PROCEEDINGS AND PAPERS

of the

Sixty-Third Annual Conference of the

California Mosquito and Vector Control Association, Inc.

January 22 thru January 25, 1995

Held at
RED LION HOTEL
SACRAMENTO, CALIFORNIA

Stephen L. Durso, Editor

CALIFORNIA MOSQUITO and VECTOR CONTROL ASSOCIATION, INC.
8633 Bond Road
Elk Grove, California 95822

Published December, 1995

Printed by
Graphics Diversified
Sacramento, California

1995

CALIFORNIA MOSQUITO and VECTOR CONTROL ASSOCIATION, INC.

Donald A. Eliason, Executive Director
Charles P. Hansen, Treasurer
Linda M. Sandoval, Office Manager

BOARD OF DIRECTORS

PRESIDENT .... Charles H. Dill
PRESIDENT ELECT .... John R. Stroh
VICE PRESIDENT .... Lue Casey
PAST PRESIDENT .... Allen R. Hubbard
PRES. TRUSTEE BOARD .... Patricia A. Gillies

COASTAL REP. .... Ken Carden
SACRAMENTO VALLEY REP. .... David B. Whitesell
N. SAN JOAQUIN VALLEY REP. .... Claude L. Watson
S. SAN JOAQUIN VALLEY REP. .... David G. Farley
S. CALIFORNIA REP. .... David C. Martinez

TRUSTEE CORPORATE BOARD

PRESIDENT .... Patricia A. Gillies
VICE PRESIDENT .... Chester C. Miller
SECRETARY .... Grant W. McCombs
PAST PRESIDENT .... William L. Boynton

COASTAL REP. .... Milton Wallace
SACRAMENTO VALLEY REP. .... (Vacant)
N. SAN JOAQUIN VALLEY REP. .... Gerard Fondse
S. SAN JOAQUIN VALLEY REP. .... Patricia Gillies
S. CALIFORNIA REP. .... Leo F. Kohl

CORPORATE MEMBERS

Alameda County MAD
Alameda County VCSD
Antelope Valley MVCD
Burney Basin MAD
Butte County MVCD
Carpinteria MAD
Coachella Valley MAD
Coalinga-Huron MAD
Colusa MAD
Compton Creek MAD
Consolidated MAD
Contra Costa MAD
Delano MAD
Delta VCD
Durham MAD
East Side MAD
El Dorado County Vector Control - CSA3
Fresno MVCD
Fresno Westside MAD
Glenn County MVCD
Goleta Valley VCD
Greater Los Angeles County VCD
Kern MVCD
Kings MAD
Lake County MAD
Los Angeles County West VCD
Madera County MVCD
Marin/Sonoma MAD
Merced County MAD
Moorpark MAD
Napa County MAD
Northern Salinas Valley MAD
Northwest MVCD
Orange County VCD
Owens Valley MAP
Pine Grove MAD
Sacramento-Yolo MVCD
San Bernardino County VCP
San Gabriel Valley MAD
San Joaquin County MVCD
San Mateo County MAD
Santa Clara County VCD
Santa Cruz MVCD
Shasta MVCD
Solano County MAD
Sutter-Yuba MVCD
Tehama County MVCD
Tulare MAD
Turlock MAD
West Side MVCD
West Valley VCD

DONORS/CONTRIBUTORS TO THE 1995 ANNUAL CONFERENCE

BEECOMIST SYSTEMS
BIOQUIP PRODUCTS, INC.
BUTTE COUNTY M.V.C.D.
EHMANN OLIVE COMPANY
FENNIMORE CHEMICALS
LUNDBERG FAMILY FARMS
R.V. SPECIALTIES, INC.
SANDOZ AGRO, INC.
TARGET SPECIALTY PRODUCTS
VALENT U.S.A. CORP.

EXHIBITORS AT THE 1995 ANNUAL CONFERENCE

ABBOTT LABORATORIES	Kirk Azevedo	(209) 826-3830
AMERICAN CYANAMID COMPANY	William Jany	(201) 831-2684
BEECOMIST SYSTEMS	Ed Kutzner	(800) 220-0787
BIOQUIP PRODUCTS, INC.	Richard P. Fall	(301) 324-0620
CHEMINOVA, INC.	Bob Richard	(201) 305-6600
FENNIMORE CHEMICALS	H.B. Munns	(209) 295-3540
J.T. EATON & CO., INC.	Janice Richards	(406) 642-6757
POINTS WEST ARGO, INC.	Tom Tomlinson	(916) 671-6905
SANDOZ AGRO, INC.	Margie Koehler	(714) 969-7387
TARGET SPECIALTY PRODUCTS	Dennis Candito	(800) 767-0719
VALENT U.S.A. CORP.	Pam Knoepfli	(702) 626-8431
VAN WATERS & ROGERS	Jeff Smith	(916) 371-7600
WILBUR-ELLIS CO.	P. Parises	(209) 982-4337
WITCO CORP., GOLDEN BEAR PRODUCTS	Carter Knowles	(805) 393-7110
ZANUS CORP.	David G. Sullivan	(415) 591-7783

TABLE OF CONTENTS

CONFERENCE DEDICATION

PUBLIC HEALTH AND DISEASE PREVENTION

BIOLOGICAL AND ECOLOGICAL STUDIES

GENERAL

C.M.V.C.A. REPORTS

WILLIAM C. REEVES NEW INVESTIGATOR AWARD

California Mosquito and Vector Control Association, Inc.

Volume 63 January 22 thru January 25, 1995

CONFERENCE DEDICATION

DEDICATION OF THE 63RD ANNUAL C.M.V.C.A. CONFERENCE TO WILLIAM E. HAZELTINE

Allan R. Hubbard

1994 President
California Mosquito and Vector Control Association

William E. Hazeltine
1926 - 1994

William E. Hazeltine died Saturday November 5, 1994. When Bill passed away, the people of this nation lost a public servant who devoted his career to the protection of their health and well-being. We lost a colleague, mentor and friend. Bill was a complex, multifaceted person, like a gem with many of the facets not readily apparent at first glance nor when viewed from a particular angle.

A native Californian, Bill was born in San Jose on September 4, 1926. Bill earned his Bachelor of Arts degree at San Jose State College, continued his graduate studies at the University of California at Berkeley and received his Doctorate from Purdue University. As an entomologist, his interests varied from insect control to systematics. Bill authored over 23 publications which reflected this diversity, from proposing schemes on the classification of Bumble Bees based upon genitalia to the impact of environmental and legislative constraints on vector control operations. Bill enjoyed the collection of beetles belonging to the tribe Melolonthini, and he was considered by some to be an expert on the genera *Polyphylla* and *Pleocoma*. When two species belonging to these genera were in the process of being listed as threatened, he collected boxes of specimens to prove the proposed listings were not based on the best available data. One tenet of Bill Hazeltine's was that science should not be compromised nor manipulated regardless of the nobility of the cause. He had a keen sense of recognizing such schemes and worked hard to expose them.

One facet of Bill's personality was his interest in political activities, and he was active at many levels. Bill was on a first name basis with city managers and Congressmen alike. He often corresponded with both the Reagan and Bush administrations. From his activities at the local level serving on the school board and as chairman of the planning commission, to providing expert testimony to congressional committees at the national level, Bill's efforts to improve and maintain the high standards of public health were recognized throughout the country. He received the American Mosquito Control Association's Meritorious Service Award and was an Honorary Member of the California Mosquito and Vector Control Association. Bill continually spoke and wrote about the social implications of efforts to fabricate environmental purity, an aspect not usually considered with such altruistic endeavors.

Bill's career started in the Navy during World War II. As a registered professional entomologist, he started working in the field for Agricultural Specialties Company, headquartered in Dallas. From 1961-64 he was the manager of the Lake County Mosquito Abatement District, where he initiated some of the first applications of parathion for control of the Clear Lake Gnat. In 1964, he moved to Kansas to perform field research for the Chemagro Corporation. Bill moved back to California in 1966 where he managed the Butte County Mosquito Abatement District for 26 years. Bill was well known for always taking the high road, maintaining honesty and integrity throughout his life. Although sometimes brusque and painfully honest, he was respected by both his supporters and adversaries.

Bill was a member of the Trinity Presbyterian Church and belonged to a number of organizations. He was active in the Entomological Society of America, the Society for Vector Ecology, the American Mosquito Control Association, the California Mosquito and Vector Control Association, and the American Association for the Advancement of Science. Bill also served on the Board of Scientific and Policy Advisors for the American Council on Science and Health. After his retirement, Bill worked as an environmental consultant and served as an unpaid adviser for the California Mosquito and Vector Control Association and the American Mosquito Control Association, providing testimony and representing these organizations before federal and state legislative committees on issues concerning the protection of public health. He worked endlessly on the technical aspects of legislation concerning pesticide registration for the protection of public health; and constantly pointed out the seldom considered public health effects of wetland preservation and the Endangered Species Act.

Bill will be greatly missed by all of us. As Dr. Chester Ward said, "Physically he is gone, but from an intellectual standpoint his contributions will remain for many years to come."

Thoughtfully submitted by,

Scott E. Monsen
James A. Camy

Butte County M.V.C.D.
5117 Larkin Road
Oroville, California 95965

CURRENT STATUS OF VECTOR-BORNE DISEASES IN THE UNITED STATES

William K. Reisen[1]

Arbovirus Research Program
School of Public Health
University of California
Berkeley, California 94720

ABSTRACT

Temporal and spatial patterns in the number of reported human cases of vector-borne diseases are described for the United States since 1965. The epidemiology of four additional diseases (Hantavirus pulmonary syndrome, murine typhus, Ehrlichiosis and Dengue fever) is presented to emphasize their potential importance to public health in California. Trends in human ecology and demography in California indicate a potentially increasing risk of infection with vector-borne zoonoses.

This paper summarizes recent trends in the prevalence of vector-borne diseases in the United States. The number of cases of reportable diseases such as Lyme disease and malaria was obtained from the cumulative summary for 1994 in the Morbidity and Mortality Weekly Report (CDC 1995). Surveillance data for mosquito-borne arboviral diseases was provided by the staff of the Division of Vector-borne Infectious Diseases, Centers for Disease Control and Prevention, Ft. Collins, Colorado. Information on other diseases was obtained through the scientific literature or by direct correspondence with specific investigators.

The data are limited to reported cases. Most were confirmed by laboratory tests, but to be included a physician had to 1) clinically recognize a probable vector-borne illness, 2) request and submit the proper samples for laboratory tests, and 3) report the results to local or national health authorities. It is unknown how well this system actually works, and therefore these data may underestimate the total number of cases. This is especially true of diseases such as mosquito-borne encephalitis where most infections do not produce clinical illness and where the clinical presentation is extremely variable. In contrast, for diseases such as Lyme disease where clinical diagnosis and laboratory confirmation are difficult and media focus has increased the awareness by the medical community, reporting may overestimate the number of cases.

The provisional number of vector-borne disease cases recognized in the United States through December 24, 1994 is summarized in Table 1. Additional reports most likely will be forthcoming or corrections made. Therefore, the totals for 1994 should be considered "provisional". Information on new or poorly recognized pathogens such as *Ehrlichia* or non-reportable diseases such as relapsing fever are scanty and were not included in this table. With the exception of malaria, all these pathogens are vector-borne zoonoses that are maintained by transmission among wild mammals or birds and only occasionally are transmitted to humans, which usually are "dead end hosts".

CASE PREVALENCE

Lyme disease remains the most frequently reported vector-borne disease in the United States, with 11,144 cases listed in the MMWR through December 24, 1994; 83 cases were reported from California. Consistent with trends over the past decade, most (79%) cases were concentrated in the northeastern states of New York, New Jersey, Pennsylvania and Connecticut (Fig. 1).

[1]Address correspondence to: Dr. William K. Reisen, Arbovirus Field, Station, 4705 Allen Road, Bakersfield, California 93312.

Table 1. Provisional number of vector-borne disease cases in the United States and California during 1994.

Disease	Number of cases U.S.	California
Lyme disease [1]	11,144	83
Malaria [2]	1,055	195
Rocky mountain spotted fever [1]	437	0
Tularemia [1]	85	2
Plague [1,2]	14	1
LaCrosse encephalitis [3]	44	0
St. Louis encephalitis [3]	18	0
Eastern equine encephalomyelitis [3]	1	0
Western equine encephalomyelitis [3]	2	0

[1] CDC (1995); [2] K. Gage, CDC, personal communication. [3] R.S. Nasci, CDC, personal communication.

Here, this pathogen remains a major public health problem of affluent suburbia where homes interface with woodlots. The marked temporal increase in cases from the mid- to late-1980s represents a combination of the expanded distribution and abundance of the tick vector due to increases in deer populations, the associated expansion in the distribution of the bacterial pathogen, increased awareness by the medical community, and improved case definition, diagnosis and reporting. Since 1989 the number of cases have stabilized at between 8,000 and 12,000 per year, with an incidence of 3.2 cases per 100,000 individuals in 1993. The highest incidence in the United States in 1993 was in Connecticut at 41.3 cases per 100,000 individuals.

Lyme disease cases in the United States may be divided into three geographical groups: 1) cases transmitted by *Ixodes scapularis* in the New England and Middle Atlantic states, 2) cases transmitted by *Ixodes pacificus* in California, and 3) the rest of the United States where the epidemiology is poorly understood and many of the clinical diagnoses are not confirmed by the isolation of *Borrelia burgdorferi*. Recently, Oliver et al. (1993) isolated *B. burgdorferi* from *I. scapularis* in Georgia, indicating that the Group 1 area with confirmed pathogen presence could be expanded to the southeastern states.

The number of Rocky Mountain spotted fever cases continued to decline in 1994 (Fig. 2), with 437 cases reported as of December 24, 1994 (CDC 1995). Cases were distributed widely throughout the United States, with most (47%) cases reported from North Carolina, Georgia, Oklahoma and Ohio. However, 53% of the remaining cases in 1994 were distributed among 33 additional states, indicating the wide distribution of this rickettsia.

A total of 1,055 cases of malaria were reported from the United States through December 24, 1994, of which 195 were from California (CDC 1995). As the circumtropical transmission of malaria continues

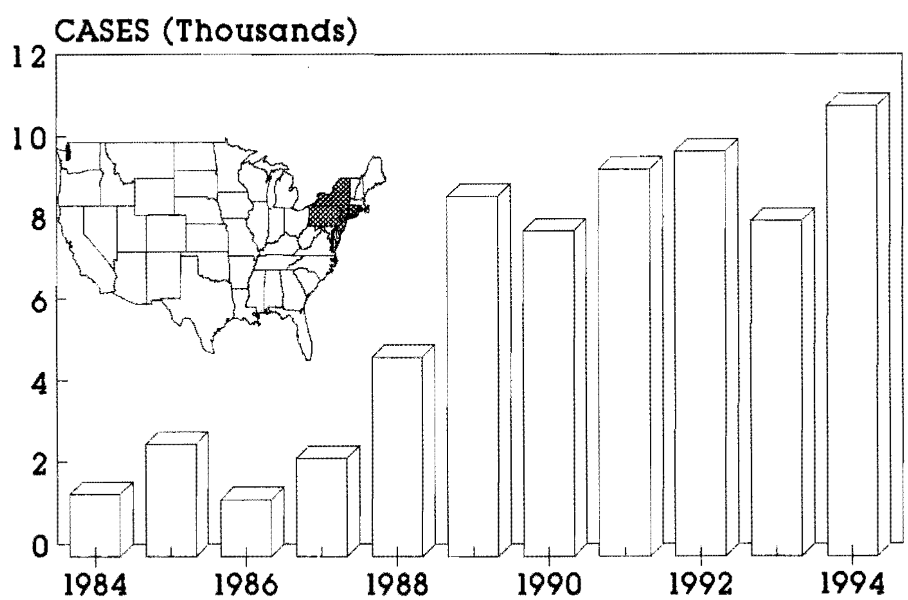


Figure 1. Annual changes in the number of reported Lyme disease cases in the United States. Map shows the four states with the highest number of cases in 1994 (CDC 1995).

