseminations. Male reproductive effort (females inseminated per male) increased as a function of male longevity, i.e., males living longer inseminated more females.

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ATTEMPTED INSERTION OF A RECESSIVE AUTOSOMAL GENE INTO A SEMI-ISOLATED POPULATION OF *CULEX TARSALIS* COQ. (DIPTERA: CULICIDAE)[1]

William K. Reisen, Martha E. Bock, Marilyn M. Milby and William C. Reeves[2]

ABSTRACT

A total of 158,957 *Cx. tarsalis* adults carrying a recessive, autosomal mutant, carmine eye, were released on 21 occasions from 5 April to 15 May 1982 at a semi-isolated area in the arid foothills of Kern County, California. The persistence of the released genotype was monitored by examining the progeny of field-mated females from the target population, backcrossing field males to homozygous carmine laboratory females and examining their progeny, and searching for homozygous carmine larvae. Phenotypic frequencies declined rapidly following the vernal rise of the target population, although males heterozygous for carmine were recovered throughout the sampling period. Assortative swarming and mating behavior among the released genotype may have precluded the insertion of the released gene into the target population gene pool. Reduced larval fitness and a high immigration rate may have contributed to the failure of the released genome to be maintained at a high level.

[1]Funded by Research Grant AI-3028 from the National Institute of Allergy and Infectious Diseases, General Research Support Grant I-S01-FR-0441 for the National Institutes of Health and by special funds for mosquito control research appropriated annually by the California Legislature.

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A FIELD LARVAL LIFE TABLE FOR *ANOPHELES FREEBORNI*

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ABSTRACT

Field studies were carried out in a large, laser-planed commercial rice field in Yuba County chosen for its atypically high density population of *Anopheles freeborni*. Removal-replacement counts by instar were performed on a sample grid of 480 points on each of 13 days, divided into three periods of sequential sampling between July 28 and September 16. This period corresponds to the usual time frame of the peak population counts and declining numbers in the seasonal population cycle for *An. freeborni*, bracketing the period wherein the population growth appears as exponential. The sample grid was shifted after the seventh sample day so that half the points were sampled throughout, the other half seven and six times respectively, to control for location phenomena and possible disturbance by the sampling protocol. Relative sampling efficiency per instar per individual were calibrated utilizing isolation columns stocked with four different densities of *An. freeborni* larvae marked with neutral red dye. Counts were analyzed with a modification of the Service-Lokhani method in differential form to determine the intrinsic rate of increase for the local population and adjusting the **M** values of the Service-Lokhani model appropriately to eliminate steady-state assumptions which are inapplicable to this species. Estimated survivorship shows 5% survival from 1st instar to pupa in spite of a rich community of predatory forms. The population did not exhibit the anticipated precipitous decline but rather showed oviposition continuing beyond the time of rice harvest into late October, as determined at an alternate experimental test site kept in rice for an additional month.

THE EFFECT OF BAY SIR 8514 AGAINST SELECTED ORGANISMS ASSOCIATED WITH MOSQUITO BREEDING HABITATS

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ABSTRACT

The acute toxicity of technical material of a candidate mosquito larvicide, BAY SIR 8514, (2-Chloro-N-[[[4-(trifluoromethoxy) phenyl] amino] carbonyl] benzamide) varied greatly among species of organisms. Water fleas (*Ceriodaphnia* spp.) were considerably more sensitive (LC_{50} = 0.00005 ppm) than sideswimmers [*Hyalella azteca* (Saussure), LC_{50} = 0.0002 ppm], and early instars of copepods (*Cyclops vernalis* Fischer, LC_{50} = 0.012 ppm), whereas the compound caused 0% mortality of seed shrimp (*Cyprois* spp.) at 0.02 ppm. Among aquatic insects tested, 1st instars of *Hydrophilus triangularis* Say were the most susceptible (LC_{50} = 0.0006 ppm), followed by mayfly nymphs (*Callibaetis* spp. LC_{50} = 0.003 ppm). Dragonfly nymphs [*Anax junius* (Drury) 0% mortality at 0.02 ppm] demonstrated a tolerance. Mosquitofish fry [*Gambusia affinis* (Baird and Girard)] showed no effect when exposed to a 0.2 ppm concn for 10 days.

Simulated field tests with BAY SIR 8514 (technical material) at the rate of 0.02 ppm suppressed the water flea and sideswimmer population densities but they recovered again. Copepod population densities may have been affected but the damage was minor. Seed shrimp were not affected. Multiple applications of BAY SIR 8514 (25% WP) at the rate of 56 g AI/ha for a 2 month period did not harm mosquitofish colonies in the tanks.

The data obtained from field tests indicate that a 0.5% sand G formulation at 11.2 g AI/ha and a 35 g/liter EC at 112 g AI/ha treatments severely affected water fleas but only slightly affected copepod populations. Both populations recovered within a week. Seed shrimp and aquatic insect populations were not affected by the treatments.

INTRODUCTION.–The insect growth regulator BAY SIR 8514, (2-Chloro-N-[[[4-(trifluoromethoxy) phenyl] amino] carbonyl] benzamide) is a substituted benzamide that inhibits the terminal polymerization step in chitin synthesis during metamorphosis of insects (Hajjar and Casida 1978). It is biologically very active against a variety of pestiferous insects such as mosquitoes (Herald et al. 1980, Miura and Takahashi 1979, Mulla and Darwazeh 1979, Schaefer et al. 1978), the house fly (Chang 1979), chironomid midges (Ali and Lord 1980a, Johnson and Mulla 1981), the Clear Lake gnat (Colwell and Schaefer 1981), a sciarid fly, *Lycoriella mali* (Fitch) (Argauer and Cantelo 1980) and the spruce budworm (Retnakaran 1980). However, toxicity information for evaluating its effects on nontarget and predacious organisms of the target insects is lacking.

Reported here are results of the toxicity studies and field tests conducted to evaluate effects of BAY SIR 8514 on selected aquatic organisms commonly associated with mosquito breeding habitats in the San Joaquin Valley of California.

MATERIALS AND METHODS.–The toxicity study of the compound against nontarget organisms was conducted in the laboratory and treatment impact was investigated outdoors in artificial containers, ponds and irrigated pastures.

Toxicity Tests. The test organisms were obtained from laboratory colonies or from the Tracy Experimental Plot (Miura et al. 1978) and only acclimatized immature organisms (ambient temperature 21° ± 1°C for two days) were used.

The evaluation techniques were those by Miura and Takahashi (1973), except where noted otherwise. In brief, the stock solutions (wt/v) in acetone were prepared from technical material and serial dilutions were prepared with deep well water as needed. Usually each concn was run in triplicate and each test was repeated 2 to 3 times.

Outdoor Tests in Artificial Containers. A simulated field test against zooplankton was conducted outdoors in aquaria. A mixture of *Ceriodaphnia* spp., *Moina* spp., *Alona* spp., *Cyprois* spp., *Cyclops vernalis* and *Hyalella azteca* were maintained in a 20-liter glass aquaria containing 16 liters of water. One aquarium was tested with 0.02 ppm of technical material in acetone and the other was left as an experimental check. Pre- and posttreatment population censuses were made by sampling at the corners of each aquarium with a 36-ml dipper.

Colonies of mosquitofish, Gambusia affinis (Baird and Girard) were established in two metal tanks (ca. 200-liter capacity, 112 x 76 cm surface area) containing 160 liters of water. The fish in one tank were treated three times with BAY SIR 8514 25% WP at the rate of 56 g AI/ha; the other tank was left as a check. Pre- and posttreatment population counts were taken twice a week with modified minnow traps (Miura and Takahashi 1975).

Field Tests. Field tests were conducted in 0.02 ha ponds at the Tracy Experimental Plot. BAY SIR 8514 was applied with a hand sprayer using a 35 g/liter EC formulation for *Aedes nigromaculis* (Ludlow) control, a 0.5% sand G formulation, applied by a granule spreader was used for *Culex tarsalis* Coquillett control. The rate used for both formulations ranged from 112 to 1.12 g AI/ha.

Population densities of nontarget organisms in treated plots were sampled prior to and at intervals after treatment. The planktonic organisms were collected by a 450 ml dipper, 10 dip samples were taken from each plot and processed in the laboratory (Miura and Takahashi 1975, 1976). The nectonic insects were collected weekly using modified minnow traps (Miura and Takahashi 1975).

RESULTS AND DISCUSSION.–**Toxicity Tests.** The acute toxicity of technical material of BAY SIR 8514 varied greatly among organisms (Table 1). Generally planktonic crustaceans especially immature water fleas (Cladoceran, *Ceriodaphnia* spp.) and sideswimmers (*H. azteca*) were highly sensitive (LC_{50} = 0.00005 ppm and 0.0002 ppm respectively). The LC_{50} for mixed instars (nauplii and early instars) of copepods (*C. vernalis*) was 0.012 ppm and mixed populations of late instars and adults was 0.02 ppm, whereas seed shrimp (*Cyprois* spp.) tolerated the compound at levels as much as 0.02 ppm. Among aquatic insects tested, 1st instars of *Hydrophilus triangularis* Say were the most susceptible (LC_{50} = 0.0006 ppm), backswimmers (*Notonecta unifasciatus* Gerin) nymphs were moderately susceptible. While dragonfly (*Anax junius* Drury) nymphs demonstrated a high tolerance. No mortality was observed by exposing mosquitofish fry in a 0.02 ppm concn for a 10 day period.

Outdoor Tests in Artificial Containers. Figure 1 shows effects of BAY SIR 8514 (0.02 ppm) on crustacean population densities. Water fleas were most susceptible; the density declined to zero after the treatment and remained undetectable for 40 days and gradually recovered to the pretreatment density level. The sideswimmer, *H. azteca*, population suffered but it did not become zero. The copepod may have shown some response to the treatment, but no prolonged adverse effects were observed. The seed shrimp, *Cyprois* spp., population density was not affected. The population of mosquitofish in the tank was treated three times at the rate of 56 g AI/ha during a two month period, but no deleterious effect was observed (Figure 2).

Field Tests. Table 2 summarizes effects of the sand G treatment (11.2 g AI/ha) on planktonic organisms and some small immature insects. Water fleas were most severely affected; the population disappeared in the water sample taken immediately after treatment and held in the laboratory for observations, the population in the treated pond also was severely suppressed and remained at undetectable levels for 10 days posttreatments. Copepod and mayfly populations may have suffered slightly in the posttreatment water sample, but in the treated pond, no detectable population change was observed.