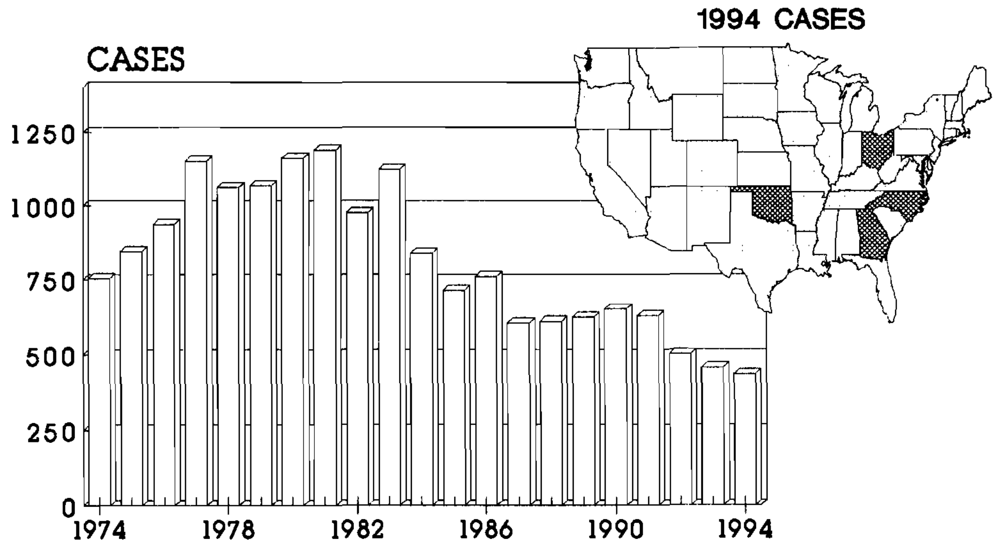


Figure 2. Annual changes in the number of reported Rocky Mountain spotted fever cases in the United States. Map shows the four states with the highest number of cases in 1994 (CDC 1995).

unchecked with as many as 300 to 500 million clinical cases and countless additional parasite carriers, the importation of malaria parasites into the United States can only be expected to continue, if not increase (Fig. 3). The most recent analysis of cases available was for 1991, when 1,016 were recorded (CDC 1994a). Of these cases, 43% were *Plasmodium vivax*, 39% *P. falciparum*, 6% *P. malariae*, and 2% *P. ovale*; while 9% were not specifically identified. Overall, 577 cases were in United States citizens who acquired their infections mostly from Africa and Asia and 439 cases were in travelers or immigrants from other countries. Of particular interest was the was the fact that only 38% of the *P. vivax* and 87% of the *P. falciparum* infections were recognized

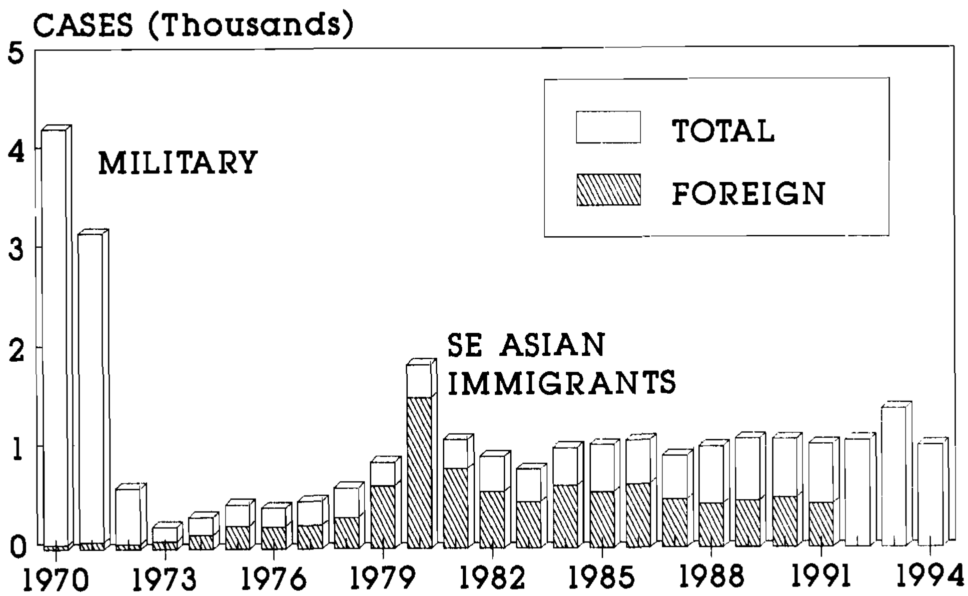


Figure 3. Annual changes in the number of malaria cases reported in the United States among American citizens and foreign travelers and immigrants (CDC 1994a, 1995).

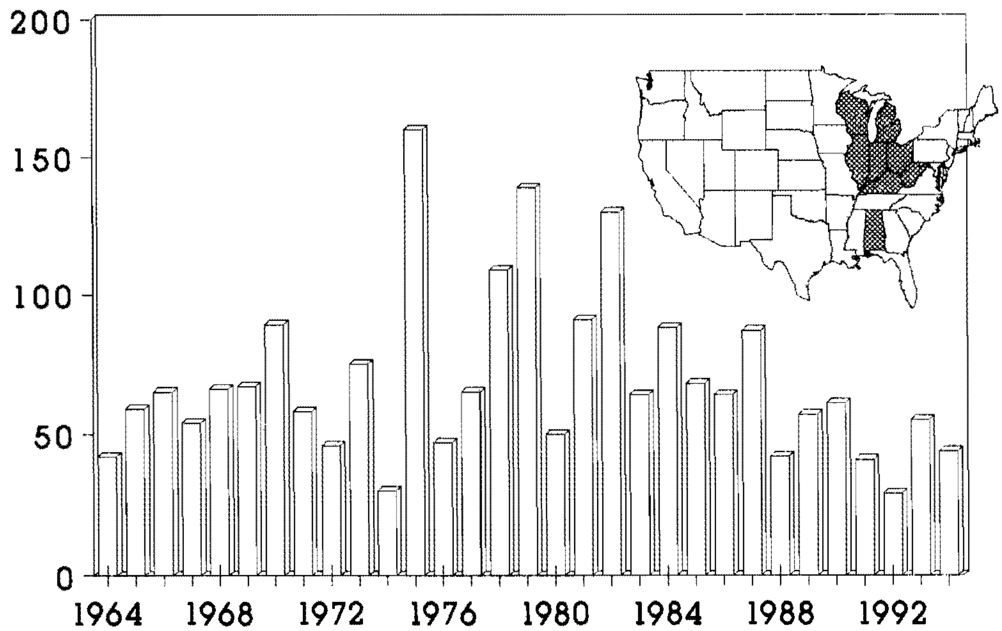


Figure 4. Annual changes in the number of LaCrosse encephalitis cases reported in the United States. Map shows all states reporting cases during 1994 (R.S. Nasci, CDC, personal communication).

within the first month of entry into the United States. This means that a substantial number of infected individuals relapsed, recrudesced, or went undiagnosed while living in the United States. These individuals may provide a source of infection for local anophelines, many of which remain focally abundant and susceptible to infection. In 1991, 8 cases were acquired within the United States, 5 congenitally and 3 by possible local transmission associated with migrant workers. One of these three cases was a resident of Yuba City, California.

The number of reported human cases of arboviruses seem to be on the decline in the United States. I emphasize the terms "reported" and "seem". There were 7,932 cases of aseptic meningitis reported in the United States through December 24, 1994, including 1,295 from California (CDC 1995). Most likely, some of these cases were caused by arboviruses, but were not definitively diagnosed because physicians did not collect appropriate specimens or request appropriate tests.

California group viruses were the most frequently reported arbovirus infection, with 44 clinical cases detected as of November 17, 1994 (R. Nasci, CDC, personal communication). No cases were from California. Historically most cases were reported from upper midwest states; however, during 1994, 32 (73%) of the cases occurred in West Virginia (Fig. 4). Two confirmed cases of LaCrosse encephalitis were reported from Alabama, expanding the known geographical distribution of this virus. A single clinical case of Jamestown Canyon was reported from Rhode Island.

Historically, most cases of St. Louis encephalitis (SLE) virus have occurred in the Ohio River drainage, Texas and Florida, with large outbreaks at 12 to 14 year intervals (Fig. 5). During 1994, single cases of SLE occurred in Mississippi and Florida, and a limited outbreak of 16 cases with one fatality occurred in New Orleans (D.B. Francy, CDC, personal communication). Most of the New Orleans cases were clustered in and around public housing projects where faulty plumbing resulted in the pooling of polluted water under housing units and the production of large *Culex quinquefasciatus* Say populations. In addition, surveillance programs in Texas and southern California detected widespread enzootic transmission without associated human cases.

Two presumptive human cases of western equine encephalomyelitis (WEE) and one human case of eastern equine encephalomyelitis (EEE) were reported during 1994 from Wyoming and Florida, respectively (R.S. Nasci, CDC, personal communication). This was consistent with the downward trend in the number of human alphavirus cases over the past 30 years (Fig. 6). Historically, most of the WEE cases have been reported from Colorado, Texas, North Dakota and California, with increases at about 10 year intervals. Most of EEE cases have been reported from Florida, Georgia, New Jersey and Massachusetts, but without marked temporal

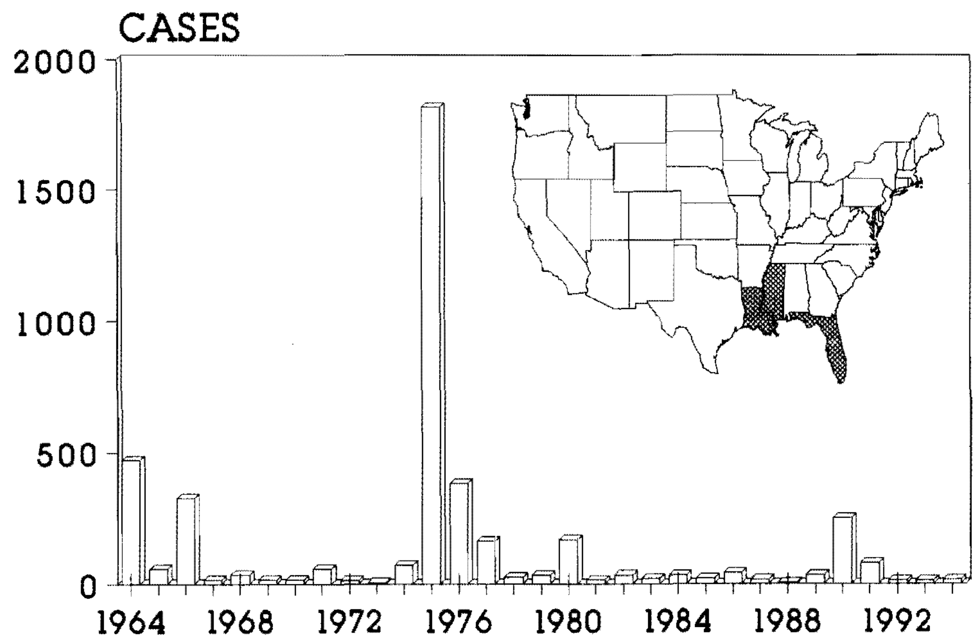


Figure 5. Annual changes in the number of St. Louis encephalitis cases reported in the United States. Map shows all states reporting cases during 1994 (R.S. Nasci, CDC, personal communication).

changes. WEE virus was extremely active in California during 1993 and 1994, when extensive seroconversions in sentinel chickens were recorded in the Sacramento Valley. These increases in enzootic transmission were associated with a single equine case in 1993 and no human cases. EEE virus was active epizootically as indicated by the occurrence of 122 horse cases from 11 states during 1994. Both alpha viruses killed emus in

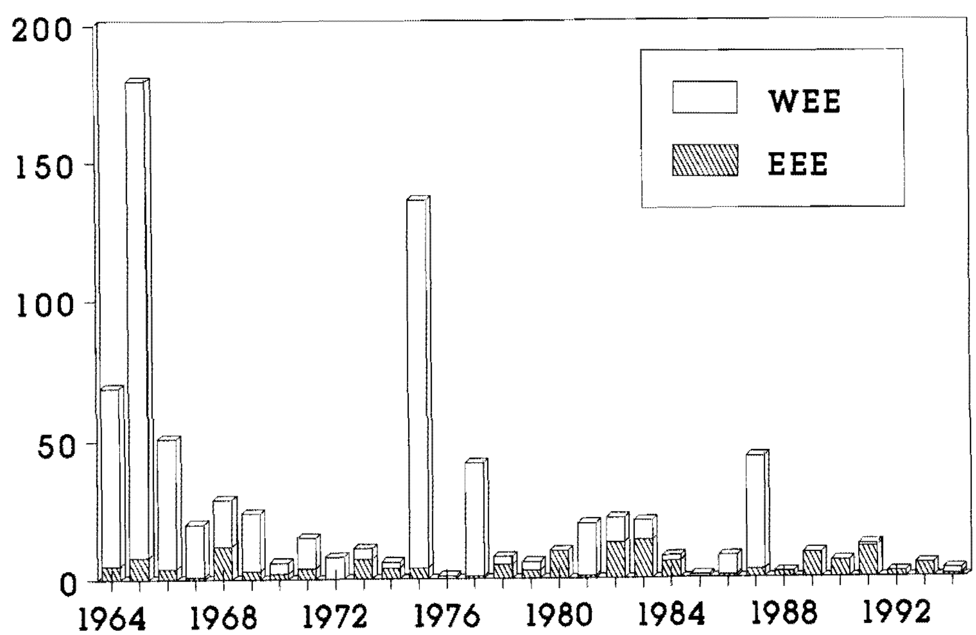


Figure 6. Annual changes in the number of western equine encephalomyelitis (WEE) and eastern equine encephalomyelitis (EEE) cases in the United States.

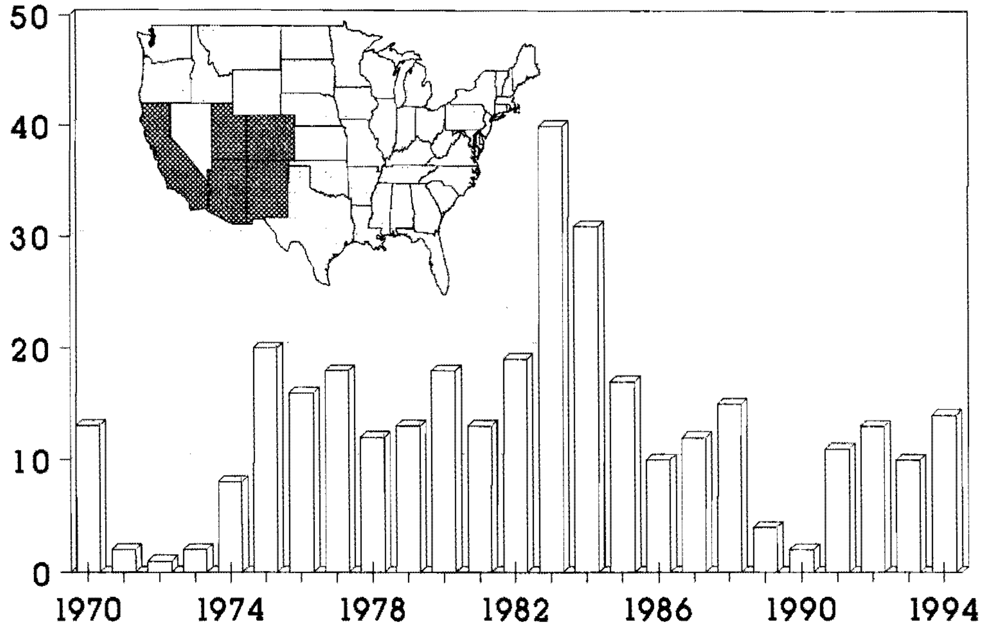


Figure 7. Annual changes in the number of plague cases reported in the United States. Map shows all states reporting cases during 1994 (K. Gage, CDC, personal communication).

California and Alabama, Georgia, Florida and New Jersey, respectively.

In 1994, 14 confirmed and 3 presumptive cases of plague were reported from the United States (K. Gage, CDC, personal communication). Similar to the historical distribution of cases, 5 cases were from New Mexico, 5 from Arizona, 2 from Colorado, 1 from Utah, and 1 from California (Fig. 7). In these areas the bacteria is maintained epizootically in prairie dogs, ground squirrels and wood rats. One case of primary pneumonic plague was acquired from an infected cat. Recent trends in plague cases included (CDC 1994b): 1) increased geographical distribution to states north and east of California and the southwest, 2) increased risk of infection associated with peri-domestic rodents, particularly in the southwest, and 3) increased risk of infection associated with pets, especially cats, either by bringing infected fleas or the infection itself into the home. Pneumonic transmission by infected cats is especially serious, because the infected human rapidly develops pneumonic disease and can transmit the infection directly to other humans.

EMERGING DISEASES

Pathogen drug resistance, vector insecticide resistance, explosive human population increases, the decay of public health services and surveillance, the potential rapid transport of infected humans and domestic animals, and the lack of immunological experience of human populations with many infectious agents have combined to bring new or resurgent diseases to the medical forefront in the United States as well as the rest of the world. Recent reports (Institute of Medicine 1992, CDC 1994c) have addressed this issue on the national level. Vector-borne diseases have been prominent in these reports that recommend renewed interest in surveillance and research.

Four diseases have had or may have an increasingly serious impact on human health in the United States and possibly California: 1) Hantavirus pulmonary syndrome, 2) murine typhus, 3) Ehrlichiosis, and 4) Dengue fever. These diseases have been recognized recently as a new infection of humans (Hantavirus pulmonary syndrome, Ehrlichiosis), have changed their ecology and/or etiology (murine typhus), or may be re-introduced (Dengue fever).

An outbreak of acute respiratory disease occurred in the 4 corners area of the southwest during May-June 1993. This new disease, Hantavirus pulmonary syndrome, was caused by a virus in the family Bunyaviridae and genus *Hantavirus* that was maintained by direct contact among deer mice populations which had up to a 30% antibody prevalence rate. By December 1993, 53 cases with a 60% fatality rate were reported to the CDC from 14 states (CDC 1994d), indicating that this pathogen was widespread. Although not insect-borne, Hantavirus surveillance in rodents has become the responsibility of vector ecologists and seriously has impacted enzootic plague surveillance and other activities that involve the live-trapping and handling of rodents. In 1994, Hantavirus was isolated from the lung tissue of *Peromyscus maniculatus* and was characterized partially (Elliot et al. 1994). The name Muerto Canyon has been proposed to the American Committee on Arthropod-borne Viruses. This virus has been found in *P. maniculatus* collected in Orange County, California (J.P. Webb, OCVCD, personal communication) and may be an important public health concern wherever residential communities interface with large *P. maniculatus* populations.

In classical murine typhus, the pathogen, *Rickettsia typhi*, is maintained in an urban cycle involving rodents and their fleas. Recently, an outbreak of murine typhus was described from Los Angeles, California, involving opossums and cat fleas (Sorvillo et al. 1993). Cat flea infestations averaged 101 *Ctenocephalides felis* per opossum. A similar opossum-cat flea transmission cycle was recognized in south Texas during 1993 (Schreifer et al. 1994). These discoveries were of public health importance because using polymerase chain reaction methodology, Schreifer et al. (1994) showed that a second rickettsia, ELB agent, also was being transmitted among opossums by cat fleas and was the causative agent of 1 of 5 cases clinically diagnosed as murine typhus. The identification of human illness due to infection with ELB agent is especially alarming, because previously Azad et al. (1992) reported that this rickettsia is extremely common in cat flea colonies and is maintained transovarially. Cat fleas are the most common flea on domestic animals in California, and the possibility that a proportion of flea populations are infected vertically with the ELB agent is alarming and deserves follow-up investigation.

The *Ehrlichia*, another rickettsia, are recognized as an expanding health problem throughout the United States (J. Olson, personal communication). The original outbreak in the mid1980s was attributed to *Ehrlichia chafeensis* which first was cultured and isolated in 1990. Between 1985 and 1992, 297 cases were identified in 27 states (CDC 1994c). However, active surveillance in Georgia has estimated that the attack rate focally could be as high as 5.3 cases per 100,000 individuals, or 6 times the Rocky Mountain spotted fever rate. The transmission cycle is poorly understood, but may involve *Amblyoma americanum* ticks and perhaps deer which are susceptible to the rickettsia. If a tick-deer cycle is necessary for this zoonoses, then areas of California experiencing Lyme disease cases also may be susceptible to the introduction of this or closely related pathogens.

Although *E. chafeensis* is not known from California, one case of a new ehrlichia has been recognized in California. This *Ehrlichia* infects the granulocytes and resembles an *Ehrlichia* infecting domestic animals. *Ixodes* ticks are suspected as the vector, but further investigation is required.

Dengue is a mosquito-borne arbovirus maintained by horizontal transmission among humans by *Aedes aegypti* (L.) and *Aedes albopictus* Skuse. Dengue and dengue hemorrhagic fever pose a potential public health problem for the United States, because 1) suitable vectors, *Ae. aegypti* and *Ae. albopictus*, are well established throughout much of the eastern United States, 2) infected travelers repeatedly import dengue virus, and 3) large epidemics now occur annually in Central and South America.

The ability to lay diapausing eggs has allowed *Ae. albopictus* to expand the area of the United States that must be considered receptive to Dengue virus introduction (Gubler and Trent 1994). *Aedes aegypti* remains indigenous throughout the southeastern states and has expanded its distribution throughout Central and South America where eradication programs have not been sustained. Because dengue can be transmitted vertically within mosquito populations, imported mosquitoes also may mean imported virus.

At present neither *Aedes* species is established in California, although introductions of *Ae. aegypti* have occurred in port areas in the past and several laboratories in the state maintain colonies. It is conceivable that environmental changes in southern California may have made areas of the Los Angeles basin suitable for colonization by either *Aedes* species. Reisen et al. (1990) reported that ≈50% of houses in the communities of Norwalk, Los Angeles County, and Rossmoor, Orange County, had containers with water on their property, but only 10% were positive for mosquitoes. These data indicate that the container-breeding niche would seem to lie vacant, awaiting the importation of an efficient colonizing species such as *Ae. aegypti*.

Dengue viruses are continually active in large areas of Asia, the Pacific, and Latin America. During 1986-1992 the CDC tested sera from 788 residents of 47 states diagnosed with clinical dengue, of which 20% were positive, with all 4 serotypes represented; 45% of these patients recently had visited Latin America (Rigau-Perez et al. 1994). These imported cases could provide a source of infection for local mosquitoes. Since 1981, epidemics of dengue hemorrhagic fever have occurred annually within different countries in Central and South America (Gubler and Trent 1994). The implementation of North American Free Trade Agreement which promotes the movement of people and commerce between the United States and Mexico can only increase the rate of case importation and the risk of local transmission.

In summary, vector-borne diseases continue to impact human health throughout the United States. Because most of these diseases are zoonoses and their recorded prevalence is low, transmission probably will continue relatively unchecked (Reeves 1991). The on-going Lyme disease epidemic in the northeastern United States demonstrates the ease with which a previously unknown pathogen can be amplified and become a major health problem in a suburban environment (e.g., Dennis 1991). Several trends in demography and behavior may combine to enhance human exposure to vector-borne diseases in California (Reeves and Milby 1989). These trends seem rooted in the desire of the suburban populace to reside in close proximity with nature, altered only by their perceptions and to their specifications.

1. Housing Location: The explosive increase in the human population of California has required the expansion of housing into foothill terrain and agricultural areas (Reeves 1991). Residences surrounded by undeveloped chaparral and scrub vegetation in southern California and the Central Valley bring humans into close proximity with feral rodents and their ectoparasites, potentially facilitating infection with zoonoses such as Hantavirus pulmonary syndrome, plague and Lyme disease.
2. Landscaping: In residential areas, irrigation has changed native vegetation from desert scrub or chaparral to broadleaf deciduous forest. Residential fruit trees and associated harborage have resulted in an explosive increase in roof rat populations (R.P. Meyer, OCVCD, personal communication) which essentially fill the niche normally reserved for tree squirrels. Vegetative harborage in greenbelts and highway right-a-ways encourage other species such as opossums and skunks which bring zoonoses, including murine typhus and rabies, into the peridomestic environment. In addition, extensive irrigation and landscaping in upper socioeconomic level neighborhoods increase the abundance of potential arbovirus vectors such as *Culex quinquefasciatus* and *Cx. stigmatosoma* Dyar that are less abundant in natural settings or neighborhoods without extensive irrigation (Reisen et al. 1990).
3. Wetland Reclamation: Conservationists plan to establish a series of wetlands throughout California using secondary or tertiary treated sewage effluent. Many of these wetlands will be established in areas previously endemic for mosquito-borne encephalitides that have been largely eliminated by the draining of marshes and the development of agriculture. Undoubtedly these wetlands will develop dense stands of tules and cattails which, in turn, will bring black bird and mosquito populations into close proximity with human populations. Increasing the abundance of both vertebrate hosts and vectors may be associated with increases in arboviruses such as SLE. Comparable conditions in the Los Angeles basin, for example, have produced ecological settings that have permitted the maintenance of SLE virus, which now appears to be endemic.
4. Wildlife Enhancement: Legislation to preserve the habitat and populations of wildlife seriously has impacted the surveillance and control of vectors. For example, the presence of endangered fairy shrimp in vernal pools impedes the surveillance and control of *Culex tarsalis* larvae in riparian systems during late winter and early spring. The nesting of yellow headed blackbirds in tules which grow in urban sumps in the San Joaquin valley precludes vegetation removal and treatment for mosquitoes during the nesting season. In addition, projects that enhance wildlife such as the establishment of greenbelts within or adjacent to residential areas bring humans into closer contact with not only wildlife, but also the zoonoses they carry.
5. Increased Outdoor Recreation: Not only has housing patterns brought wildlife and associated zoonoses close to human habitation, the increased use of national and state parks and other wildlife areas has increased the exposure of non-immune urban residents to zoonoses. In California, this exposure may range from mosquito encephalitis viruses encountered while fishing along the Colorado River to sylvatic plague in montane camp grounds.

Although these trends in human demography and behavior will not be reversed by vector control agencies, it is critical that community planners as well as managers of wetlands and wildlife areas recognize the potential threat to public health and make every effort to establish a dialogue with vector control agencies.

ACKNOWLEDGMENTS

I especially thank Dr. D.J. Gubler and the staff of the Division of Vector-Borne Infectious Diseases, Ft.

Collins, for providing unpublished surveillance data, reprints and for reading the manuscript. Dr. W.C. Reeves improved an earlier version of this manuscript.

REFERENCES CITED

Azad, A.F., J.B. Sacci Jr, W.M. Nelson, G.A. Dasch, E.T. Schhmidtmann and M. Carl. 1992. Genetic characterization and transovarial transmission of a typhus-like rickettsia found in cat fleas. Proc. Natl. Acad. Sci. 89: 43-46.

CDC. 1994a. Malaria surveillance summary. U.S. Government Printing Office, Washington, DC. 10 pgs.

CDC. 1994b. Human plague - United States, 1993-1994. Morbidity and Mortality Weekly Report. 43(13):242-246.

CDC. 1994c. Addressing emerging infectious disease threats. A prevention strategy for the United States. United States Dept Hlth Human Svcs, Publ. Hlth. Svc., Centers for Disease Control and Prevention, Atlanta, GA 46 pgs.

CDC. 1994d. Update: hantavirus pulmonary syndrome - United States. Morbidity and Mortality Weekly Report. 43:45-58.

CDC. 1995. Cases of selected notifiable diseases, United States, weeks ending December 24, 1994. Morbidity and Mortality Weekly Rept. 43(51&52): 963-966.

Dennis, D.T. 1991. Lyme disease. Tracking an epidemic. J. Am. Med. Assoc. 266:1269-1270.

Elliot, L.H., T.G. Ksaizek, P.E. Rollin, C.F. Spiropoulou, S. Morzunov, M. Monroe, C.S. Goldsmith, C.D. Humphrey, S.R. Zaki, J.W. Krebs, G. Moupin, K. Gage, J.E. Childs, S.T. Nichol, and C.J. Peters. 1994. Isolation of the causative agent of hantavirus pulmonary syndrome. Am. J. Trop. Med. Hyg. 51: 102-108.

Gubler, D.J. and D.W. Trent. 1994. Emergence of epidemic dengue/dengue hemorrhagic fever as a public health problem in the Americas. InfectiousAgents & Dis. 2:383-393.

Institute of Medicine. 1992. Emerging infections: Microbial threats to health in the United States. Washington, DC, National Academy Press.

Oliver Jr, J.H., F.W. Chandler Jr., M.P. Luttrell, A.M. James, D.E. Stallknecht, B.S. McGuire, H.J. Hutcheson, G.A. Cummins and R.S. Lane. 1993. Isolation and transmission of the Lyme disease spirochete from the southeastern United States. Proc. Natl. Acad. Sci. 90:7371-7375.

Reeves, W.C. 1991. Population growth in California and impact on disease transmission. Proc. Calif. Mosq. Vector Control Assoc. 59:10-13.

Reeves, W.C. and M.M. Milby. 1989. Changes in transmission patterns of mosquito-borne viruses in the U.S.A. pp. 121-141. *In*: Service, M.W. [ed.] Demographic Changes and Vector-Borne Diseases. CRC Press, Inc., Boca Raton, Florida

Reisen, W.K. , R.P. Meyer, C.H. Tempelis and J.J. Spoehel. 1990. Mosquito abundance and bionomics in residential communities in Orange and Los Angeles Counties, California. J. Med. Entomol. 27:356-367.

Rigau-Perez, J.G., D.J. Gubler, A.V. Vorndam and G.G. Clark. 1994. Dengue surveillance - United States, 1986-1992. Morbidity and Mortality Weekly Rept. 43:7-19.

Schreifer, M.E., J.B. Sacci Jr, J.P. Taylor, J.A. Higgins and A.F. Azad. 1994. Murine typhus: updated roles of multiple urban components and a second typhus like Rickettsia. J. Med. Entomol. 31:681-685.

Sorvillo, F.J., B. Gondo, R. Emmons, P. Ryan, S.H. Waterman, A. Tilzer, E.M. Andersen, R.A. Murray and R.A. Barr. 1993. A suburban focus of endemic typhus in Los Angeles County: Association with seropositive cats and opossums. Am. J. Trop. Med. Hyg. 48:262-273.

THE PUBLIC HEALTH IMPORTANCE OF MOSQUITO-BORNE ARBOVIRUSES IN CALIFORNIA: A HISTORICAL PERSPECTIVE

Bruce F. Eldridge

Department of Entomology
University of California
Davis, California 95616

The year 1952 was quite a year in California. The winter of 1951-52 was one of the wettest on record and snowfall in the Sierra Nevada was so heavy that on January 13th, Southern Pacific's streamlined *City of San Francisco* got stuck at Yuba Gap, stranding 226 passengers and crew for 72 hours until rescuers broke through the snow drifts with snow plows operating on U.S. Highway 40. On July 21st, a powerful earthquake, centered in the town of Tehachapi, and recording 7.5 on the Richter Scale, shook the entire southern half of the state, causing a number of deaths and extensive property damage. In addition, that year saw the last really big epidemic of mosquito-borne encephalitis in California, which occurred in the Central Valley from Tehāma County in the north to Kern County in the south. How big was it? Between June and October of that year there were 375 confirmed human cases of western equine encephalomyelitis (WEE) and 45 human cases of St. Louis encephalitis (SLE). There were an additional 393 cases of encephalitis for which the etiology was not determined. The case rate in Kern County was estimated at 50 cases per 100,000 people (Reeves 1990).

Since 1952, the annual number of confirmed cases of WEE has declined steadily, and there has been an overall reduction in the number of SLE cases as well, although not as great as in the decline of WEE. In 1993, several surveillance indicators suggested a high level of WEE viral activity in the Sacramento Valley, yet there were no human cases confirmed that year. I would like to put forth some possible reasons for the apparent decline in the incidence of mosquito-borne encephalitis in California, and then make some comparisons between the early 1950s and the 1990s in terms of WEE and SLE ecology.