Figure 3 shows temporal population density trends of crustaceans and mayfly nymphs in the sand G treated plot. The aforementioned water fleas were the most severely affected; the density of pretreatment counts fluctuated between ca. 100 and 500 per 10 dips, but on the 4th day posttreatment the count became 0 for a week. This is in contrast to the results of the outdoor test in artificial containers, in the container, the population was eliminated for forty days posttreatment (Figure 1); quicker recovery of the density in the treated plot is probably due to reinfestation through periodic flooding from an open ditch system. Copepod and mayfly nymphs may have suffered from the treatment but the population densities were not altered noticeably. No deleterious effect on seed shrimp was observed.

Table 1. Biological activity of BAY SIR 8514 against organisms associated with mosquito breeding habitats.

Test organism	Stage	LC_{50} or mortality (%)	Concn (ppm)	Exposure (h)	Replicates	No. org./ container
Ceriodaphnia spp.	Immature	LC_{50}	0.00005	48	3	27+
C. vernalis	Adult	LC_{50}	.02	168	2	30,40
C. vernalis	Immature	LC_{50}	.012	168	2	30
Cyprois spp.	Immature	0	.02	168	3	15+
H. azteca	Immature	LC_{50}	.0002	168	3	20
Calibaetis	Nymph	LC_{50}	.003	96	25	1
N. unifasciatus	Nymph	ca. 40	.02	192	3	10
H. triangularis	1st instar	LC_{50}	.0006	240	20	1
A. junius	Nymph	0	.02	96	1	10
G. affinis	Fry	0	.02	240	3	10

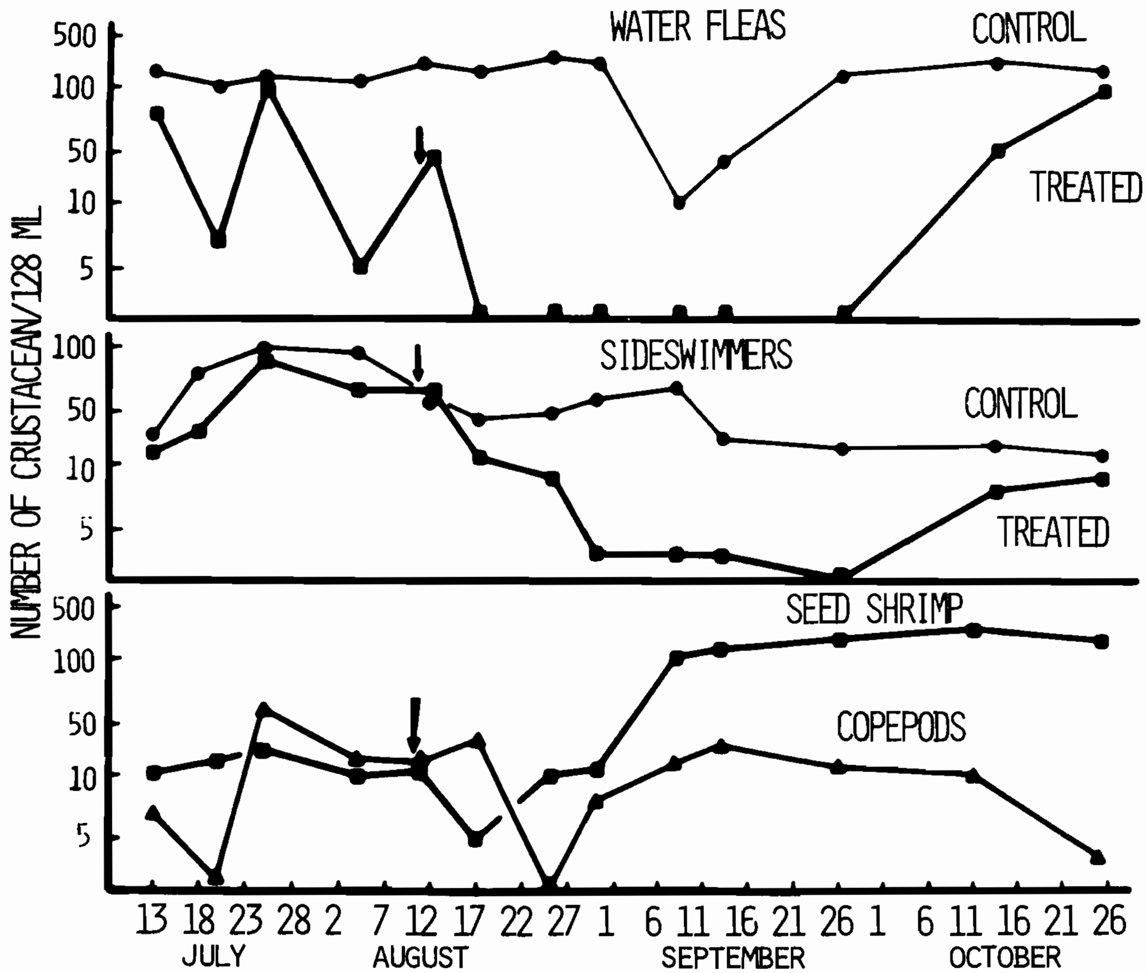

Figure 1. Outdoor tests in 20 liter aquaria: Effects of BAY SIR 8514 (0.02 ppm) on the selected crustacean populations (arrows indicate application days. Treatment control of seed shrimp and copecod populations are not shown, use pretreatment counts as control).

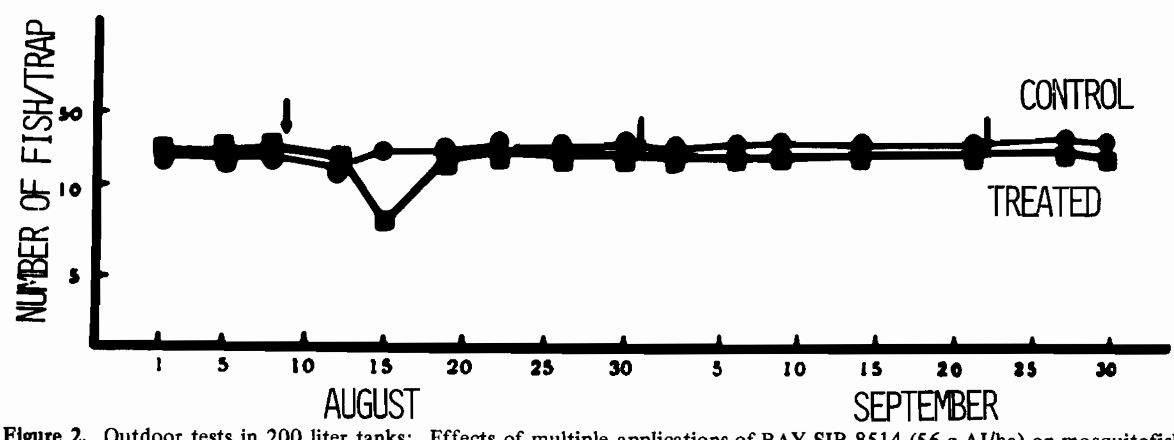

Figure 2. Outdoor tests in 200 liter tanks: Effects of multiple applications of BAY SIR 8514 (56 g AI/ha) on mosquitofish populations (arrows indicate application days).

Table 2. Effects of BAY SIR 8514 (0.5% sand G) on nontarget organisms applied at 11.2 g AI/ha against *Cx. tarsalis.*

Organism	August 18	21	22	23	28
	No. of organisms in the pretreatment water (4500ml)[a]				
Water fleas	46	44	43	42	41
Copepods	16	13	13	13	14
Ostracods	5	5	5	5	5
Mayfly N[b]	1	1	1	1	1
Belostoma sp.	1	1	1	1	0
	No. organisms in water (4500ml) collected immediately after treatment[a]				
Water fleas	24	0	0	0	0
Copepods	17	13	13	9	9
Ostracods	22	19	19	15	7
Mayfly N	3	3	2	1	0
Belostoma sp.					
	No. organisms in water (4500ml) from daily field collection				
Water fleas	46	9	0	0	0
Copepods	16	34	45	23	21
Ostracods	45	430	403	79	17
Mayfly N	1	51	12	54	1
Belostoma sp.	1	0	0	0	0

[a] Water samples (pretreatment and immediately after treatment) were held in the laboratory and organisms were observed daily.

[b] N = nymphs.

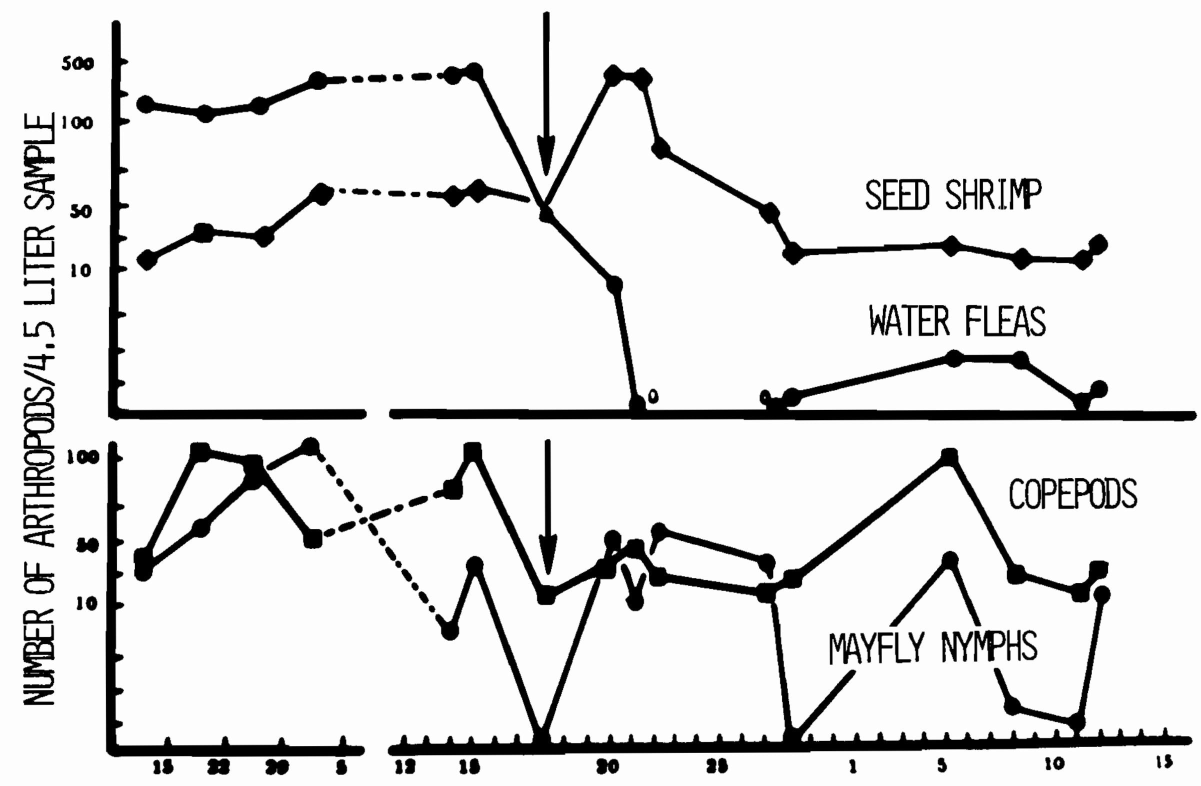

Figure 3. Effects of BAY SIR 8514 0.5% sand G formulation (112 g AI/ha) on crustacean and mayfly nymph populations in ponds (arrows indicate application days. Use pretreatment counts as control).