TRENDS IN CONFIRMED MOSQUITO-BORNE ARBOVIRUS CASES SINCE 1952

Let us first examine the situation for mosquito-borne arbovirus disease nationally. After 1952, the number of cases of WEE reported in the United States dropped, then gradually declined to a relatively low number of cases, with numbers never again reaching the epidemic peaks recorded in 1952 and some earlier years (Fig. 1). Reported cases of SLE also diminished, but there continued to be periodic outbreaks. In 1975 an epidemic of SLE occurred in the eastern United States with nearly 2,000 confirmed cases. Numbers of cases returned to endemic levels during the 1980s, then in the 1990s there were several outbreaks of SLE and eastern equine encephalomyelitis (EEE) in the southeastern United States.

In California, the number of confirmed cases of WEE and SLE during the latter half of this century show a pattern similar to that seen nationally (Fig. 2). Very few cases of WEE have been confirmed since 1970, and there have been only small outbreaks of SLE since that time.

Within the last decade, WEE has virtually disappeared in California, and the only noteworthy arbovirus event was a 1989 SLE outbreak of 29 confirmed cases which occurred in Kern, Tulare and Kings counties (Fig. 3).

In the summer of 1993, high levels of WEE activity were in evidence based on sentinel chicken conversions and on isolations from mosquito pools. Most of this activity was centered in the Sacramento Valley. There was a single equine case of WEE confirmed, and several cases in emus, an imported bird being raised commercially in California. However, in spite of this level of activity, there were no confirmed human

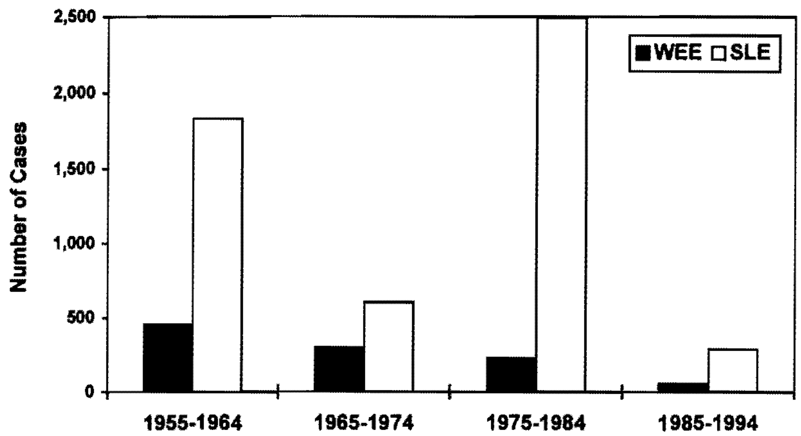


Figure 1. United States cases of WEE and SLE by decade, 1955-1994
(Data from CDC Morbidity and Mortality Weekly Reports annual summaries).

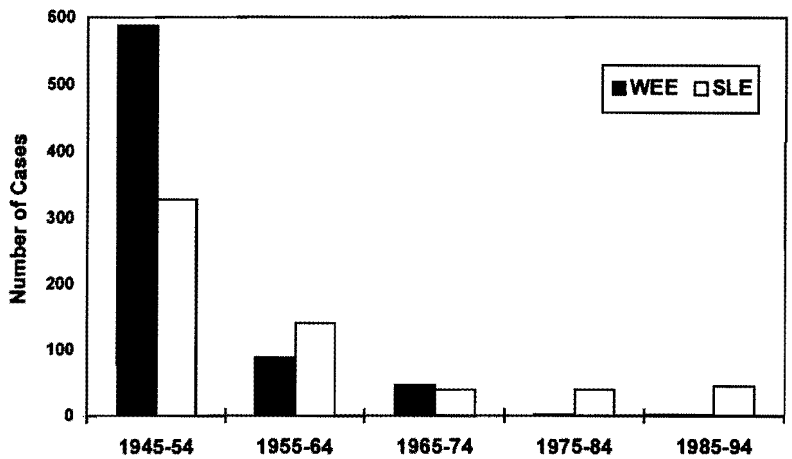


Figure 2. California cases of WEE and SLE by decade, 1945-1994
(Data from California Department of Health Services).

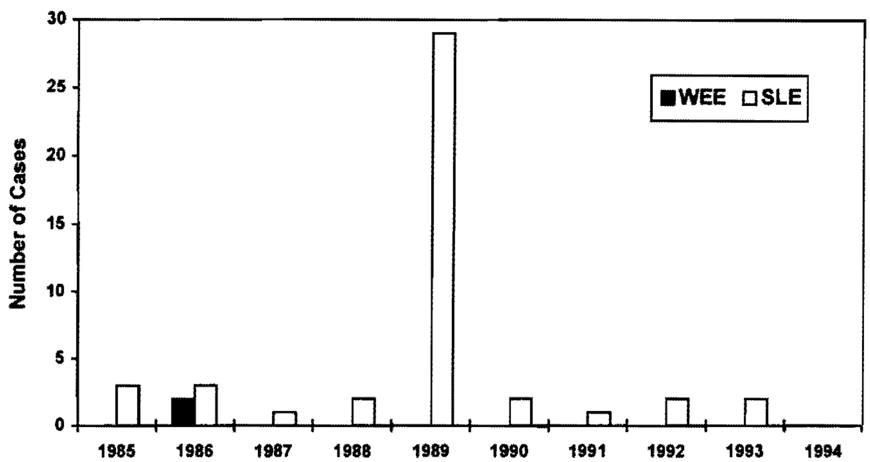


Figure 3. California cases of WEE and SLE reported annually, 1985-1994
(Data from California Department of Health Services).

of WEE. This is very puzzling. Can it be that in a year in which nearly every flock of sentinel chickens seroconverted in that area, that not a single person came down with a case of arboviral disease? This seems unlikely, but why have there been no confirmed cases? Let us dig a little deeper.

First of all, let us consider some possible reasons for the recent lack of confirmed human cases of WEE in California. Possible reasons are:

1. The abundance of vector mosquitoes is considerably lower than it was 40 years ago, because of mosquito abatement operations, or because of a reduction in mosquito habitat.
2. People are not exposed and bitten by vector mosquitoes to the extent they were 40 years ago.
3. The WEE viral activity has become greatly reduced over the past 40 years, so that virus rarely gets into vector mosquito populations.
4. The WEE virus has changed over the years, and is now less virulent for people.
5. *Culex tarsalis* Coquillett has changed over the years, and present populations are less efficient vectors of WEE.
6. People are being infected, and some portion of those infected do show symptoms, but human case detection is less efficient that it used to be.

Let's examine each of these possibilities in more detail.

1. The abundance of vector mosquitoes is considerably lower than it was 40 years ago, because of mosquito abatement operations, or because of a reduction in mosquito habitat.

Olson et al. (1979), in their well-known study of factors predisposing human WEE cases in California, concluded: "*Culex tarsalis* female populations, as measured by New Jersey light trap indices, correlated positively with the incidence rates of encephalitis in humans, ...". This relationship is evident from an inspection of data shown in Table 1. Olson et al. concluded that critical urban levels of *Cx. tarsalis* associated with significant human SLE or WEE incidence ranged from 6.4 (for rural mosquito abatement districts with large resident human populations) to 62.4 (for urban MADs with relatively small human populations). This study led to the reporting of mosquito abundance in California in three categories. The categories are: Urban (traps located more than 1 mile inside of a densely populated urban area), suburban (traps located 1/4 to 1 mile inside of a densely populated urban area) and rural (traps located less than 1/4 mile inside of a densely populated urban area). Reeves et al. (1990) later concluded that 1.0 female per trap-night in urban areas may be significant in terms of transmission of WEE to human beings.

Table 1. Statewide comparison between female *Culex tarsalis* trapped by month in California, and the number of human WEE cases (data from Olson 1977).

Month	Female mosquitoes per trap-night	WEE cases
May	0.6	0
June	3.0	1
July	6.3	16
August	8.7	27
September	7.6	14
October	1.5	0

Unfortunately, available mosquito records from the 1950s do not allow a direct comparison with today's levels in terms of the classification used in recent years. However, allowing for this, it still appears that levels of *Cx. tarsalis* of a magnitude comparable to 1952 were reached in 1993 in the Sacramento Valley. An examination of the Adult Mosquito Occurrence Report for Weeks 25-28 of the vector-borne disease surveillance year (June 27 - July 17) for the Sutter-Yuba MAD shows levels of nearly 100 adult females per trap-night in rural areas, and about half as many females per trap-night in suburban areas (Fig. 4). Data from the same period reported by the Sacramento-Yolo MVCD also show high densities of vector mosquitoes (Fig. 5).

2. People are not exposed and bitten by vector mosquitoes to the extent they were 40 years ago.

This is a difficult question to answer, and certainly one which can not be quantified, but common sense suggests that human exposure to mosquito bites has indeed been reduced. Gahlinger et al. (1986), in fact, suggest that television and air conditioning in the Central Valley have played a definite role in the reduction of human WEE cases. Reeves and Milby (1989) discuss a number of other factors which may affect the degree of human-mosquito contact. One of these factors is the urbanization of the state, with the proportion of people living in urban areas increasing dramatically over the past 40 years.

3. Overall viral activity has become greatly reduced over the past 40 years, so that virus rarely gets into vector mosquito populations.

Most available evidence suggests that this is not a

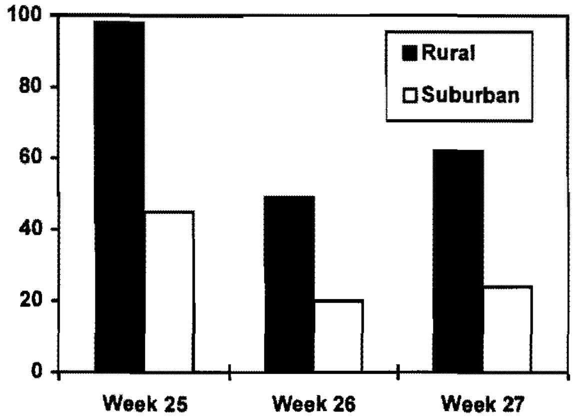


Figure 4. Mosquitoes trapped during surveillance Weeks 25-27, Sutter-Yuba Mosquito and Vector Control District, 1993. Y-Axis represents number of female *Culex tarsalis* per trap-night captured in New Jersey light traps (Data from Adult Mosquito Occurrence Report, California Department of Health Services).

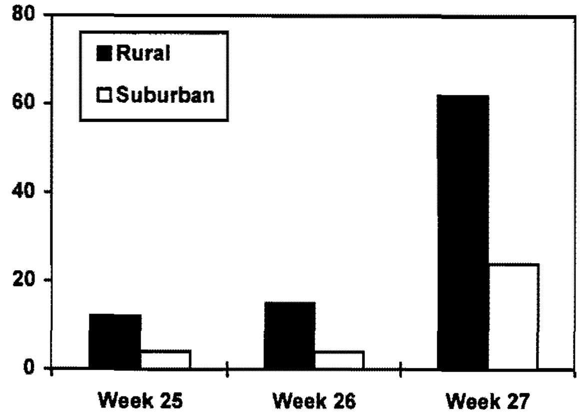


Figure 5. Mosquitoes trapped during surveillance Weeks 25-27, Sacramento-Yolo Mosquito and Vector Control District, 1993. Y-Axis represents number of female *Culex tarsalis* per trap-night captured in New Jersey light traps (Data from Adult Mosquito Occurrence Report, California Department of Health Services).

reasonable possibility. In 1993, about 4,395 pools of mosquitoes were submitted for testing statewide, with at least 180 positive for WEE virus (0.8 isolates per thousand individuals tested), 35 for SLE virus (0.16 per thousand). A total of 157 flocks of chickens yielded over 17,000 blood samples. Of these, 281 showed evidence of WEE antibodies, 91 for SLE virus. How do these rates compare with 1952?

In Kern County in 1952, WEE virus-positive pools began showing up in May (Table 2). In contrast, in 1993, there were no WEE-positive pools before July. Viral isolation rates in 1993 never reached the level reported for Kern County in 1952, but the rates for 1993 are for all California counties, and if pools submitted from counties where no viral activity occurred are removed, the 1993 rates would be higher. Certainly, on the basis of these figures, the case can not be made for absence or scarcity of the virus in mosquitoes in 1993.

4. The WEE virus has changed over the years, and is now less virulent for people, or less infectious for mosquitoes.

This is one area that definitely needs to be examined. Dr. James Hardy of the School of Public Health at Berkeley is already beginning comparisons of recent isolates of SLE and WEE with those isolated many years ago, looking both at virulence in animal models as well as specific DNA sequences. Dr. Hardy will be presenting data on animal virulence in a later paper.

5. *Culex tarsalis* has changed over the years, and present populations are less efficient vectors of WEE.

It is known that vector competence of mosquitoes for arboviruses varies within populations, and that some environmental factors, such as salinity, are related in some way. It was suspected at one time that insecticide resistance might also be related to vector competence, but no one has ever been able to demonstrate this conclusively. However, I know of no evidence to suggest that there has been any shift in vector competence for WEE in California populations of *Cx. tarsalis* resulting in an overall diminishment of vector efficiency. But recent observations by Hardy and Reeves (1990) on temporal and spatial oscillations in vector competence of *Cx. tarsalis* for WEE and SLE viruses suggests that more research in this area is needed.

6. People are being infected, and some portion of those infected do show symptoms, but human case detection is less efficient than it used to be.

This is a question which is being explored in detail by the Encephalitis Task Force. Dr. Michelle Jay will cover most of the details relating to this, but I do want to emphasize several points. One is that the case definitions used for reporting various diseases under surveillance vary considerably. For example, reporting of a case of aseptic meningitis requires only a laboratory test which rules out bacteria or fungi from a spinal fluid sample. Reporting of a case of primary encephalitis is based only

Table 2. WEE isolation rates (isolates per 1,000 mosquitoes tested) in California for Kern County (1952) and statewide (1993). Data for 1952 from Reeves and Hammon (Reeves and Hammon 1962); for 1993 from California Department of Health Services Arbovirus Surveillance Bulletin.

Month	Kern County (1952)	Statewide (1993)
May	0.50	0.00
June	4.70	0.00
July	2.20	0.03
August	1.50	1.39
September	0.30	1.53
October	—	0.63

on symptomology and case history. Arboviral encephalitis, on the other hand, is not reported without laboratory confirmation of the viral pathogen involved. Every year in California thousands of cases of aseptic meningitis and primary encephalitis are reported and appear in CDC's Morbidity and Mortality Reports.

Could there have been cases of WEE among the reported cases of these diseases? In 1989, the 28 cases of SLE were confirmed retrospectively by an epidemiologist who happened to be available to the State Department of Health Services at the time, and was alerted to an abnormally high number of reported cases of aseptic meningitis in the Los Angeles Basin. Were there cases of WEE among the reported cases of these diseases in 1993? Nobody knows. Most cases of aseptic meningitis are caused by enteroviruses such as coxsackie and echo viruses, but nearly all epidemiological studies of these diseases include a group of unknown etiology, and when serum samples are tested retrospectively, arboviruses are often found to be responsible for a proportion of the cases (Deibel et al. 1979). The "take-home" message here is that primary encephalitis and aseptic meningitis are catch-all categories for diseases caused by a variety of pathogens, whereas arboviral encephalitis is a specific category requiring laboratory confirmation for reporting.

CONCLUSIONS

Having gone over briefly six possible reasons for the virtual disappearance of confirmed cases of WEE and a reduction of SLE cases in California, what can we say with certainty? Actually, very little. We can say that *Cx. tarsalis* and mosquito-borne viruses which cause encephalitis still exist in California, and apparently in some areas of the state in some years, such as 1993, at levels nearly as high as they ever have been. We have little to offer in the way of objective data on the degree of human-mosquito vector contact which exists today in comparison with 40 years ago, although circumstantial evidence would suggest that it is less. We badly need more research on vector competence of various California populations of *Cx. tarsalis*, and on viral strain variation. We certainly need better human case identification, confirmation and reporting.