Seasonal abundances of insect population densities in the sand G treated plot are shown in Figure 4. Density change during the pre-and posttreatment were evaluated by the family units for notonectids (backswimmers; *N. unifasciatus* and *Buenoa scimitra* Bare), adult dytiscids (predacious water beetles; mostly *Laccophilus* spp. and *Thermonectus* spp.) and adult hydrophilids (water scavenger beetles; mostly *Tropisternus* spp. and *H. triangularis*). All collections of aquatic beetle larvae were combined and treated as one unit. No adverse treatment effect was observed among those insect groups by examining the pre-, and posttreatment density changes. The toxicity test in the laboratory indicated that the newly hatched *H. triangularis* larvae were considerably sensitive (LC_{50} = 0.0006 ppm), but the results of the field test did not show any noticeable adverse effect on the larval population, although *H. triangularis* larvae were abundant in the treated plot. Notonectid bugs may have learned to avoid traps when the traps are used repeatedly; ca. 70 to 90 bugs were trapped at the early trappings, but only 10 to 25 bugs were collected at each subsequent trapping even though the population growth was observed in the ponds, trapping records did not reflect this increase (Figure 4).

Table 3 shows the results of the BAY SIR 8514 (EC) treatment at the rate of 112 g AI/ha on nontarget organisms in an irrigated pasture. Here again water flea populations were severely damaged. Copepod and mayfly populations may have been affected but the densities were never depressed severely. Other insects i.e., dragonfly and corixid nymphs and chironomid larvae were also collected but the collection numbers were too small to draw conclusions.

The results obtained during this study are in general agreement with those of Ali and Lord (1980b), Mulla and Darwazeh (1979) and Schaefer et al. (1978). However, the circumstantial data (i.e., no toxicity tests of organisms) of Ali and Lord (1980b) on copepods do not agree with the present findings where copepods were slightly affected by BAY SIR 8514 even at a rate as high as 112 g AI/ha.

In summary, BAY SIR 8514 does not appear to have any

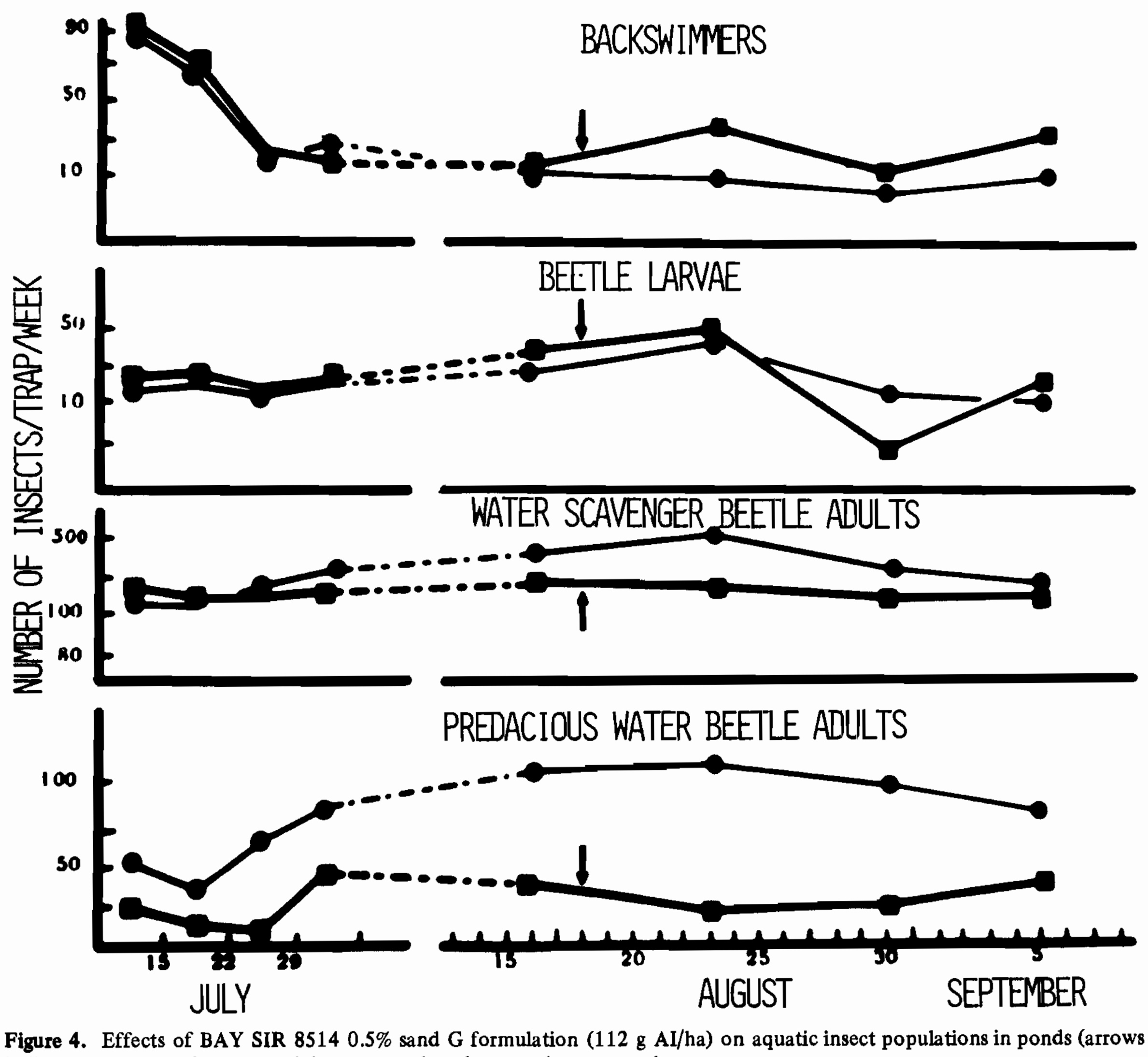

Figure 4. Effects of BAY SIR 8514 0.5% sand G formulation (112 g AI/ha) on aquatic insect populations in ponds (arrows indicate application days: round dots - control, and square dots - treated.

Table 3. Effect of BAY SIR 8514 (35 g/liter, EC) on nontarget organisms applied at 112 g AI/ha against *Ae. nigromaculis*.

Organism	July				
	5	6	7	8	9
	No. organisms in water (4500ml) from treated pasture				
Water fleas	6	4	0	0	0
Copepods	44	13	26	8	11
Mayfly N[a]	2	59	89	6	3
Dragonfly N	0	1	0	0	0
Corixid N	0	7	0	1	3
Chironomid L[b]	0	1	2	4	0
	No. organisms in water (4500ml) from check pasture				
Water fleas	16	22	66	188	9
Copepods	48	109	90	70	102
Mayfly N	15	31	127	29	21
Dragonfly N	0	2	5	2	0
Corixid N	0	0	1	2	0
Chironomid L	0	1	1	4	1

[a] N = nymphs.

[b] L = larvae.

long lasting adverse effects on nontarget aquatic organisms associated with mosquito breeding habitats. Therefore, development and use of such compounds for mosquito control will open a new strategy for integrated pest management programs.

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NONTHERMAL AEROSOLING TO CONTROL *ANOPHELES FREEBORNI* AND PREVENT SECONDARY TRANSMISSION OF *PLASMODIUM VIVAX*

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Cases of *Plasmodium vivax* malaria among Punjabi immigrants in the Sutter-Yuba area continue to concern local health and mosquito control officials. Although the number of cases in 1982 was below the peak number in 1979 and 1980, the possibility of secondary transmission persists. Adulticiding with nonthermal fog is the primary method of preventing secondary transmission of imported malaria. This study was designed to test the efficacy of adulticiding procedures presently employed in controlling adult *Anopheles freeborni* populations in Sutter County, California.

Sentinel cage tests were conducted to determine the potential impact of spray operations. Using malathion under normal spray conditions (i.e., 6-8 oz/min), mortality of caged *An. freeborni* was high (100% to 0.25 mi; 85% at 0.5 mi). The impact of insecticidal treatments on the wild population was estimated by collecting resting mosquitoes from 6 x 4 x 4 ft. red boxes prior to and after treatment. Initially, there appeared to be little difference between population growth in the treated zone and that in a nontreated, control area. After four weekly treatments, however, population growth in the treated zone declined while the population in the nontreated area continued to increase. Movement of mosquitoes into or out of the treated and the control areas may have influenced these population trends, but this possibility was not investigated.

Because older peach orchards provide a habitat attractive to adult *An. freeborni*, a sentinel cage test was conducted to determine the effectiveness of nonthermal fogging in an orchard environment. A young (ca. 5% canopy cover) and an old (ca. 70% canopy cover) orchard were used for this test. In the young orchard, mortality was observed at all distances tested (over 90% to 100 m; 35% at 400 m). Mortality in the old orchard was negligible in cages close to the spray line (5% at 10 m), and completely absent to all other distances tested. Wind speed during the orchard spray test was low (0-1 mph); an increase in wind speed might significantly alter the spread of insecticide through the orchard.

EVALUATION OF UNSATURATED FATTY ACIDS AS MOSQUITO OVIPOSITIONAL REPELLENTS

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Gravid mosquitoes sometimes use certain volatile organic compounds as olfactory indicators to search for suitable ovipositional sites or to avoid unsuitable sites. Our studies showed that six lower carboxylic acids (acetic, propionic, isobutyric, butyric, isovaleric, and caproic), isolated and identified from an organic infusion, were ovipositionally repellent to gravid *Culex* mosquitoes (Hwang et al. 1980). Further studies revealed that some medium-chain carboxylic acids, such as octanoic, nonanoic, and decanoic acids, were more repellent than the lower carboxylic acids (Hwang et al. 1982).