Does it really matter? Some may argue that since clinical treatment of encephalitis or aseptic meningitis, whatever the cause, does not vary, then the specific diagnosis is really not that important. However, from the standpoint of preventive medicine, of land use planning, and of public policy, this question is of the utmost importance. Decision makers in government are continually weighing perceived public health threats posed by mosquitoes against perceived gains in habitat improvement. If the public health threat is imperfectly known, how good are the decisions being made? At this very moment, the fate of synergized pyrethrum as a mosquito adulticide is being pondered by an U.S. EPA-industry task force. Shouldn't they have available accurate information on the public health significance of vector-borne diseases such as WEE?

A comparison of today's epidemiological features of WEE and SLE in California with those of 40 years ago do not provide all the answers needed to explain why there have been so few confirmed human cases of WEE and SLE in recent years. Nevertheless, I believe it is important to all of us to continue to study these viruses and their public health importance, and to continue to maintain the best and most comprehensive surveillance program we can.

REFERENCES CITED

Deibel, R., S. Srihongse and J.P. Woodhall. 1979. Arboviruses in New York State: An attempt to determine the role of arboviruses in patients with viral encephalitis and meningitis. Am. J. Trop. Med. Hyg. 28:577-582.

Gahlinger, P.M., W.C. Reeves and M.M. Milby. 1986. Air conditioning and television as protective factors in arboviral encephalitis risk. Am. J. Trop. Med. Hyg. 35:601-610.

Hardy, J.L. and W.C. Reeves. 1990. Experimental studies on infection in vectors. Chapter 5, pp. 147-253, *In:* Reeves, W.C. (ed.). Epidemiology and Control of Mosquito-Borne Arboviruses in

California, 1943-1987. California Mosquito and Vector Control Association, Sacramento, CA.

Olson, J.G., W.C. Reeves, R.W. Emmons and M.M. Milby. 1979. Correlation of *Culex tarsalis* population indices with the incidence of St. Louis encephalitis and western equine encephalomyelitis in California. Am. J. Trop. Med. Hyg. 28:335-343.

Reeves, W.C. 1990. Epidemiology and Control of Mosquito-Borne Arboviruses in California, 1943-1987. California Mosquito and Vector Control Association, Sacramento, CA. 508 pp.

Reeves, W.C., M.M. Milby and W.K. Reisen. 1990. Development of a statewide arbovirus surveillance program and models of vector populations and virus transmission. Chapter 10, pp. 431-459. *In:* Reeves, W.C. (ed.). Epidemiology and Control of Mosquito-Borne Arboviruses in California, 1943-1987. California Mosquito and Vector Control Association, Sacramento, CA.

Reeves, W.C. and W.M. Hammon. 1962. Epidemiology of the Arthropod-Borne Viral Encephalitides in Kern County, California, 1943-1954. University of California Press, Berkeley, CA.

Reeves, W.C. and M.M. Milby. 1989. Changes in transmission patterns of mosquito-borne viruses in the USA. Chapter 8, pp. 121-141. *In:* Service, M.W. (ed.). Demography of Vector-Borne Diseases. CRC Press, Boca Raton, FL.

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:18-22

SURVEILLANCE FOR ARTHROPOD-BORNE VIRUS ACTIVITY AND HUMAN DISEASE IN CALIFORNIA DURING 1994

Kevin F. Reilly, Michael S. Ascher, Dale V. Dondero, Barryett Enge, Franklin Ennik, Lucia T. Hui, William K. Reisen[1], Donald A. Eliason[2], Lal S. Mian[3], Jieyan Lin, Robert A. Murray, Marilyn M. Milby[1], James L. Hardy[1], Sally B. Presser[1], Robert E. Chiles[1], William C. Reeves[1]

Division of Communicable Disease Control
California Department of Health Services
P.O. Box 942732
Sacramento, California 94234-7320

The California Arbovirus Surveillance Program is a cooperative effort conducted by the California Department of Health Services' Division of Communicable Disease Control (CDHS); the University of California, Berkeley's Arthropod-Borne Virus Research Unit; the California Mosquito and Vector Control Association; local mosquito and vector control agencies; local health departments, physicians and veterinarians; and other interested parties. The surveillance program is multifaceted and utilizes the following tools: Mosquito population monitoring and testing for arboviruses; serological monitoring of sentinel chicken flocks in areas of the state with historical evidence of arbovirus activity; serological monitoring of domestic animal species that may show clinical illness with arbovirus infection; and serological testing of patients suffering from fever, neurological symptoms and other signs of viral meningitis or encephalitis.

The 1994 surveillance program started in April with the delivery of sentinel chickens to cooperating local agencies, and the beginning of light trapping and adult mosquito occurrence data collection. Twenty-five weekly bulletins and adult mosquito abundance reports were faxed and mailed to all surveillance program participants starting on May 13. Positive serology and mosquito pool results were communicated immediately to the submitting agency.

Human Disease Surveillance.

In 1994, 39 human sera and cerebral spinal fluid (CSF) specimens from patients suffering symptoms of viral meningitis or encephalitis were tested by the Department's Viral and Rickettsial Disease Laboratory (VRDL) for antibodies to St. Louis encephalitis (SLE) and to western equine encephalomyelitis (WEE). None of these sera showed IgM antibody or a four-fold rise in total antibody between two titers to meet the diagnostic requirements as a case of arboviral encephalitis.

A drop in submissions for human arbovirus testing has been of great concern to CDHS. Even active surveillance efforts in 1993 involving seven referral hospitals in endemic areas of the state failed to elicit a large number of submissions for arbovirus serology. One theory behind the lack of submissions is the limited information the clinician receives back from test results. In the past, VRDL was only able to detect evidence of infection with SLE or WEE. Most "summer encephalitis" patients that present to a physician with fever, neurological symptoms, and a negative CSF prompting a physician to consider aseptic meningitis or arboviral encephalitis are suffering from enterovirus infections (the family of viruses that includes polio, Coxsackievirus and echoviruses). Recently, VRDL has developed the capability to do serological testing for enteroviruses. The value here is that physicians can receive diagnostic information on most patients where sera or CSF is

[1] Arbovirus Research Unit, School of Public Health, University of California, 140 Warren Hall, Berkeley, California 94720.

[2] California Mosquito and Vector Control Association, 8633 Bond Road, Elk Grove, California 95624.

[3] San Bernardino County Vector Control Program, 2355 East 5th Street, San Bernardino, California 92415

submitted for a rule-out of "aseptic meningitis". A majority of these patients will test positive for enterovirus. Culture can then provide the definitive diagnosis. The possibility of giving the patient a diagnosis in most cases may make the submission of sera a much higher priority for physicians. The arbovirus surveillance system benefits by having more sera to test for WEE and SLE at the same time.

A single human case of SLE was retrospectively confirmed in 1994 in a 35-year-old white male resident of Moreno Valley, Riverside County. The man was hospitalized on September 12 with a ten day history of progressive headache, fever and stiff neck. The patient was admitted to the intensive care unit for six day with severe neurological signs and was finally discharged from the hospital ten days following admission. The patient continued to have residual neurological signs for several months. The patient traveled to the California-Arizona border on the Colorado River in the week prior to onset of illness. He camped at Park Moabi in San Bernardino, and his wife remembered many mosquito bites at that time. A sentinel chicken flock in Needles (10 miles South of Park Moabi) had birds seroconvert to SLE on August 16 and August 30, and the Arizona Health Department collected four positive *Culex tarsalis* Coquillett pools on the Arizona side opposite Needles on August 3. Convalescent sera and CSF collected from the patient in early October tested positive for IgM and IgG against SLE by a southern California reference laboratory. A small amount of CSF was available from the reference lab for confirmatory testing at VRDL. Although the IgG reactivity was confirmed, the quantity of CSF was insufficient for IgM testing.

The case came to the attention of public health authorities some time after initial onset of illness. Anecdotally, friends and other members of the patient's family also traveled to Park Moabi on the Colorado River in late August, and reportedly suffered with "flu-like" illness in that time frame. These persons were not subsequently bled due to the time delay and the inability to interpret any detectable titers.

Mosquito Testing.

Mosquitoes captured with CDC style carbon dioxide (CO_2)-baited traps, modified Reiter gravid traps and other methods conducted by 30 local mosquito control agencies around the state were pooled and tested for arboviruses at VRDL using standard methods. Of 1,859 mosquito pools (80,892 mosquitoes) tested for both SLE and WEE (Table 1), only 8 pools were positive for WEE and none were positive for SLE. The positive *Cx. tarsalis* mosquito pools were collected from July 26 - August 10 in Lake (4) and Sacramento Counties (4).

Chicken Serosurveillance.

One hundred sixty-two sentinel chicken flocks were maintained by local vector and mosquito control agencies in 1994. Over 18,900 individual chicken sera were serologically tested for WEE and SLE by VRDL and by the University's Arbovirus Research Unit. Forty-nine birds tested positive for WEE and 117 birds tested positive for SLE (Table 2). The first seroconversions to both SLE and WEE appeared relatively late in the year in sera collected on July 18, 1994. Examination of the distribution of chicken flocks that tested seropositive in 1994 (Figs. 1 and 2) indicate that SLE activity was confined to Southern California, primarily in the desert in Imperial and Riverside Counties while WEE activity was found in that same area, and in the northern Sacramento valley extending into the bay area.

There was significantly less WEE activity detected by the surveillance program in 1994 than in 1993 (25 seroconverted flocks with 49 birds in 1994 versus 51 seroconverted flocks with 372 birds in 1993). In 1994, a significant proportion of flocks that seroconverted to WEE did so in a single bird, or relatively few birds. Concerns arose over the fact that single birds within the flocks were showing serological evidence of infection, even over prolonged periods of exposure time. To check this, titers on six single bird seroconversions were monitored for several bleedings following seroconversion. In five of the six birds, seropositivity persisted therefore validating the original test. The single bird that did not have a reproducible significant titer was borderline sero-positive to begin with, indicating that WEE was present in several locations around the state in 1994, but in a more limited extent than in 1993.

Equine and Ratite Disease Surveillance.

A total of 12 sera and brain tissue specimens from horses displaying neurological signs were submitted by practicing California veterinarians for arboviral testing at VRDL in 1994. None of these specimens were positive on arbovirus serology or antigen testing.

The California Veterinary Diagnostic Laboratory System tests sera from ratites (the Ostrich and Emu family of flightless birds) for evidence of WEE infection. Sera are tested at the University of Texas at Amarillo using a virus neutralization method. No ratites showed evidence of seroconversion between two titers in 1994. Several birds had low titers (<1:128) on single sera that were tested, but the diagnosis was not confirmed.

Table 1. Mosquitoes and mosquito pools tested by VRDL for WEE, SLE and CE viruses in 1994, by submitting agency and mosquito species.

Agency	*Cx. tarsalis*		*Cx. pipiens*		*Cx. stigmatosoma*		*Ae. melanimon*		*Cx. erythrothorax*		*Cx. quinquefasciatus*		Totals	
	pools	mosq.	pools	mosq.	pools	mosq.	pools	mosq.	pools	mosq.	pools	mosq.	pools	mosq.
ALAM	11	433	1	14									12	447
CNTR	146	6925											146	6925
DLTA	13	591											13	591
FRNO	11	327											11	327
GLNN	39	1920											39	1920
GLUY		0			1	10							1	10
GLVY	3	44							2	100			5	144
JCVC	18	717	4	160					14	700			36	1577
KERN	124	5453	1	50									125	5503
KGNS	5	237											5	237
KNGS	4	157											4	157
LAKE	79	3658			5	218	36	1713					120	5589
LONG	42	2012									47	2227	89	4239
LOSA	120	5668			6	177	5	189			37	1802	168	7836
MADR	8	400			2	100							10	500
MARN	5	187	2	100									7	287
NWST	111	5522			15	738	1	50			89	4446	216	10756
ORCO	21	419									101	2616	122	3035
SACR	277	12802					30	1243					307	14045
SANB	52	2155											52	2155
SAND	38	1892											38	1892
SANJ	25	1095											25	1095
SBOV	14	238			4	51					6	93	24	382
SGVA	15	406			2	30					32	1178	49	1614
SHAS	23	977											23	977
SOUE	54	1954	2	100							29	823	85	2877
SUYA	71	3185											71	3185
TECO	8	285											8	285
TRLK	22	1083			2	87	1	50					25	1220
VENT	23	1085											23	1085
Totals	1382	61827	10	424	37	1411	73	3245	16	800	341	13185	1859	80892

Current information suggests that clinical illness in ratites with WEE is mostly limited to young birds less than 6 weeks of age. Anecdotally, the use of equine arboviral encephalitis vaccine in ratites continues to some extent in the ratite raising community. Unfortunately, previous vaccination with WEE and Eastern Equine Encephalitis vaccine creates difficulties in interpreting serological results (as is seen in horses). However, the use of ratite serological testing as a surveillance tool for arbovirus activity continues to be of interest.

Acknowledgments.

Special thanks to Sarah Ball for assistance in surveillance data management. We are grateful to staff members of the VRDL and the Diseases Investigation and Surveillance Branch, CDHS; the Arbovirus Field Station and the Arbovirus Research Unit, School of Public Health, University of California, Berkeley; participating local vector and mosquito control agencies; local health departments; the Department of Food and Agriculture, Animal Health Branch; and physicians and veterinarians who submitted specimens from suspect clinical cases.

Special thanks to the California Mosquito and Vector Control Association for financial support to conduct laboratory testing.

Table 2. WEE and SLE Seropositive Chickens for 1994 by Location and Incident Seroconversion Date.

County	City	Location	Incident Seroconversion Date								Total
			7/22	8/5	8/19	9/2	9/16	9/30	10/14	10/28	
Imperial	Brawley	Cadys Road			1	3	2		1		7
Imperial	El Centro	Christopher				2					2
Imperial	Calexico	Dog Pound				1					1
Imperial	Orita	Green				4					4
Imperial	Bonds Corner	Keefer		2	3	1	1	4			11
Imperial	Seeley	Rio Bend RV Park	1				3	1	2	1	8
Imperial	Calexico	Rockwood				3	3	1	2		6
Imperial	Westemoreland	Rosario			1	6	2				11
Imperial	Salton Sea NWR	Salton Sea NWR			2	3	1				7
Imperial	Wister NR	Wister NR				2	1	4	1	1	9
Imperial	Holtville	Zenos				1			2		4
Los Angeles	Glendora	615 Los Cerritos								1	1
Riverside	Mecca	Adohr Farms					1	7	1		9
Riverside	Mecca	Dex-O-Tex					2	5			7
Riverside	North Shore	El Rancho								3	3
Riverside	Valerie	Fish				1					1
Riverside	North Shore	Gordon					2	2			4
Riverside	Mecca	Hayes					1	1			2
Riverside	Valerie	Jessup					1	2			3
Riverside	Valerie	Kono						4			4
Riverside	Mecca	Mecca						1			1
Riverside	North Shore	Salton Sea State Park				6		2			8
San Bernardino	Needles	Sewage Plant			1	2		1			4
		SLE Totals	1	2	8	35	19	36	7	9	117
Butte	Gridley	Grey Lodge		1	1						2
Butte	Chico	River Road		2							2
Butte	Biggs	Theback Ranch		1							1
Chuchill	Fallon	Tarzyn Road						1		1	2
Colusa	Colusa	Grussenmeyer Ranch			2		1				3
Glenn	Willows	N.E. Willows				3		1			4
Imperial	Bonds Center	Keefer						1			1
Imperial	Seeley	Rio Bend RV Park			1	2		2			5
Imperial	Salton Sea NWR	Salton Sea NWR					1				1
Imperial	Holtville	Zenos			1						1
Lake	Upper Lake	MAD Fish Pond		2	1						3
Marin	Novato	Marin Sanitary Dist.		1							1
Sacramento	Hood	G. Whitney	1								1
Sacramento	Hood	N. Stone Lake				3					3
Sacramento	Freeport	N. Stone Lake					1				1
Sacramento	Hood	S. Riggs				1					1
Sacramento	Franklin	T. Johnson			1						1
San Joaquin	Holt	Bacon Island			1						1
San Joaquin	Thornton	Midsection Road					1				1
Solano	Cordelia	Cordelia			1		1				2
Solano	Suisan	Grizzly Island					1				1
Tehama	Red Bluff	MAD Office		1	3	2					6
Yolo	Woodland	Merritt						1	1		2
Yuba	Maysville	Alberto's					1				2
Yuba	Olivehurst	Senior Citizen's Cntr.									1
		WEE Totals	1	8	12	13	7	6	1	1	49

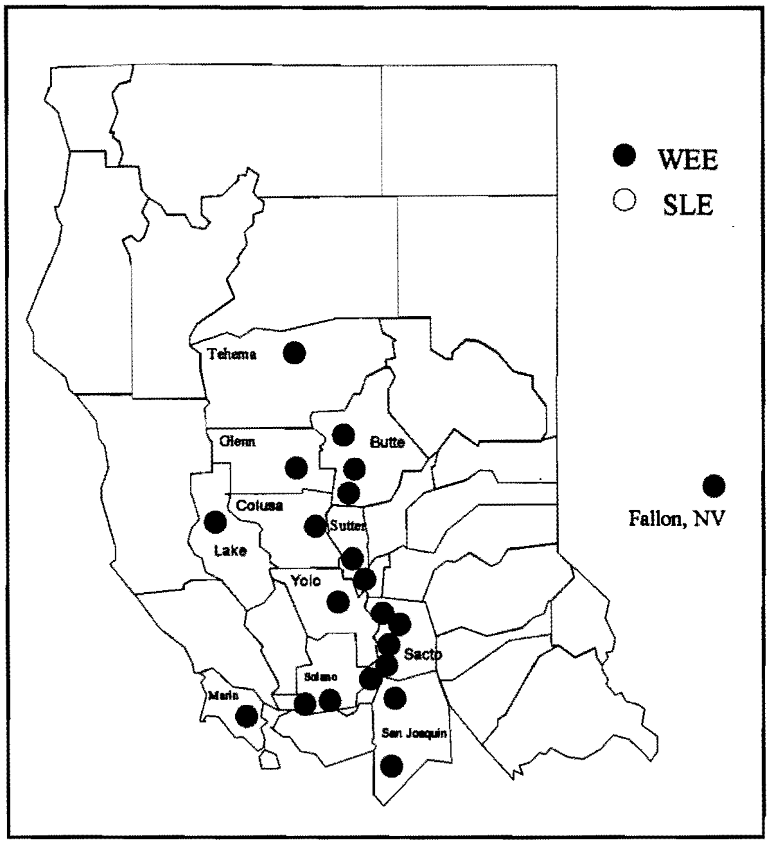


Figure 1. WEE and SLE seropositive chicken flocks from northern California during 1994.