In the present study, we evaluated higher carboxylic acids for their ovipositional repellency against *Cx. quinquefasciatus* Say and investigated their structure-activity relationship

In conducting bioassay tests, the olfactometer disclosed earlier (Kramer and Mulla 1979) was used to determine the ovipositional repellency of test compounds. In the olfactometer, five gravid mosquitoes were given a choice between a treated dish and a control dish in replicated tests. The ovipositional activity was determined by the ratio of the numbers of egg rafts in the treated and control dishes and expressed as ovipositional activity index (0AI) calculated according to the formula $0AI = (N_T - N_S) / (N_T + N_S)$. N_T represents the number of egg rafts in the treated dishes, and N_S represents that in the control. All index values fall within the range of +1 to -1. A negative 0AI indicates that more ovipositions take place in the control than in the treated dish, thus signifying the treated dish contains a repellent. On the other hand, a positive 0AI means the treated sih contains an attractant.

Figure 1 and 2 give the names and the structures of 18 unsaturated fatty acids evaluated. Oleic acid and γ-linolenic acid were the most active in repelling ovipositing mosquitoes showing significant 0AI of -0.34 to -0.35 at 1×10^{-5}M. In addition to these 18 unsaturated fatty acids, eight straight-chain saturated fatty acids from C_{15} to C_{21} were evaluated and found to have no ovipositional activity.

Stearic acid is a typical example of a saturated fatty acid which was found to be ovipositionally inactive. Oleic acid has a double bond at the 9-position and showed a good repellency. A fatty acid with a triple bond, such as 9-octadecynoic acid, did not show any ovipositional activity. Therefore, it was apparent that a double bond was essential in a fatty acid to show ovipositional repellency.(Figure 3).

In discussing the relationship between the repellency and the structures of the unsaturated fatty acids, the following criteria are taken into consideration: (1) the geometric isomerism about the double bond, (2) the number of double bonds, (3) the position of the double bonds, and (4) the chain length of fatty acids.

Oleic acid and elaidic acid are geometric isomers. Oleic acid which has *cis*-configuration was one of the most repellent compounds among those evaluated; however, its *trans*-isomer, elaidic acid was not active at all. A similar relationship could be found between palmitoleic and palmitelaidic acids, between *cis*- and *trans*-vaccenic acids, and between linoleic and linolelaidic acids. All *cis*-acids were highly repellent, but all *trans*-acids were inactive or weakly active. Consequently, it

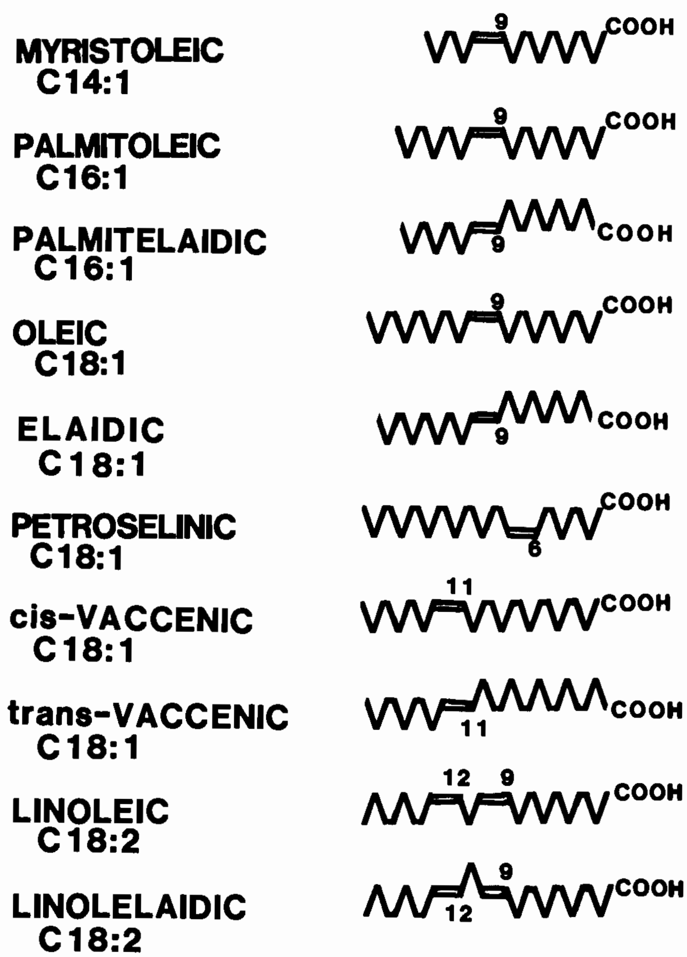

Figure 1. Structures of unsaturated fatty acids evaluated for ovipositional repellency. Part 1.

was concluded that a double bond with *cis*-configuration was essential for a fatty acid to show good repellency.

Oleic acid and γ-linolenic acid were equally active and were more active than linoleic or linolenic acid. These acids are all C_{18} acids and have 1, 2, or 3 double bonds, but an increase in the number of double bonds did not necessarily increase the repellency.

In C_{20} acids, (*Z*)-11-eicosenoic and arachidonic acids were more active than homo-γ-linolenic acids. There was no clear-cut relationship between the number of double bonds and the repellency in C_{20} acids either.

Erusic and (*Z,Z,Z,Z,Z,Z*)-4,7,10,13,16,19-docosahexaenoic acids were equally active despite the former being monounsaturated and the latter being polyunsaturated.

Oleic, *cis*-vaccenic and petroselinic acids are all C_{18} acids with the double bond in different positions. When the double bond moved from 9- to 11- to 6-position, the ovipositional repellency decreased. It seems that a *cis*-double bond at the 9-position was essential in attaining the high ovipositional repellency in C_{18} fatty acids.

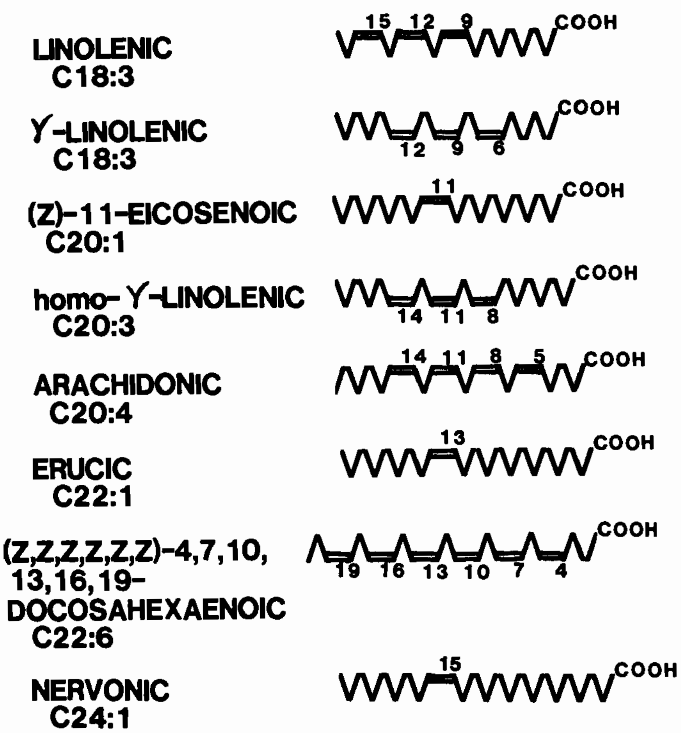

Figure 2. Structures of unsaturated fatty acids evaluated for ovipositional repellency. Part 2.

Linoleic acid has 2 double bonds at 9- and 12- positions and was moderately repellent. If an additional double bond is present at the 15-position as in the case of linolenic acid, the activity did not change. But, when the additional double bond was located at the 6-position as in the case of γ-linolenic acid, the activity increased. It seemed that the position of double bonds was crucial in determining the repellency of an unsaturated fatty acid.

Myristoleic, palmitoleic, and oleic acids are C_{14}, C_{16}, and C_{18} acids, respectively, and have the same feature in having the double bond at the 9-position. As the chain length shortened from oleic acid to palmitoleic acid to myristoleic acid, the activity decreased in this order. Oleic, (*Z*)-11-eicosenoic, erucic, and nervonic acids are C_{18}, C_{20}, C_{22}, and C_{24} acids, respectively, and share a similar structural characteristic in the position of the double bond if the numbering starts from the *ω*-end, or the methyl end. In these 4 compounds, when the chain length elongated in the α-end, or the carboxyl end, as from oleic acid to (*Z*)-11-eicosenoic acid to erucic acid to nervonic acid, the activity decreased. Accordingly, a chain length of 18 seems to contribute to a higher dregree of repellency.

Among the unsaturated fatty acid tested, oleic acid, which seemed to meet all the criteria discussed above, was the most ovipositionally repellent to gravid females of *Cx. quinquefasciatus*.

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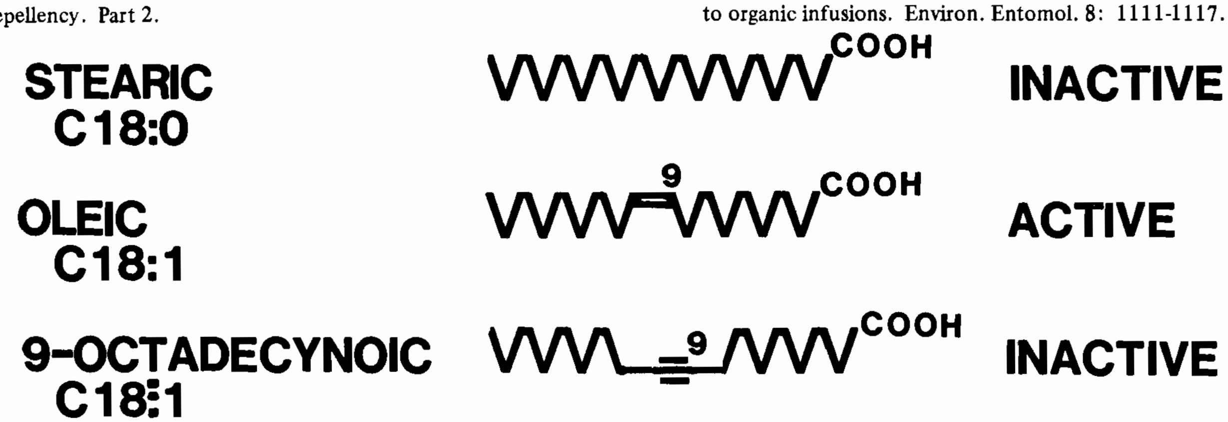

Figure 3. Only fatty acids with double bonds were ovipositionally active. Fatty acids with single bonds or triple bonds were not active.