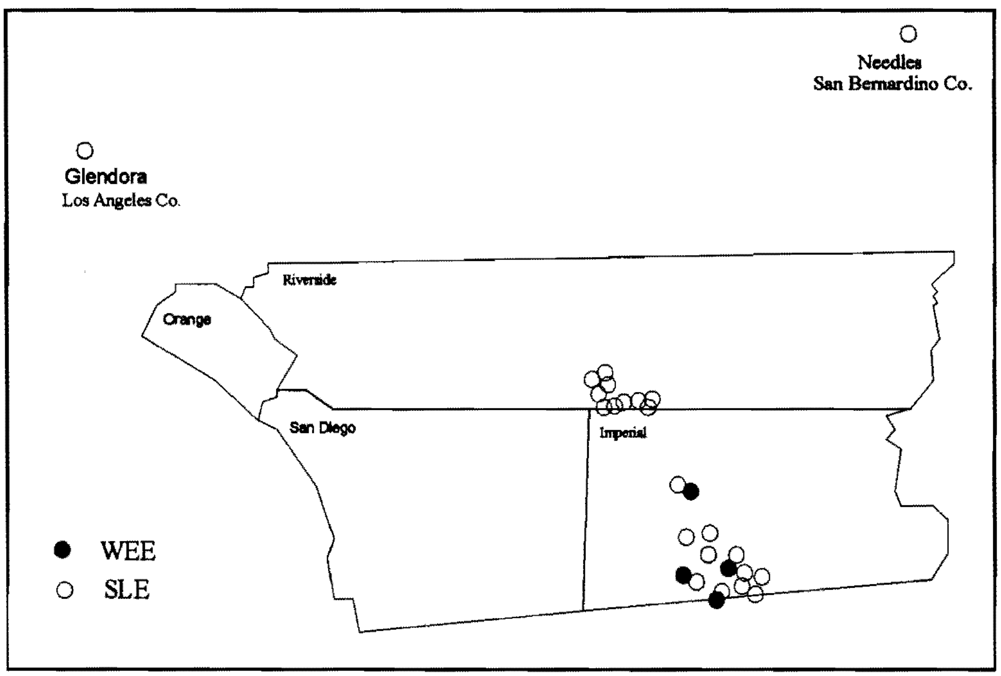


Figure 2. WEE and SLE seropositive chicken flocks from southern California during 1994.

MOSQUITO-BORNE ENCEPHALITIS SURVEILLANCE IN WESTERN LOS ANGELES COUNTY DURING 1991-1994

Jean G. Kovaltchouk, Diana G. Garcia, Robert F. Cummings, Greg Pierson,
Howard M. Muramoto and Robert Saviskas

Los Angeles County West Vector Control District
6750 Centinela Avenue
Los Angeles, California 90230

ABSTRACT

During 1991 through 1994, the Los Angeles County West Vector Control District tested 3,235 peridomestic bird sera for antibodies to Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses; 110 sera (3.3%) tested positive for SLE antibodies and none tested positive for WEE antibodies. Also during that time, 1,679 mosquito pools comprised of 74,301 mosquitoes were tested; seven pools collected in September 1991 tested positive for WEE and none tested positive for SLE. Two to six sentinel chicken flocks were tested semi-monthly for viral activity. In September and October 1992, three chickens in one flock seroconverted for SLE antibodies and in November 1993, one chicken seroconverted for WEE antibodies.

The Los Angeles County West Vector Control District (LACWVCD) covers about 600 square miles of the western portion of Los Angeles County. Half of this area is rural, mountainous and under the influence of mosquito sources such as streams, marshes and duck clubs. Mosquito species typically found in these sources are *Culex tarsalis* Coquillett, *Culex erythrothorax* Dyar, *Anopheles hermsi* Barr and Guptavanij, *Anopheles franciscanus* McCracken, *Culiseta inornata* Williston and *Aedes squamiger* Coquillett. The other half of the district is home to over three million Los Angelinos. It is highly residential, but also commercial with some manufacturing. Mosquito breeding sources in this half are numerous and varied. They can be the petroleum-base polluted waters of two oil refineries, of numerous oil extraction sumps, or of oil and tar natural ground seepage. Sources can also include underground drainage channels rich in decaying vegetation, backyard spare tires, fish ponds or swimming pools. Mosquito species commonly encountered in these habitats are *Cx. tarsalis, Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar and *Culiseta incidens* Thomson.

The Los Angeles County West Vector Control District is part of the statewide mosquito-borne encephalitis surveillance program, a program which, aside from monitoring human and equine arbovirus infections, also tests mosquitoes and sentinel chickens submitted by collaborating mosquito and vector control districts. In addition to participating in the statewide program, LACWVCD also implemented a peridomestic bird testing program to monitor levels of arboviral infection, albeit not necessarily recent infections, in the avian population. Since, in urban areas, peridomestic birds are the reservoir for Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses, LACWVCD recognizes the need to sample the peridomestic bird population to monitor infection levels and occurrences of viral amplification. We also hoped to evaluate the use of a peridomestic bird testing program as a warning system to detect amplification of mosquito-borne arboviruses. Although the study was not designed to correlate infection levels in the peridomestic birds with evidence of infection in the mosquitoes or evidence of transmission in the sentinel chickens, we tried to infer such correlation in our evaluation of the results.

MATERIALS AND METHODS

Peridomestic Bird Testing Program.

From September 1991 to December 1994, up to six modified Australian Crow traps were used to collect peridomestic birds. During the study, the number of traps used was progressively increased and some traps were moved to different locations. By 1994, the trapping sites were: the Botanical Gardens in Palos Verdes, the Playa del Rey salt flats, the Chevron Oil refinery in El Segundo, the Mobil Oil refinery in the city of Torrance, the La Brea tar pits in the city of Los Angeles, and a cemetery in the city of Calabasas. All traps were placed in or near residential areas where peridomestic birds could be found. Although an occasional mourning dove (*Zenaida macroura*) was tested at the onset of the program, the study concentrated on house finches (*Carpodacus mexicanus*) and english sparrows (*Passer domesticus*). Birds were captured, tagged, bled and released. Disposable, 26 gauge syringes were used to extract approximately 0.1 cc of blood from the birds' jugular vein. The blood samples were poured into test tubes holding 0.9 cc bovine solution and analyzed with the hemagglutination inhibition (HI) test for antibodies to SLE and WEE viruses.

Mosquito Testing Program.

Female mosquitoes were collected using carbon dioxide (CO_2)-baited CDC style traps and modified gravid female mosquito traps, generally, 3-4 nights per week throughout the season. Trapping was performed at random locations, next to wild bird traps and sentinel chicken flocks to hopefully correlate virus amplification in wild birds or virus transmission in sentinel chickens and in areas of high mosquito activity, especially at foci of *Culex tarsalis* mosquitoes. Three locations with high mosquito activity were continually monitored:

1) The La Brea Tar Pits (naturally occurring oil and tar sumps in which rain water and landscaping water accumulates and where vegetation has, at times, reduced the efficacy of mosquito suppression efforts, produces large numbers of *Cx. tarsalis* while the surrounding residential areas produce other mosquito species).
2) The City of Torrance (prior to annexation into the vector control district in 1991, this city produced large numbers of *Cx. quinquefasciatus* from flood control channels and flood control sumps and *Cx. tarsalis* from an oil refinery, oil extraction sumps and a small preserve).
3) The western part of the City of Malibu (west Malibu was affected by *Cx. tarsalis* mosquitoes immigrating from duck clubs and marshy areas 18 miles west of the collection sites (Saviskas 1992)).

Female mosquitoes were anesthetized with triethylamine, sorted to species and pooled into freezer vials. The vials were placed on dry ice and shipped to the California Department of Heath Services, Viral and Rickettsial Disease Laboratory (VRDL) for testing.

Sentinel Chicken Program.

In 1991, two flocks of 15 sentinel chickens each were used. By 1994, the number of flocks had increased to six and the number of chickens per flock had decreased to ten, except for two flocks (Palos Verdes and Culver City) which held five chickens each. The flock locations were: The Palos Verdes Botanical Gardens, the Mobil Oil refinery in the city of Torrance, the headquarters of Los Angeles County West Vector Control District in the city of Culver City, the La Brea tar pits in the city of Los Angeles, the Las Virgenes Water District headquarters in Calabasas, and Charmlee Park in Malibu.

Chickens were bled monthly in 1991 and semi-monthly from April 20, 1992 through December 1994. The blood sera were tested by the VRDL by IgG capture ELISA. The State of California, Environmental Management Branch's "1991 Revised Protocol for Sentinel Chicken Serum Submission" was followed in the preparation of the sera until April 1994 when the more practical "filter paper technique" protocol was implemented (Reisen 1993).

RESULTS

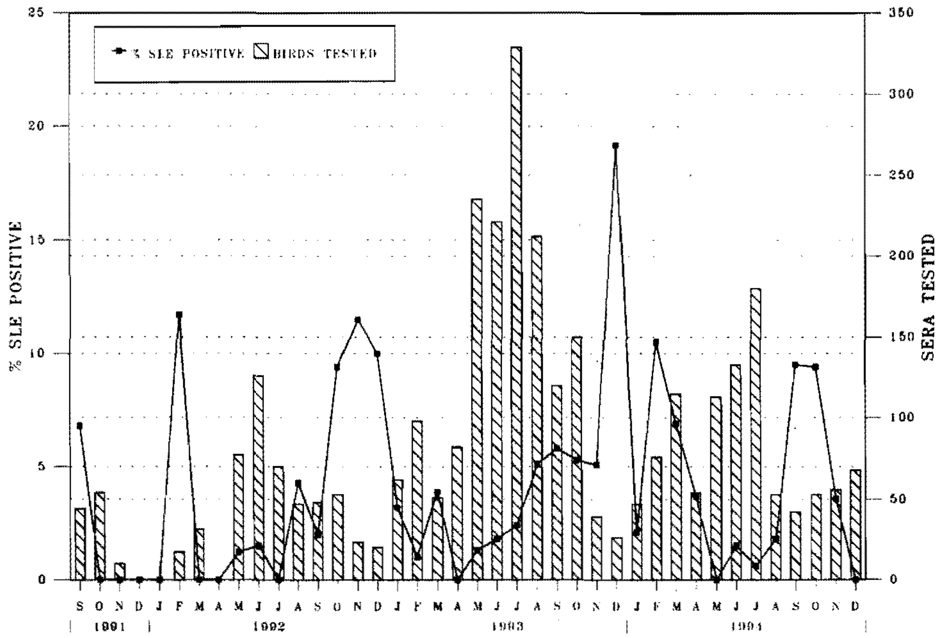
Peridomestic Bird Testing Program.

During the four years, 3,235 bird sera were tested. Overall, no sera tested positive for WEE and 110 sera (3.3%) tested positive for SLE. The actual positive percentages by species were: 3.3% (92 of 2,803) house finches, 3.2% (14 of 435) english sparrows and 4.3% (4 of 92) mourning doves. Thirty of the 37 surveillance months showed some level of infection ranging from 0.6-19.2% (Fig. 1). For the years 1992-1994, infection levels for the months of June and July ranged from 0-2.5% with a mean of 1.3% and infection levels for August to November ranged from 1.8-11.5% with a mean of 6.1%.

It should be noted that serology experts suggested that, because of various protein inhibitors present in avian sera, HI test protocols developed for human sera may need to be modified when used on avian sera. Comparative HI testing with kaolin sera extraction, as used with human sera, versus protamine sulfate precipitation-sera extraction will be investigated.

Mosquito Testing Program.

A total of 74,301 mosquitoes was collected, sorted and submitted in 1,679 pools to the VRDL for arbovirus testing (Fig. 2). The breakdown by species was: 43,436 (973 pools) *Cx. quinquefasciatus*, 29,478 (651 pools)



	S 1991	O	N	D
% SLE POSITIVE	6.8	0	0	0
BIRDS TESTED	44	34	10	0

	J 1992	F	M	A	M	J	J	A	S	O	N	D
% SLE POSITIVE	0	11.7	0	0	1.2	1.5	0	4.3	2	9.4	11.5	10
BIRDS TESTED	0	17	31	0	77	126	70	47	48	53	23	20

	J 1993	F	M	A	M	J	J	A	S	O	N	D
% SLE POSITIVE	3.2	1	3.9	0	1.3	1.8	2.4	5.1	5.8	5.3	5.1	19.2
BIRDS TESTED	62	98	51	82	233	221	329	212	120	150	39	26

	J 1994	F	M	A	M	J	J	A	S	O	N	D
% SLE POSITIVE	2.1	10.5	6.9	3.7	0	1.5	0.6	1.8	9.5	9.4	3.6	0
BIRDS TESTED	47	76	115	54	113	133	180	53	42	53	56	68

Figure 1. Peridomestic bird sera tested for SLE and WEE arboviral antibodies during 1991-1994, suggesting virus amplification during late summer, fall and winter months.

Cx. tarsalis, 1,198 (50 pools) *Cx. stigmatosoma*, and 189 (5 pools) *Ae. squamiger*. Most *Cx. quinquefasciatus* were collected with oviposition traps and were gravid (Cummings 1992). Seven pools, three *Cx. quinquefasciatus* pools collected on September 24, 1991 in the city of Torrance and four *Cx. tarsalis* pools collected in the western part of Malibu on September 25, 1991 tested positive for WEE. These mosquitoes were collected in or near uncontrolled areas which were subjected to large populations of *Cx. quinquefasciatus* and *Cx. tarsalis*. No mosquito pool tested positive for SLE virus.

Sentinel Chicken Program.

Virus transmission to sentinel chickens was observed twice between 1991 and 1994. Seroconversion for SLE antibodies was detected in three of eight chickens (37.5%) of the La Brea tar pits flock in September and October of 1992. The bleeding dates corresponding to the seroconversions were September 22, 1992 (1 hen) and October 5, 1992 (2 hens). However, 2,044 mosquitoes (49 pools) collected at the La Brea tar pits around that time tested negative for arboviruses. Also, no peridomestic bird was captured in the tar pits bird trap in early to mid- September 1992. Other Los Angeles County vector control agencies also confirmed seroconversions in their sentinel flocks near the same period, but some of their seroconversions were observed as early as July 15, 1992 (Brisco et al. 1993).

The second chicken seroconversion was for WEE antibodies and was observed in one chicken from the Charmlee park flock in November of 1993. This is the same location where four WEE-positive *Cx. tarsalis* pools were collected in 1991. However, 2,676 *Cx. tarsalis* (54 pools) collected near that site in September, October and November of 1993 tested negative for arboviruses and peridomestic birds were not captured at that location.

DISCUSSION

Data collected by the LACWVCD mosquito-borne encephalitis surveillance program indicates that there is continuous SLE activity in the western part of the Los Angeles metropolitan area. Some level of avian infection was detected during 30 of the 37 surveillance months, supporting the theory that SLE is a continuous epizootic condition in the avian population of urban Southern California (Gruwell et al. 1988).

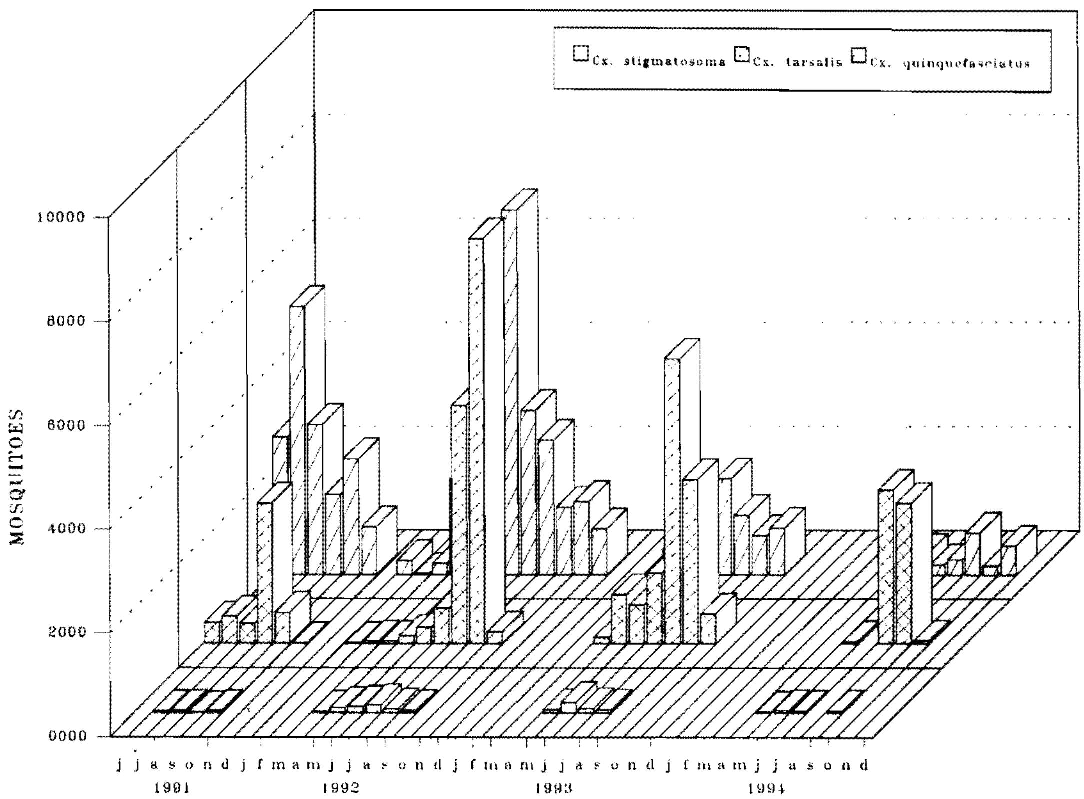


Figure 2. Adult mosquitoes tested for SLE and WEE arboviral infections during 1991-1994.

We observed that the number of peridomestic birds seroconverting for SLE antibodies increased in late summer, fall and winter months with seroconversions for SLE antibodies in the sentinel chickens of the La Brea tar pits detected during the months of September and October in 1992, the seven WEE-positive mosquito pools were collected in September of 1991 and the single WEE-positive sentinel chicken seroconverted in November of 1993. So, two statements could be suggested. First, it seems there is a temporal correlation between SLE virus amplification in the peridomestic birds and SLE virus transmission, hence the suggestion that a peridomestic bird testing program could be used as a warning system in an arbovirus surveillance program, and secondly, we can presume a somewhat autumnal period for both SLE and WEE virus transmission in the western part of the Los Angeles metropolitan area.

However, because we could not coincide observed virus amplification in the peridomestic birds of a specific locale with seroconversions in the sentinel chickens or with virus infected mosquitoes from that same locale during the same time period, a simultaneous spatial and temporal correlation can not be made.

ACKNOWLEDGMENTS

The authors express their gratitude to J.P. Webb and C. Fogarty for their support and expertise. Special thanks are also extended to D. Muth, W.K. Reisen and B. Chiles for offering their thoughts and expertise on arboviruses and serological methodologies.

REFERENCES CITED

Brisco, A., N. Gergis, G. VanGordon, F. Hall and K. Fujioka. 1993. Arthropod-borne encephalitis virus surveillance in the San Gabriel Valley, Los Angeles County during 1992. Proc. Calif. Mosq. Vector Control Assoc. 61:7-10.

Cummings, R. 1992. The design and use of a modified Reiter gravid female trap for mosquito-borne encephalitis surveillance in Los Angeles County, California. Proc. Calif. Mosq. Vector Control Assoc. 60:170-176.

Gruwell, J.A., B.L. Brown and J.P. Webb, Jr. 1988. Passeriform birds as a surveillance method for arbovirus activity in rural and suburban sites in Orange County, California, 1987. Proc. Calif. Mosq. Vector Control Assoc. 56:58-68.

Reisen, W.K. 1993. Evaluation of new methods for sampling sentinel chickens for antibodies to WEE and SLE viruses. Proc. Calif. Mosq. Vector Control Assoc. 61:33-36.

Saviskas, R. 1992. The relationship between the isolation of western equine encephalomyelitis virus in Los Angeles County and the long distance migration of *Culex tarsalis*. Proc. Calif. Mosq. Vector Control Assoc. 60:57-59.

MOSQUITO ABUNDANCE AND ARBOVIRAL ACTIVITY IN SAN BERNARDINO COUNTY DURING 1994

Lal S. Mian, Christian N. Nwadike, Robert G. Prochaska
and Minoo B. Madon[1]

San Bernardino County Vector Control Program
Department of Environmental Health Services
2355 East Fifth Street
San Bernardino, California 92410-5201

ABSTRACT

Of the 9,101 mosquitoes collected in New Jersey light traps and CO_2-baited CDC traps in San Bernardino County during 1994, 80.2% were from the desert region (Needles) and 19.2% from the valley region (including <0.1% from the mountains). In the desert region, the dominant species were *Culex tarsalis* (56.9%), *Aedes vexans* (34.4%) and *Culiseta inornata* (6.8%). In the valley region, *Culiseta incidens* (24.7%), *Culex stigmatosoma* (24.5%), *Cx. tarsalis* (19.7%), *Anopheles franciscanus* (12.7%), *Culex quinquefasciatus* (11.8%) and *Cs. inornata* (5.6%) were found in significant numbers. All 76 pools of culicine mosquitoes submitted from the desert and valley regions tested negative for both Saint Louis encephalitis and western equine encephalomyelitis viruses.

The Needles sentinel chicken flock showed a first SLE seroconversion in mid-August followed by three more SLE seroconversions in late August and mid-September. The single human SLE case was in an out-of-county resident who possibly contracted the disease in the Needles area during August.

As part of the state-wide encephalitis virus surveillance (EVS) program in California, the San Bernardino County Vector Control Program (SBCVCP) has carried out EVS and other mosquito control activities in both the valley and desert areas of San Bernardino County for several years. Geographically, the county consists of three distinct regions; the desert, mountain, and valley regions. Demographically, the valley region houses over 80% of the nearly 1.5 million county population with the remaining scattered over various parts of the desert and mountain regions. Historically, cases of both Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) have been reported in the desert and valley regions from time to time.

After experiencing 26 human cases of SLE in southern California during 1984, the only human case of encephalitis (SLE) in California during 1987 was reported from San Bernardino (Emmons et al. 1988). Of the two cases reported state-wide in 1988, one was from the same San Bernardino site (Emmons et al. 1989). Recently, two of the three SLE cases reported state-wide in 1993 were found to have been contracted in San Bernardino County (Emmons et al. 1994). During the same period, both SLE and WEE virus activities were reported in the desert region, especially Needles, and adjourning areas along the Colorado River. Due to the periodic incidence of encephalitis disease, mosquito control and EVS activities have been routinely carried out in the desert and valley regions of this county. Data generated in routine EVS activities are appraised here in relation to mosquito abundance and arboviral activity in San Bernardino County during 1994.

MATERIALS AND METHODS

EVS procedures described by Mian and Prochaska (1990) were continued in these studies as follows:

[1] California Department of Health Services, Vector-Borne Disease Section, 2151 East D Street, Suite 218B, Ontario, California 91764.

Adult Mosquito Population Dynamics.

The abundance of various mosquito species was monitored on a weekly basis through a number of New Jersey light traps. In the valley and mountain regions, the traps were stationed at seven different locations; Yucaipa, Highland, San Bernardino, Colton, Fontana, Ontario, Upland, and Jenkin Lake in the mountains. Within the valley region there were two trap sites each in urban, suburban and rural environments. In the desert region (Needles area), one trap each was operated in urban, suburban and rural areas along the Colorado River.

Adult mosquitoes collected weekly in all traps were counted, sexed, and identified to species with the Adult Mosquito Occurrence Reports submitted to the California Department of Health Services.

Arboviral Activity in Female Mosquitoes.

Arboviral activity in local mosquito populations was monitored in all three regions, using dry ice (CO_2)-baited CDC traps to collect host-seeking adult female mosquitoes. Eight or more such traps were operated twice a month in the valley area, once a month in the Colorado River (desert) region and two times during the season (May through October) in the mountain area. Female mosquitoes collected overnight were anesthetized using triethylamine (TEA), counted, identified to species and sex; they were pooled by species with 10-50 adults per labeled vial. All pools (vials) were stored in dry ice in the field or in a deep freezer at -60°F in the laboratory before being shipped in dry ice-packed containers by overnight express mail to the Viral and Rickettsial Disease Laboratory (VRDL) in Berkeley.

Arboviral Activity in Sentinel Chickens.

Both wild and domestic birds are known to play a significant role in the epidemiology of mosquito-borne encephalitides by acting as reservoir hosts for the encephalitis virus(es). Therefore, one sentinel flock consisting of ten white leghorn chickens was maintained in both the valley area in Colton and the desert area in Needles. The valley flock was stationed near a horse ranch at the northeastern corner of Meridian Avenue and Olive Street in the city of Colton. This site is located in the general area where the SLE human cases occurred in 1987 and 1988. The desert flock was maintained at the sewage treatment facility in the city of Needles. New Jersey light traps were regularly operated at both flock sites. Using the comb prick method, blood samples were taken from all sentinel chickens onto prelabeled filter paper strips on a bi-weekly basis during April through October 1993. These samples were then mailed to the State's VRDL for detection of arboviral activity.

RESULTS AND DISCUSSION

Of the 9,101 mosquitoes collected at all sites during the season, 80.2% were trapped in the desert area with only 19.8% being from the valley sites, including <0.1% from the mountain area (Table 1). The most abundant mosquito in the desert region was *Culex tarsalis* Coquillett (56.9%), followed by *Aedes vexans* Meigen (34.4%), *Culiseta inornata* Williston (6.8%), *Anopheles franciscanus* McCracken (0.8%), *Culex erythrothorax* Dyar (0.6%) and *Culex quinquefasciatus* Say (0.2%). *Culex tarsalis* was present during the summer and fall months, whereas *Ae. vexans* populations peaked during

Table 1. Percent species composition of female mosquitoes collected in New Jersey light traps and dry ice (CO_2)-baited CDC traps (and number of pools submitted) in San Bernardino County during 1994.

Species	DESERT REGION		VALLEY REGION	
	N.J. traps	CO_2 traps	N.J. traps	CO_2 traps
Aedes vexans	13.2	47.8	0.0	0.0
Anopheles franciscanus	0.4	1.0	19.7	0.0
Culex erythrothorax	1.1	0.3	1.3	0.5
Culex quinquefasciatus	1.2	<0.1	9.0	16.9 (7)
Culex stigmatosoma	0.0	0.0	31.0	12.8 (4)
Culex tarsalis	66.4	50.8 (52)	10.1	37.0 (13)
Culiseta incidens	0.0	0.0	20.2	32.8
Culiseta inornata	17.5	<0.1	8.7	0.0
Psorophora signipennis	0.2	0.0	0.0	0.0
Total Mosquitoes	2,839	4,458 (52)	1,190	614 (24)
Region Totals (%)	7,297 (80.2)		1,804 (19.8)	

September and October due to flood irrigation practices on the Arizona side of the Colorado River.

The most abundant mosquito species in the valley region was *Culiseta incidens* (Thompson) (24.7%) followed by *Culex stigmatosoma* Dyar (24.5%), *Cx. tarsalis* (19.7%), *An. franciscanus* (12.7%), *Cx. quinquefasciatus* (11.8%), *Cs. inornata* (5.6%), and *Cx. erythrothorax* (1.0%). Earlier studies in this area, indicated *Cx. tarsalis* as the most abundant species, comprising as much as 72%, 62%, 86%, 55%, 70%, 42% and 37% of the mosquitoes collected in 1986 and 1987 (Reisen et al. 1988), 1989, 1990, 1991, 1992 and 1993 (Mian and Prochaska 1990, 1991, 1992 and 1993) and 1994 (Mian et al. 1994), respectively.

A total of 76 pools of culicine mosquitoes (*Cx. tarsalis*, *Cx. quinquefasciatus* and *Cx. stigmatosoma*) were sent to the VRDL for virus study. All tested negative for both SLE and WEE viruses.

At the August 16 sentinel chicken serum sampling, the Needles flock showed a first seroconversion to SLE followed by 3 additional seroconversions during late August and mid-September. Upon confirmation of the seroconversions, the area was posted with "Encephalitis Warning" signs followed by press releases advising residents to take necessary precautions during outdoor activities especially at dusk and dawn in the affected area.

In the wake of this virus activity, mosquito source reduction activities were enhanced in the Needles area. Moreover, joint larvicidal activities were carried out by the SBCVCP and the Mohave County Health Department for the first time at several sites in the Havasu National Wildlife Refuge (U.S. Fish and Wildlife Service) in Mohave County, Arizona. The microbial larvicide containing *Bacillus thuringiensis* var. *israelensis* was effective in controlling both *Cx. tarsalis* and *Ae. vexans* populations.

During the 1994 mosquito season, there was only one case of SLE, which was reported late, after conclusion of the mosquito season. According to preliminary epidemiological investigations carried out by the senior author, the SLE case was a 35-year old male resident of Moreno Valley, Riverside County who along with his wife, another couple and five children had been to the Colorado River, 10 miles south of Needles during August 19-21, 1994. He became ill on September 2 with high fever, which developed into stiff neck symptoms on September 9. He was hospitalized September 12-22, eventually recovered completely from the illness. The probable area where he may have contracted the disease (Colorado River), had shown high SLE virus activity during that time.

ACKNOWLEDGMENTS

The authors thank Kirt Emery and Jose Tacal D.V.M., both of the San Bernardino County Department of Public Health for their collaboration. The authors also acknowledge with thanks the SBCVCP technical and professional staff for their field assistance, especially Pam Felts for typing the manuscript.

REFERENCES CITED

Emmons, R.W., M.S. Ascher, D.V. Dondero, B. Enge, W.K. Reisen, M.M. Milby, D.A. Eliason, R.A. Murray, J. Lin, F. Ennik, L. Hui, J.L. Hardy, S.B. Presser, W.C. Reeves and K. Reilly. 1994. Surveillance for arthropod-borne viral activity and disease in California during 1993. Proc. Calif. Mosq. Vector Control Assoc. 62:13-17.