CUTICULAR LIPIDS OF *HIPPELATES COLLUSOR*

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The primary function of insect cuticular lipids is to restrict water movement across the cuticle, thereby preventing insects from desiccation (Beament 1964). The insect cuticular lipids also prevent the penetration of inorganic compounds and affect the absorption of organic compounds (Beament 1964, Ebeling 1964). They act as a barrier against the invasion of microorganisms (David 1967). They may serve as kairomones for insect parasites and predators and as pheromones for chemical communications among individual insects of the same species (Evans and Green 1973, Lewis et al. 1976). It is the pheromonal function of insect cuticular lipids that we are currently most interested in.

A number of examples of cuticular lipids served as sex pheromones in dipteran insects have already been reported. Females of the little housefly, *Fannia canicularis* (L.) produces sex attractant (*Z*)-9-pentacosene (Uebel et al. 1975a). The sex pheromone of the face fly, *Musca autumnalis* DeGeer, was produced by both sexes; however, the pheromonal activity of the active compounds, (*Z*)-14-nonacosene, (*Z*)-13-nonacosene, and (*Z*)-13-heptacosene, was masked in the male by a higher proportion of nonacosane and heptacosane than were present in the female (Uebel et al. 1975b). Female *Musca domestica* L. produced (*Z*)-9-tricosene as a sex pheromone (Carlson et al. 1971). The sex pheromone of the mushroom fly, *Lycoriella mali* (Fitch), was reported to be a mixture of 15 to 26 carbon *n*-alkanes with heptadecane being the most active component which was present 5 to 6 times more in females than in males (Kostelc et al. 1975). 13,23-Dimethylpentatriacontane was present in the cuticular lipids of the tsetse fly, *Glossina pallidepes* Austen and found to be a sex stimulant pheromone for this species of insect (Carlson et al. 1982).

The eye gnat, *Hippelates collusor* (Townsend), is a pestiferous insect in southern California. Particularly in Coachella Valley of Riverside County, high populations of *H. collusor* are frequently observed during summer months. Cultural, chemical, biological, and behavioral control strategies have been adopted to suppress eye gnat populations.

According to Adams and Mulla (1967), oogenesis in female *H. collusor* could be divided into a series of 10 stages with stage 1 being the germarium and stage 10 the mature egg. Female eye gnats mated during stages 6 through 9 of ovarian development with the preferred stage at 7. Olfactometer studies demonstrated the presence of a sex pheromone in live females in stages 6 through 9. The sex pheromone was attractive to males, but it was effective only over a short distance (Adams 1966).

With the knowledge of above-described findings, we embarked upon a series of studies on the cuticular lipids of *H. collusor* with an ultimate objective of isolating and identifying the sex pheromone of this insect. In the first stage of these investigations, efforts were concentrated on the separation and identification of cuticular components of *H. collusor.*

In extracting cuticular lipids, about 200 female or male adult eye gnats of the same age were frozen at -10° C for 45 minutes and immersed in 50 ml of redistilled hexane for five minutes with occasional gentle stirring. The extracted eye gnats were removed by filtration, again immersed in hexane for another five minutes, and filtered. The filtrates were combined and evaporated to dryness to give cuticular lipids which were subjected to gas chromatographic and mass spectrometric analyses.

The gas chromoatography was carried out with a column packed with 3% Dexsil 300 on Chromosorb W. The temperature was programmed between 200° and 300° C at 4° C/min. Eleven major peaks were repeatedly obtained from this glc analysis (Figure 1). A similar chromatographic pattern was obtained with a SE-30 column. Each cuticular component eluted from the SE-30 column was trapped with dry ice and analyzed with a mass spectrometer. As a result of this analysis, 11 straight-chain alkanes from C_{26} to C_{36} were identified. The most prominent component was C_{34} alkane, tetratriacontane.

Our studies revealed that the gas chromatograms of the cuticular lipids of *H. collusor* consistently showed a similar pattern of 11 major peaks, suggesting the presence of 11 alkanes regardless of age and sex. This indicated that the differences in age and sex did not affect the qualitative composition of the eye gnat cuticular alkanes.

However, quantitative studies showed that some differences existed in the alkane contents between sexes and among various ages of *H. collusor.* The major difference was found in the contents of C_{29} alkane, nonacosane, which constituted only 40% of those of C_{30} alkane, triacontane, in male cuticular lipids but which was about equal to those of C_{30} alkane in female.

In Figure 2, the C_{29} to C_{30} ratios in the male and female cuticular alkanes were plotted against the age of *H. collusor*

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extracted. In one-day old eye gnats, the ratios between male and female were quite close (0.4-0.5) signifying that the chemical compositions of the cuticular alkanes of both sexes were qualitatively and quantitatively similar at this early age. Thereafter, the ratio for females suddenly increased to about 0.9 and fluctuated around this level until day 14. This increase in ratio indicated that the quantity of C_{29} alkane drastically increased in the cuticle of adult female *H. collusor* when they reached two days of age. The C_{29} contents in females remained high throughout the rest of their adult life. The ratio for males, on the other hand, stayed at the same low level (0.4) until the last day of this experiment.

The deficiency of C_{29} alkane in the male alkanes would make the profile of male cuticular lipids somehow different from that of female lipids. The quantitative difference in chemical composition may result in olfactory difference between sexes. Further work is currently under way to study the implication of this difference.

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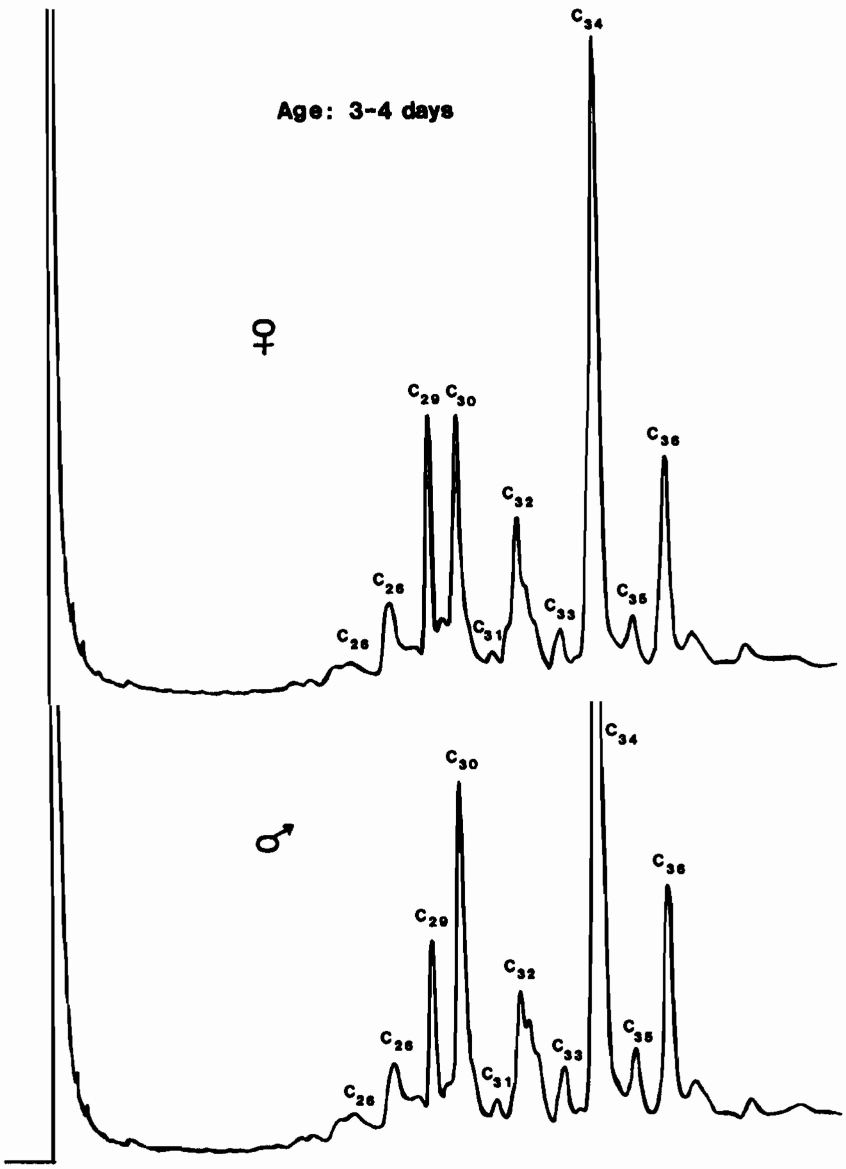

Figure 1. Typical gas chromatograms of cuticular lipids extracted from male and female *H. collusor.*

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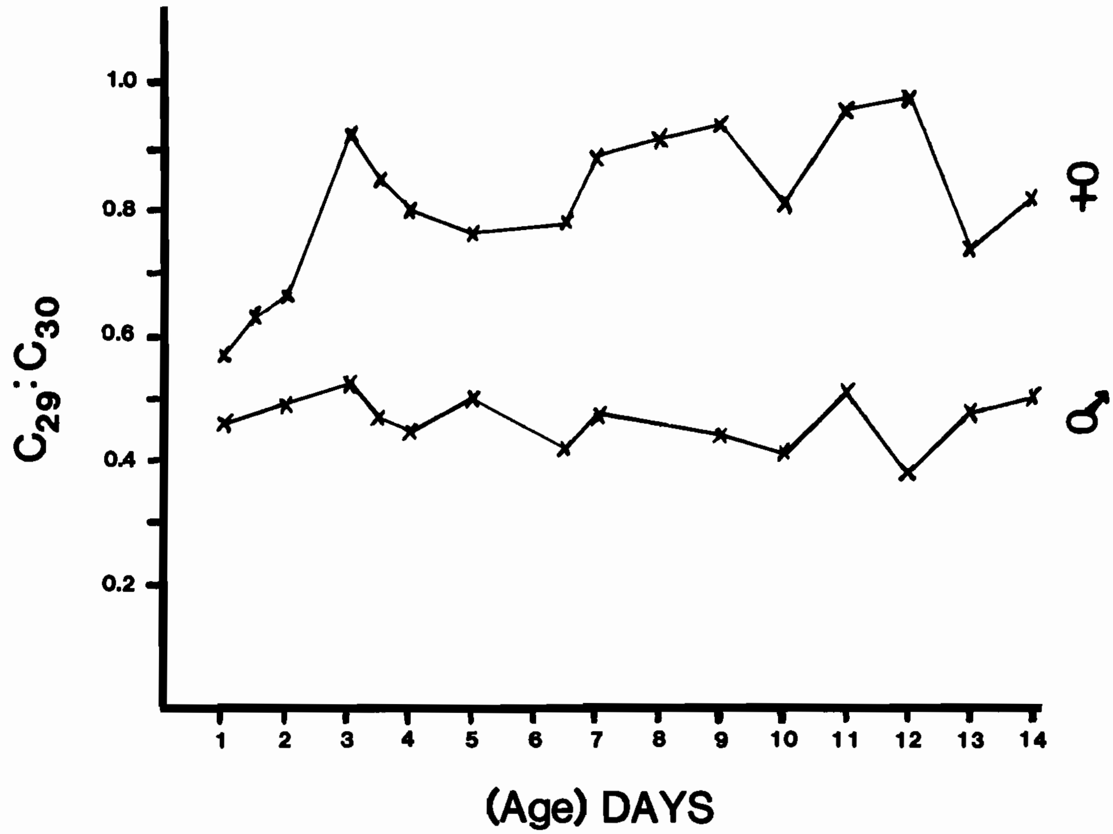

Figure 2. Relationship between the C_{29}/C_{30} ratio and the age of male and female *H. collusor.*

A SIMPLIFIED APPROACH TO OPTIMUM AEROSOL DROP SIZE BIO-ASSAY

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The review of information on optimum droplet size by Mount (1970) suggested that for ground aerosol applications, droplets of 5 to 10μ were considered optimum. For aerial sprays or aerosols he considered that 10 to 25μ is probably optimum, but finished his paper with the comment that these values need to be confirmed by additional research.

Since 1970, there have been discussions about optimum droplet size, but it was not until the tests by Akesson in late 1981 that we had a good comparison of how the California agencies' aerosol generators were performing. The measurements in those tests showed that few if any of the tested machines met the small drop-size criteria of Mount.

I suggest that with the available technology, we are in a position to reappraise the conclusions of Mount concerning optimum drop-size for best kill of adult mosquitoes. I also suggest that this kind of experimentation needs to be done in order to provide a basis for establishing priorities in application technique research.

If Mount is correct in his assessment, we are wasting money by using too much pesticide in the form of large droplets, and we should take immediate steps to reduce these expenditures by finding ways to produce smaller droplet dispersions of aerosols.

I propose the following test protocol, to look for evidence on whether there is an optimum sized insecticide droplet for adult mosquito control:

1. Discharge a pesticide aerosol cloud into a large, tightly closed room.
2. Draw a given volume of room air, containing aerosol droplets, past caged mosquitoes and a laser drop-size detector. If the detector program will give cumulative volume measurements of the droplets, then expose the mosquitoes to a uniform volume of pesticide for equal dose exposure. If the program will not measure a cumulative dose, then expose groups of mosquitoes for a uniform period of time, measuring the droplet size spectrum at each exposure.
3. Repeat the exposure/measurement sequence at time intervals, which will result in each group of mosquito being exposed to particles of continually diminishing average size, due to gravitational settling of the larger droplets during the interval between each exposure.
4. Observe the exposed mosquitoes at time intervals, to determine LT50 and LT90 values. By picking a uniform time after exposure and knowing the exposure dose and size for each group of exposed mosquitoes, a dose mortality relationship regression can be determined as well as determining when the droplet size results in significantly less mortality.
5. Repeat the experiment using other pesticide formulations, other mosquito species, and whatever ambient temperature conditions exist.

If there is a biological difference in mortality with different drop sizes, the data for each test should show a marked change in trend at some point in the test sequence and this change point should be consistent in repeated tests. This technique would also allow more precision in directly comparing different products. It might even replace, or at least confirm, data from tests in the field in which many of us have participated over the years.

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MOSQUITO CONTROL ON THE DAIRY WASTEWATER PONDS IN THE DELTA VECTOR CONTROL DISTRICT

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Early dairies in Tulare County, California, were mostly small family dairies with perhaps 10 to 25 milking cows. Many dairymen sat on a 3-legged stool, wiped the cow's udder with a damp cloth, and milked into a pail. Cows were put out to pasture except at milking time. In this early period there was usually little or no wastewater problem, since so little washwater was used. Somewhat later, from the 1930's into the 1950's, these family dairies constructed small milking barns with concrete flooring, and manure deposited there was hosed out into a ditch, a nearby irrigation canal, an idle weedy area, or into a small, shallow pond. In almost all cases weed growth was heavy and the environment was superb for the production of house mosquitoes (*Culex quinquefasciatus*). Effective control of these mosquitoes by larviciding was sometimes impossible because the sprays used could not penetrate the dense weed mass. Applications of granular insecticides were also ineffective or inefficient. Thus, while the use of water to clean the barn and the cows improved the dairyman's opportunity to provide clean milk, it nevertheless was detrimental to the human environment in that it created a severe community mosquito problem.

An intermediate step in the evolution of dairies occurred in the 1950's and 1960's. Dairies began using corrals for holding cows throughout their milking days - - these were known as dry-lot dairies. Since the cows now spent their entire time in the corral and barn enclosures, all manure was deposited there: - - about 10 to 15% in the pre-milking holding pen and the milk barn, and 85 to 90% in the corrals. There was increased use of water for washing the cows and the barn. Cows' udders were washed more thoroughly and manure could be washed out of the holding pen and the milk barn completely and with less hand labor than previously. In place of a couple of gallons of water per day per cow for simple washing, many dairies began to use 50 or up to 100 gallons for soaking the manure and dirt from the cows before milking and for washing out the holding pen and milk barn.

Planning for routine disposal - or use - of this wastewater was almost always inadequate. In most cases this manure-laden water was simply something to get rid of. The Delta Mosquito Abatement District (now known as the Delta Vector Control District), in order to improve its ability to provide effective mosquito control, began a program of dairy pond construction. These ponds were constructed sufficiently deep that no weeds could grow from the bottom, and the sides were constructed as steeply as possible to reduce the area where marginal weed growth could occur. At first it was believed that the water level would stabilize as a result of percolation into the soil and evaporation into the air. However, the manure served to seal the soil and evaporation was insignificant, so these ponds filled surprising quickly. Gradually it was realized that the only effective way to get rid of the wastewater was to pump it out, usually into a farm irrigation ditch where it could be used to help irrigate and fertilize the crops.

The District, which had been responsible for the construction (at cost to the dairymen) and the recommendations for use of a great many of the ponds, purchased a high volume pump connected to the power takeoff of a Jeep pickup. This was rented to the dairymen to pump their drain ponds. At the same time, the District encouraged the dairymen to install permanent pumps for this purpose, and many of them did so. Unfortunately, in many cases the pumping was done only when convenient or when absolutely necessary to prevent an overflow or flood.

The District carried out a weed control program against the marginal weeds. A soil sterilant was applied in early winter and a contact spray was applied during the summer. Routine insecticide spraying of dairy wastewater ponds was carried out and control of house mosquitoes proved very satisfactory. However, a major problem with manure floatage developed. When the manure entered the pond it dropped to the bottom. There it produced gases which raised some of it to the surface where it crusted over and formed floating mats. Weeds grew prolifically on it and favorable mosquito breeding areas were produced over much of the ponds. This situation made effective larviciding by spraying less effective. Fortunately, a new insecticide and a new technique of application made it possible for the District to continue to provide excellent control. Dursban was applied as a "slug" dose, the concentrate material being added at the entrance of the wastewater to the pond. The amount added was based on a calculation of the total volume of the liquid in the pond. One application was completely effective for a month or more.

During this time period corrals were usually graded flat, although manure was scraped into a large mound on which the cows could climb to escape the quagmire which developed when rains fell on the manure and mud in the heavily travelled areas. Cows became heavily encrusted with the manure and mud, increasing the need for washwater use. Further, this muck was very odorous, and communities several miles away received these strong, unpleasant odors.

The next evolutionary change of dairy farm programs began in the late 1960's and has continued to the present time. In order to improve efficiency and to be competitive, it appeared that dairies needed to get larger or cease operations. Most small grade B dairies did in fact go out of business. Also, dairies in the Los Angeles basin were being crowded out by urban expansion, and many of the dairymen chose to relocate in Tulare and Kings Counties. Requirements of the County Planning and County Health Departments had to be followed before permission to begin construction was granted. Sufficient farm land had to be obtained to receive the wastewater. There are no available sewer systems in the Valley. State Water Quality Control agencies absolutely prohibited running the wastewater into canals or ditches that would take the manure-laden water off the dairy property. Further, the nitrogen and salt-containing wastewater must not be permitted to enter underground waters.

The need to eliminate water and mud quagmires in the corrals during the rainy season stimulated dairymen to grade their corrals and thus provide for drainage, the rainwater being directed via ditch, concrete trough or pipe into the wastewater pond. Dirt required to accomplish this grading came from the area destined to become the wastewater pond. The amount of dirt required was sometimes great, and the resultant ponds were therefore large, frequently 100 feet by 400 feet or larger, and sometimes 20 or 30 feet deep. Thus the ponds now served two functions: 1) to provide dirt for grading the corrals and 2) to provide a place for receiving the wastewater.

Unfortunately, much confusion developed relative to the management of the wastewater pond and the entire manure and water disposal system. Dairymen were concerned primarily about their cows and milk production. Those who moved into the San Joaquin Valley from southern metropolitan areas sometimes had little or no experience with field farming and often were not interested in developing or maintaining a good farm irrigation program. All too frequently the wastewater pond proved to be a place to dump the wastes and forget about them until a convenient time occurred to pump them out. Further, most dairymen appeared to have no concern about all the manure solids they were dumping into the ponds, with the result that within a couple of years the water capacity was largely gone and expensive and difficult dragline operations were necessary to clean out the manure. This was true in spite of the fact that these newer ponds were far larger than most of the earlier ponds.

The new, large wastewater ponds have resulted in a tremendous increase of house mosquitoes in the Delta Vector Control District, as illustrated in Chart 1. If the ponds are clean and free of marginal weeds and floatage, there will be little if any mosquito production. Unfortunately, only a few ponds have been maintained in this condition. Weed-covered mats of floating manure have developed on most of the ponds in this District. The District today is helpless to provide effective chemical control of mosquitoes when the mats are present and the weeds are dense. Insecticide particles cannot efficiently penetrate through the dense foliage and reach the water surface where the larvae live. All the spray which lands on and sticks to the weed surfaces is useless for controlling the mosquitoes, thus the cost of adding enough spray to assure that some reaches the water is extrême. Dursban, which had been effective as a slug dose during the 1960's, has become virtually useless because of the development of resistance to it by the house mosquito, and, further, the volume of liquid in the large ponds has brought the cost of using it beyond the economic resources of the District. Obviously a new look at the dairy wastewater problem is needed.

A review has been made of the literature covering dairy wastewater disposal, and errors in fact and in concepts are all too evident. For example, University of California Circulars state: "dairies with manure only from the milking barn and holding corral going into the pond seldom have a problem with floating solids", a very untrue statement. Suggestions have been made to drain the pond regularly (monthly) during the summer, an unrealistic and usually impossible recommendation in the case of the larger ponds. Even if it could be carried out, the result might be the creation of an ever-more severe marginal weed problem, since the water level for much of the time would be below the area where weed sterilants were applied during the winter. Also, wind, which can play an important role in preventing egg-laying and larval survival, would be less effective if the water level is far below the top of the banks.

Another recommendation stressed by the University and by the U.S. Soil Conservation Service is that the slope of the banks should be shallow, a 45 degree grade or even flatter. This plan would simply add a greater area where marginal weeds can grow. Fencing has been stressed in some recommendations, without regard to the low actual hazard or to the need for routine visits by the mosquito abatement districts for inspection and control.

For some reason stress has been given to fly production in dairy ponds, as though this would give added impetus to the dairymen to do something about it, yet the only fly normally produced in such a pond is the innocuous rat-tailed maggot (drone fly).

Manure separation to keep most solids out of the wastewater pond has not been given proper emphasis in the literature. Also, the adding of fresh water to the pond to dilute the wastewater and make it more suitable for use for crop irrigation needs additional study. Above all, a plan needs to be developed to tie the entire wastewater system into the routine irrigation of agricultural crops.

Staff members of the Delta Vector Control District have observed a variety of dairy wastewater systems, a few of which are virtually free of mosquito production, a few of which are in a deplorable condition with more or less uncontrollable mosquito production, and the others at various levels in between. Two factors are evident in problem-free systems: 1) a well-planned system, and 2) good management. As might be expected, some dairies have potentially good facilities but because of inadequate management create major mosquito problems, whereas other dairies with less than desirable facilities have excellent management and do not cause the District significant problems. Above all, the District recognizes that

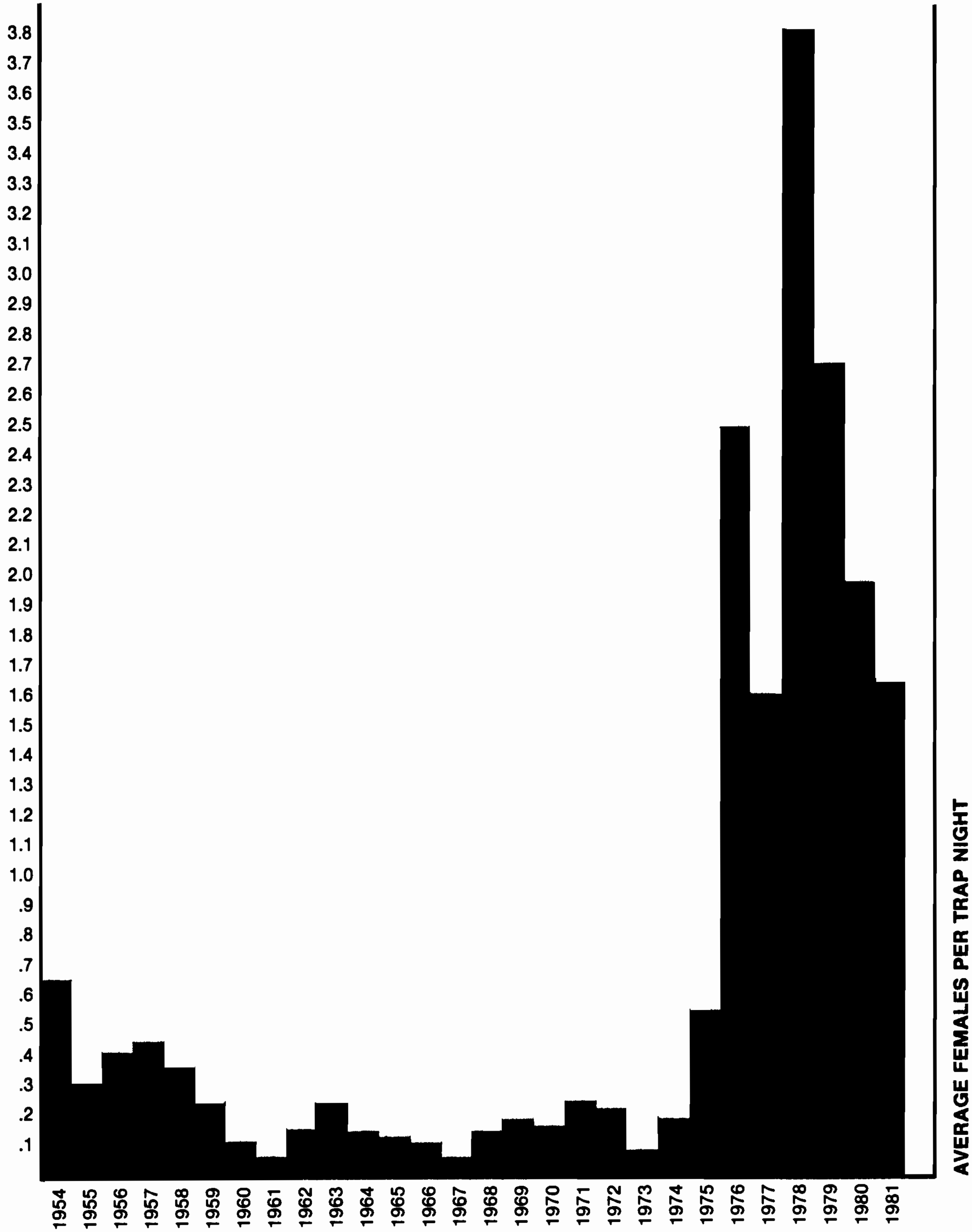

CHART I. *CULEX QUINQUEFASCIATUS* ANNUAL LIGHT TRAP SUMMARIES

more knowledge is needed relative to what facilities are best or at least adequate, and what management programs should be considered optimal.

In 1982 the District encouraged the University of California Mosquito Control Laboratory at Fresno to make a study of dairy wastewater systems to determine scientifically what facilities and management practices should be recommended. A thoroughly revised Circular on this subject is needed. Following these studies and the production of a meaningful Circular, mosquito abatement districts will be in a good position to work closely with each dairyman to assist him to provide the help which the districts need in order to obtain reasonably effective mosquito control without adversely affecting the total program of the dairyman.

REQUIREMENTS FOR APPRAISAL OF THE TRUE ROLE OF PARASITIC INSECTS IN THE NATURAL CONTROL OF SYNANTHROPIC DIPTERA

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ABSTRACT

Proper sampling of the natural breeding habitat of synanthropic Diptera is essential to accurately appraise the role of parasitic insects in natural control.

Different species of synanthropic Diptera have different favored habitats as exemplified by the oviposition preferences of the face fly, *Musca autumnalis* De Geer, and horn fly, *Haematobia irritans* (L.) in field dung of cattle versus the barnyard accumulated excrement habitat sought after by the common house fly, *Musca domestica* L., stable fly, *Stomoxys calcitrans* (L.), and the poultry fly, *Fannia canicularis* (L.). Because their breeding habitats are so different (Snowball 1941), these two groups of Diptera are usually assigned to different categories of synanthropy (Legner *et al.* 1974, Povolny 1971).

For each category of host synanthropy, there are also different groups of associated natural enemies (Legner *et al.* 1974). Predatory arthropods appear to be of principal importance in the natural regulation of Diptera breeding in isolated deposits of cattle dung in pastures (Hammer 1941, Legner 1978, Mohr 1943, Poorbaugh *et al.* 1968), while both predatory and parasitic arthropods interact to regulate populations of Diptera breeding in accumulated animal wastes and garbage (Legner 1971; Legner & Olton 1970, 1971; Legner *et al.* 1974, 1975). Although some natural enemy species overlap into both the pasture and accumulated dung habitats, there are many species which are mostly confined to either one or the other habitat (Legner & Olton 1970, Poorbaugh *et al.* 1968). Parasitic insects, in particular, tend to confine their activities to the larger accumulations of dung (Legner & Olton 1971, Legner *et al.* 1974.).

An important requirement for appraising the value of parasitic insects in the natural control of synanthropic muscoid Diptera is to extract samples from the natural, undisturbed habitat. The immature hosts (larvae and pupae) must be removed directly from the habitat in which they were naturally formed, admittedly entailing painstaking labor. Changing the breeding situation to facilitate collection as, eg., gathering pasture deposited dung into piles in order to concentrate pupation sites of horn and face flies, attracts those parasitic species which range in accumulated dung for their hosts. Consequently, as most parasites of synanthropic Diptera are not host specific but habitat specific, the pasture breeding horn and face flies then sustain parasitism by species of parasites that would rarely if ever find these hosts in nature.

The importance of host habitat to parasite searching has been emphasized for many years (Flanders 1937, Laing 1937, Salt 1935, Vinson 1976). Particular attention to habitat is required when an accurate appraisal of parasite performance is desired. Simmonds (1948) concluded that, "... to avoid misleading results care must be taken to secure samples of host material in the field with due consideration to the habits of both host and parasite." In this way, the "host-exposure method" acclaimed by Bartlett and van den Bosch (1964) is not always a well suited technique, either for the qualitative or the quantitative evaluation of parasites of synanthropic flies. The artificial exposure of host pupae can, as in the case of *Hippelates* eye gnats, attract parasites that would not normally parasitize the host in nature (Bay *et al.* 1964, Legner & Bay 1965). A careful study of the breeding situation can, however, result in the development of techniques whereby the host may be exposed in a relatively natural situation (Legner & Bay 1964).

The exposure of muscoid Diptera pupae in containers within the host habitat, often called the "sentinel pupae method," is loaded with potential error (Meyer & Petersen 1982). In California, such exposures have, (1) produced parasitism by *Nasonia vitripennis* (Walker), a parasite of blowflies that is rarely secured from muscoid hosts when these are extracted naturally from the habitat (Legner 1967a); (2) excluded the parasites *Spalangia cameroni* Perkins, *Spalangia endius* Walker and *Spalangia nigroaenea* Curtis, which are often destroyed through multiple parasitism by both *N. vitripennis* and *Muscidifurax* spp., both intrinsically superior in competition to *Spalangia* spp.; ie., their larvae kill other parasites that they encounter inside a host with almost 100% efficiency. Although *N. vitripennis* is the strongest "intrinsic" competitor by virtue of its faster rate of development and gregariousness, and *Muscidifurax* spp. are intermediate in superiority, their respective searching abilities in the breeding habitat are reversed. The *Spalangia* spp. range most broadly in the habitat, the *Muscidifurax* spp. intermediate, while *N. vitripennis* searches primarily at the habitat surface and is not capable of penetrating much beyond a few centimeters for host pupae (Ables & Shepard 1974; Legner 1976b, 1977; McCoy 1965). Therefore, since each parasite species has its own special preferred portion of the breeding habitat, an evaluation of each one's performance would require positioning the "sentinel pupae" at different habitat depths for each species. For further arguments against the sentinel pupae method of host exposure see Meyer & Petersen (1982).

Parasite species also respond differently to different sizes of hosts (Legner 1969a) and densities (Legner 1967b, 1969b). A standardized size of host which is not adjusted to seasonal changes in nature, could give biased results. Although clumping of the host is frequently found in nature, the degree of clumping varies and a host exposure would have to reflect this to realistically appraise parasite activity. Clumping intensity can be expected to vary seasonally, and accurate sampling would be necessary to judge its pattern. With such sampling necessary anyway, there is no logical reason to avoid it in the first place.

A percent parasitism figure has little real value in population studies unless it is closely associated with real host densities. For example, some of the highest levels of parasitism of synanthropic Diptera are associated with relatively low host densities (Legner 1971). Spatial density samples of hosts may be obtained by sampling known quantities of habitat (Legner & Olton 1971, Legner *et al.* 1980).

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QUESTIONS CONCERNING THE DYNAMICS OF *ONTHOPHAGUS GAZELLA* (COLEOPTERA: SCARABAEIDAE) WITH SYMBOVINE FLIES IN THE LOWER COLORADO DESERT OF CALIFORNIA

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ABSTRACT

Firmly established *Onthophagus gazella* F. populations in the Coachella Valley thoroughly scatter cattle dung; but, horn fly, *Haematobia irritans* (L.) densities remain unacceptably high. Interferences with physical controls and resident natural enemies are suggested.

The extraordinary dung scattering capability of the scarab beetle, *Onthophagus gazella* F., was soon recognized following its importation to Hawaii from southern Africa in 1958 to aid in the control of horn fly, *Haematobia irritans* (L.) (Legner 1978). This beetle was later introduced in Australia where it quickly became established and successfully reduced the volume of bovine feces that previously tended to accumulate in certain pastures (Waterhouse 1974). However, although laboratory studies have shown that the presence of these beetles can interfere with horn fly development, reducing adult fly emergence (Blume *et al.* 1973, Bornemissza 1970), there has been no conclusive evidence that fly densities are correspondingly reduced in the field (Legner 1978a, Macqueen 1975).

At first, this apparent ineffectiveness in horn fly control appears enigmatic. At four irrigated pastures in the Coachella Valley near Thermal, where *O. gazella* was introduced in May 1975 with the liberation of 120 pairs secured directly from Hawaii, dung scattering and partial burying by adult beetles begins within an hour of its deposition. Scarab beetles that remain dormant in the soil, in some cases for 6 months during the hot months, become active immediately following renewed irrigation and cattle stocking. Cattle on these pastures are often stocked at densities exceeding 25 / ha., and the amount of dung that is scattered and buried daily by the 1-cm long beetles is enormous. By October, beetle density can reach 40 or more per fresh dropping, a density approaching the 50 that Macqueen (1975) believed would be required for field fly control to result. Although ranchers are very pleased with the manner in which the cattle dung becomes incorporated into the soil, eliminating the need for mechanical mixing, there is an obvious lack of horn fly control as the cattle sustain continuously high horn fly denstities, often exceeding 1,000 per head in autumn. These densities are similar to those attained in pastures where mechanical means are employed to disperse the dung between irrigations. Therefore, the dynamics of *O. gazella* with symbovine flies (Legner *et al.* 1974) is in need of further investigation.

One explanation for the large numbers of horn flies on the cattle in pastures containing *O. gazella* is the possible immigration of flies from neighboring ranches, though the pastures in question are isolated, and it would be difficult to accept immigration as causing the high numbers that are continuously present. In fact, horn fly immatures are easily extracted from shredded dung pads in these fields.

A preliminary study to measure the production of horn flies from dung in pastures where *O. gazella* is active, was initiated on November 8, 1982, a peak time of year for this fly's abundance. Ten random samples were taken of dung pads shredded by established *O. gazella* populations in the Coachella Valley and compared to 10 unshredded samples from control pastures in which *O. gazella* was absent. Samples were placed into emergence sleeve cages in a greenhouse, incubated at 26-29° C., 50-55% RH and 14:10-h L:D photoperiod.

The average number per pad and oven-dry weights of horn fly adults produced from dung collected in both kinds of pastures was calculated. An average of 12.33 horn fly adults was produced from dung pads in which *O. gazella* beetles were highly active (40 per pad). This figure was higher than one control and lower than another (Table 1). There were no significant differences in the oven-dry weights of adults. Further sampling would be required to obtain definite quantified differences, but a significant horn fly production from *O. gazella*-inhabited dung is clear.

Possible mechanisms to explain horn fly breeding in pastures where *O. gazella* was active may be found in the dynamics of the scarab beetles with horn flies and their natural enemies in the dung habitat. When *O. gazella* scatters and buries cattle dung, a great quantity of fresh horn fly larval

Table 1. Emergence of ***Haematobia irritans*** adults from 10 randomly-sampled dung pads in Coachella Valley and Imperial Valley irrigated pastures with and without ***Onthophagus gazella*** populations present. Sampled 8 November 1982.[1,2]

Pasture Type	Avg. No. Adult Flies emerged per pad	s	Avg. O.D. wt. ($x 10^{-4}$ g.)	s
O. gazella present	12.33	3.06	7.20	0.35
Control I (*O. gazella* absent)	52.00	22.63	6.05	0.21
Control II (*O. gazella* absent)	6.00	4.24	9.10	0.85

[1] dung pad size = 1,495 cc (s = 374 cc).

[2] ca. 40 *O. gazella* adults present per pad.

habitat is incorporated into the soil before it has had a chance to fully aerate and decompose (compost). Horn fly oviposition begins in fresh dung prior to scarab beetle activity (within minutes of deposition), and some eggs hatch before beetle disturbance. The ensuing larvae may either remain in the pad or disperse at large through the soil, finding ample food to complete their development. Horn fly larvae are known to develop satisfactorily in the soil just below a cattle dropping (Legner 1978b), so that with the dung dispersed, a major behavioral change in the fly is not required. In this way, horn fly development may be actively favored by fly larvae encountering greater amounts of food material sealed from oxygenation and rapid decomposition in the soil, in a manner similar to *Hippelates collusor* Townsend, whose population density soars when natural food material is cultivated into the soil (Legner 1970, Legner et al. 1966).

Another consideration is habitat destruction for natural enemies. Although horn flies are a continuous and vexing problem of cattle in California, a number of natural enemies do forage in their breeding habitat (Poorbaugh *et al.* 1968), and may contribute some natural control. Any disturbance of this control might guarantee the survival of an even greater number of horn flies. Some of the principal staphylinid predators of horn flies in the Coachella Valley, *Philonthus discoideus* Gravenstein and *Philonthus longicornis* Clark (Legner, unpublished data), are practically eliminated from dung in pastures where *O. gazella* has been active, probably because the dung scattering activity of this scarab reduces moisture content in the breeding habitat to a level unsuited for staphylinid larval development. This may be similar to the effects of cultivation on the natural breeding habitat of *Hippelates* eye gnats, which causes a marked reduction in the effectiveness of natural enemies (Legner & Olton 1969).

A senario of horn fly production in pastures containing well established populations of *O. gazella* may be hypothesized as follows: some reduction of horn flies may initially be caused by the dung scattering activities of beetles as shown in laboratory studies (Blume *et al.* 1973, Bornemissza 1970). However, surviving fly larvae may encounter unlimited food which is distributed by the scarabs throughout the soil, and they may escape the natural predation of several predators because of habitat alteration. The net result could be a greater horn fly abundance than in pastures where *O. gazella* is absent. However, as mechanical pasture renovation also results in a high fly abundance, probably from the same causes, the difference is negligible and might be an argument for the use of scarabs, as less energy is required to maintain productive pastures. Fly control, nevertheless, is not achieved to a satisfactory level.

On range land where cattle are usually not stocked at densities exceeding 5-7 cattle per ha., and where mechanical dung mixing is not employed, horn fly densities are characteristically much lower. Dung under the lower herd densities generally decomposes at rates which are fast enough to preclude harmful accumulations in pastures (Legner 1978, McKinney & Morley 1975). The introduction of new predatory natural enemies here may afford a positive means for lowering horn fly densities. However, introducing scarabs such as *O. gazella* could result in habitat disruption to the point of predator exclusion, and increased fly densities.

The need for expanded scientific investigations into the interactions between dung beetles and symbovine flies with their natual enemies remains paramount.

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CONTRIBUTIONS: Articles are original contributions in the field of mosquito and related vector control providing information and benefit to the diverse interests in technical development, operations and programs, and management documentation. Papers on controversial points of view are accepted only as constructive expositions and are otherwise generally dissuaded, as is the case with an excessive number of papers on one subject or by one author where imbalance might ensue. Although preference is given to papers of the conference program, acceptability for publication rests on merit determined on review by the editors and the Publications Committee.

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The complete scientific name of an organism must be given the first time it is used. Terms commonly abbreviated in specific fields should be given in full the first time used, followed with the abbreviation. The abbreviation alone is acceptable in further usage in the paper. Common latin abbreviations (et al., e.g., i.e., etc.) are not italicized. Use of the metric system is encouraged. The bi-letter system of generic abbreviations is used for Culicidae.

All manuscripts will be edited to improve communications, if needed. Editors are biased against verbosity or needless complexity or jargon. Grammar will be corrected if necessary. Articles needing extensive editing or not conforming to style and instructions will be returned to the author for correction.

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ABSTRACTS: Only technical papers need be accompanied by an abstract, which should not exceed 3% of the length of the article. When an abstract is submitted for publication in lieu of a paper, the abstract length may be extended. If possible, the journal where the paper is to be published in full should be stated.

TABLES: Tables are typed on separate sheets placed in correct sequence in the text and should be limited to those strictly necessary. Tables made up by the author in the form of line drawings for photocopy are acceptable. Graphs and line drawings should be prepared with regard to the ultimate printed size of one column (3¼″) or page width of seven inches, as applies to columns of table data.

Submitted figures as maps and charts should not exceed 8½ X 11″ (22 X 28 cm), with labels and line weight adapted to the published size.Total page space for tables and figures must necessarily be limited by the editors.

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