Emmons, R.W., D.V. Dondero, C.S. Chan, M.M. Milby, J.W. Walsh, W.C. Reeves. L.T. Hui, F. Ennik, J. Griffin, E.V. Bayer and R.A. Murray. 1988. Surveillance for arthropod-borne viral activity and disease in California during 1987. Proc. Calif. Mosq. Vector Control Assoc. 56:1-10.

Emmons, R.W., D.V. Dondero, C.S. Chan, M.M. Milby, L.T. Hui, E.V. Bayer, F. Ennik, L. Pitstick, J.L. Hardy, S. Presser, W.C. Reeves, R.A. Murray and J.W. Walsh. 1989. Surveillance for arthropod-borne viral activity and disease in California during 1988. Proc. Calif. Mosq. Vector Control Assoc. 57:6-12.

Mian, L.S., C.N. Nwadike, R.G. Prochaska and M.B. Madon. 1994. Mosquito abundance and arboviral activity in San Bernardino County during 1993. Proc. Calif. Mosq. Vector Control Assoc. 62:28-31.

Mian, L.S. and R.G. Prochaska. 1990. Mosquito abundance and arboviral activity in San Bernardino County during 1989. Proc. Calif. Mosq. Vector Control Assoc. 58:35-42.

Mian, L.S. and R.G. Prochaska. 1991. Mosquito abundance and arboviral activity in San Bernardino County during 1990. Proc. Calif. Mosq. Vector Control Assoc. 59:30-34.

Mian. L.S. and R.G. Prochaska. 1992. Mosquito abundance and arboviral activity in San Bernardino County during 1991. Proc. Calif. Mosq. Vector Control Assoc. 60:52-56.

Mian. L.S. and R.G. Prochaska. 1993. Mosquito abundance and arboviral activity in San Bernardino County during 1992. Proc. Calif. Mosq. Vector Control Assoc. 61:24-28.

Pfuntner, A.R. 1988. Mosquito abundance and virus activity in the Chino area, San Bernardino County, California, 1987. Proc. Calif. Mosq. Vector Control Assoc. 56:26-31.

Reisen, W.K., R.P. Myer, M.M. Milby, S.L. Durso, W.C. Heath, S.B. Presser, J.K. Hardy and R.W. Emmons. 1988. Mosquito abundance and arbovirus activity along the Colorado River during 1986-87. Proc. Calif. Mosq. Vector Control Assoc. 56:13-19.

1995 Proc. Calif. Mosq. Vector Control Assoc. **63:32-34**

PREVALENCE OF ANTIBODIES AGAINST ARBOVIRUSES IN RESIDENTS OF THE COACHELLA VALLEY[1]

William K. Reisen[2], James L. Hardy, Robert E. Chiles,
Hugh D. Lothrop and Sally B. Presser

Arbovirus Research Program
School of Public Health
University of California
Berkeley, California 94720

ABSTRACT

A total of 630 sera were collected anonymously from outpatients attending clinics in the southern Coachella Valley and tested for IgM and IgG antibodies to western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE) and California encephalitis (CE) viruses. Overall 3, 16 and <1% were positive by an IgG-enzyme immunoassay (EIA), respectively. None were positive for IgM antibody, indicating no recent infections. Only 28 and 42% of sera positive by IgG-EIA for WEE and SLE viruses were confirmed by more specific neutralization assays. Based on IgG-EIA results, SLE virus appeared to be endemic in the Coachella Valley.

Research in the Coachella Valley of California has documented widespread enzootic transmission of both western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses to sentinel chickens located south of Indio (Reisen et al. 1992, 1995a,b). Most sentinels were positioned near human habitation, including the towns of Coachella, Thermal and Mecca. However, no human or equine cases of arboviral encephalitis have been reported from the Coachella Valley by the statewide Encephalitis Virus Surveillance (EVS) program since 1984 when single cases occurred in Indio and Palm Springs (Murray et al. 1985).

The absence of identified cases in this population may be explained by: 1) humans not being bitten by infectious mosquitoes, 2) humans are bitten, but develop inapparent infections (e.g., local virus strains may not produce central nervous system disease), or 3) cases occur, but physicians fail to request arboviral-specific diagnostic laboratory tests or report cases to the EVS program. Our research explores the first of these explanations by testing sera collected from residents of the southern Coachella Valley for arboviral antibodies.

MATERIALS AND METHODS

Anonymous blood samples were solicited from outpatients attending the El Progresso del Desierto Family Health Center (EPFHC) in Coachella and Riverside County's Indio Health Center (IHC) in Indio. Patients that were having blood drawn for other purposes were requested by the phlebotomist to give an additional blood sample for our study. Data recorded included date, age, sex, ethnicity, years residence in the Coachella Valley, zip code and occupation location (i.e., indoor, outdoor or agricultural; Indio Health Center only). Sera were frozen at -70°C and shipped to Berkeley where they were screened for both IgG and IgM antibodies to WEE, SLE and California-group viruses using enzyme immunoassays (EIA). Positive samples were retested by EIA for IgM alone (an indication of recent infection) and by a plaque reduction neutralization test (PRNT) in Vero cell culture (confirmation of virus identification). Sera that reduced plague counts by ≥80% at a dilution of ≥1:20 were considered to be positive.

[1]Interim summary of research to be submitted for publication in the American Journal of Tropical Medicine and Hygiene.

[2]Address correspondence to: Dr. William K. Reisen, Arbovirus Field Station, 4705 Allen Road, Bakersfield, California 93312.

RESULTS AND DISCUSSION

From March 1993 through September 1994, 630 patients gave a blood sample. Most samples were from middle age, Hispanic females that resided in the southern valley (Table 1).

No sample was positive for IgM antibody, indicating none of the infections were recent. This was expected, because concurrent surveillance indicated that WEE and SLE viruses were transmitted focally and at low levels during the March 1993 through September 1994 sampling period. Overall, 6 (1.7%) and 12 (4.4%) of patients attending the El Progresso Family Health Center and Indio Health Clinic, respectively, had IgG antibody against WEE virus when screened by the EIA (Table 1). In contrast, 72 (20.2%) and 29 (10.6%) had IgG antibodies against SLE virus, respectively. Of the sera positive by the EIA, 5 (28%) of the WEE virus-positives and 41 (42%)of the SLE virus-positives were confirmed by PRNTs. In addition, a single sample had antibodies to California encephalitis group virus.

Table 1. Demographic characteristics and seroprevalence rates for WEE, SLE and CE viruses of outpatient participants from El Progresso Family Health Center (EPFHC) an d Indio Health Center (IHC) during March 1993 - September 1994.

Parameter	EPFHC	IHC
Number tested:	356	274
Population characteristics:		
Age (mean years)	38	31
Sex (% female)	71	69
Ethnicity (% Hispanic)	91	79
Residency (mean years)	17	11
Reside south of Indio (%)	80	80
Seroprevalence (% IgG EIA):		
WEE	1.7	4.4
SLE	20.2	10.6
CE	0.3	0.0

The higher seroprevalence rate observed for SLE virus than WEE virus was difficult to resolve, because in the Coachella Valley both viruses are transmitted by *Culex tarsalis* Coquillett (Reisen et al. 1992). SLE virus was widespread during five of the eight previous years (i.e., transmitted to sentinel chickens at >50% of monitoring sites), whereas WEE virus was widespread during only three years (Reisen et al. 1992, 1995a). These data indicate that the risk of infection may be relatively greater for SLE virus than for WEE virus.

The higher SLE virus IgG-EIA seroprevalence among patients attending the EPFHC than the IHC was attributed to their older age and longer residence history (Table 1). These data were pooled for subsequent analyses, because both groups were predominantly Hispanic women that resided in the southern Coachella Valley. Characteristic of an endemic arbovirus, SLE virus IgG-EIA seroprevalence increased as a function of age, ranging from 8.3% among 0-10 year olds to 34.1% among 61-70 year olds. SLE virus endemicity also was indicated by the increase in seroprevalence from 4.7 and 2.9% among 0-1 and 1-2 year residents to >20% among 2-6 year residents. These data indicated that most immigrants were being infected with SLE virus after moving into the Coachella Valley.

The present interpretation must be viewed with caution, because only 28 and 42% of sera positive by WEE virus and SLE virus IgG-EIA were confirmed by PRNT. The antigens used in the EIAs were broadly reactive and may have cross reacted with previous infections with Venezuelan equine encephalitis virus or dengue virus, respectively, both of which do not occur in the United States. However, this did not seem likely because 1) the seroprevalence rate among new immigrants was lower than residents living >2 years in the Coachella Valley, and 2) the percentage of EIA positives confirmed by PRNT did not decrease as a function of residence history and antibody decay. Most likely the IgG-EIA was more sensitive than our conservative PRNT. Most of the 41 PRNT positives seemed to be old infections, because only 11 (27%) had titers ≥1:40.

The total population in the study area was approximately 82,400, or about 31% of the entire Coachella Valley population. If the overall seroprevalence rates of 3 and 16% for WEE and SLE viruses were representative of this entire population, then 2,472 and 13,184 Coachella Valley residents may have been infected sometime during their life with WEE and SLE viruses, respectively. Considering that the apparent to inapparent infection rates for these viruses are usually in the order of 1:1,000 in persons >14 yrs of age (Reeves and Hammon 1962), it is not surprising that clinical CNS illness has been recognized infrequently in the Coachella Valley.

ACKNOWLEDGMENTS

We especially thank Dr. R.J. Holmes, Medical Director, and the staff of the El Progresso Family Health Center, and Ms. S.L. Crawford, Clinic Site Manager, and the staff of the Indio Health Center, for their support and technical assistance. This research was funded, in part, by a grant from the Coachella Valley Mosquito Abatement District and by special funds for mosquito research allocated annually through the Division of Agriculture and Natural Resources, University of California. Dr. W.C. Reeves critically read the manuscript.

REFERENCES CITED

Murray, R.A., L.A. Habel, K.J. Mackey, H.G. Wallace, B.A. Peck, S.J. Mora, M.M. Ginsberg and R.W. Emmons. 1985. Epidemiological aspects of the 1984 St. Louis encephalitis epidemic in southern California. Proc. Calif. Mosq. Vector Control Assoc. 53:5-9.

Reeves, W.C. and W. McD. Hammon. 1962. Epidemiology of the arthropod-borne viral encephalitides in Kern County, California 1943-1952. Univ. Calif. Press, Berkeley and Los Angeles.

Reisen, W.K., J.L. Hardy, S.B. Presser, M.M. Milby, R.P. Meyer, S.L. Durso, M.J. Wargo and E.W. Gordon. 1992. Mosquito and arbovirus ecology in southeastern California, 1986 - 1990. J. Med. Entomol. 29:512-524.

Reisen, W.K., H.D. Lothrop, S.B. Presser, M.M. Milby, J.L. Hardy, M.J. Wargo and R.W. Emmons. 1995a. Landscape ecology of arboviruses in southern California: Temporal and spatial patterns of vector and virus activity in Coachella Valley, 1990-1992. J. Med. Entomol. 32:267-275.

Reisen, W.K., J.L. Hardy and H.D. Lothrop. 1995b. Landscape ecology of arboviruses in southern California: Patterns in the epizootic dissemination of western equine encephalomyelitis and St. Louis encephalitis viruses in Coachella Valley, 1991-1992. J. Med. Entomol. 32:255-266.

PLAGUE ACTIVITY IN SAN BERNARDINO COUNTY DURING 1994

Lal S. Mian, Christian N. Nwadike, James C. Hitchcock[1]
and Minoo B. Madon[1]

San Bernardino County Vector Control Program
Department of Environmental Health Services
2355 East Fifth Street
San Bernardino, California 92410-5201

ABSTRACT

In 1994, the San Bernardino County Vector Control Program carried out 23 plague surveys at various sites in San Bernardino County. Of the 314 live trap collected rodents, 98.4% were ground squirrels, including 305 *Spermophilus beecheyi* and 4 *Spermophilus lateralis*. The remaining rodents consisted of 1 *Tamias merriami*, 2 *Dipodomys agilis*, 1 *Dipodomys merriami* and 1 *Neotoma fuscipes*. None of the 1219 fleas collected from these animals tested positive for plague. Prior to reopening the Hanna Flats Campground from last year's epizootic, both pre- and post-treatment surveys were carried out while employing control measures to lower flea and rodent populations to acceptable levels. During 1994, routine surveys detected a new epizootic at the Green Valley Lake Campground. Effective ectoparasite control was carried out with the campground closed to public use prior to reopening the site to public use.

Plague is an enzootic rodent disease communicable to humans. The disease, caused by a bacterium, *Yersinia pestis*, is transmitted to humans and other animals through the bite of fleas. Humans are exposed to the disease if they enter plague infected areas or if the disease is transmitted from feral rodents to commensal rats or cats that cohabit human environments. The occurrence of rural plague cases has been attributed to the extension of human habitations into previously wilderness areas.

Historically, plague is reported to have originated in Central Asia from where it spread to almost all continents of the world. This spread is evidenced from the first pandemic of 542 AD, which involved Arabia, Europe and North Africa. The second pandemic, the "Black Death", of the Middle Ages (1300s) covered parts of both Asia and Europe. The third and last pandemic originated in Asia (Southwest China) and spread to South Africa and South America by 1899, then to North America (San Francisco) and Australia (Brisbane and Sidney) by 1900 (Twiggy 1978, Kettle 1992).

Following the introduction of plague in North American, there have been four major urban epidemics in California: 1900-1903 and 1907-1909 in San Francisco, 1919 in Oakland and 1924 in Los Angeles. Since that time, sporadic human cases in endemic areas have been traced to wild rodents and their ectoparasitic fleas. Plague infection in wild rodents is widely distributed in California including the coastal counties south of San Francisco Bay; inter-mountain valleys of northern California; the Sierra Nevadas from Lassen Peak to the Kern Plateau; and the Tehachapi, San Gabriel, San Bernardino and San Jacinto Mountains of Southern California (Salmon and Gorenzel 1981, Anonymous 1983).

Known foci of plague epizootics are distributed throughout the mountains and foothill areas of San Bernardino County. The mountain ranges along with natural recreational lakes provide a wide variety of

[1] California Department of Health Services, Vector-Borne Disease Section, 2151 East D Street, Suite 218B, Ontario, California 91764.

camping, hiking and water sport facilities to both local and out-of-county visitors. To safeguard public health and safety in these areas, the San Bernardino County Vector Control Program (SBCVCP) in collaboration with the California Department of Health Services - Vector-Borne Disease Section (CDHS-VBDS) and the United States Department of Agriculture - Forest Service (USDA-FS), carries out routine surveillance at plague enzootic areas during the season (April through October). The data generated in routine plague surveillance during 1994 are presented here in this paper.

MATERIALS AND METHODS

In routine plague surveys, the general method described by Lang and Wills (1991) was used as follows:

In a typical daily survey, 20-25 Tomahawk #103 live traps (Tomahawk Live Trap Co., Tomahawk, WI) baited with peanut butter and rolled oats were set at appropriate shaded locations close to rodent burrows in the survey area. In a campground situation, traps were also set near picnic tables that attract wild rodents, especially ground squirrels and chipmunks. For location purposes, each trap site was flagged with orange nylon ribbon hung from an adjacent bush or tree. The traps were usually set by 10:00 a.m. and picked up in the early afternoon the same day. For smaller rodents, such as wood rats, Sherman live traps were used overnight around rodent nesting sites.

All traps with live animals were brought to a shady site for processing. Each trap with an animal inside was transferred into an 18" x 36" clear polyethylene bag (3 mil.). A ball of cotton drenched in ethyl ether was introduced into the bag and the bag was kept tightly closed with a rubber band until the animal was anesthetized (usually in 5-15 min). The animal was taken out of the bag and cage and transferred to a white enamel pan (12" x 7.5" x 2" deep) where the fleas were combed out using a stiff bristled brush. The fleas from each animal were collected in labeled 2 ml polypropylene screw cap tubes containing a 2% saline solution. Next, through cardiac puncture, a 3 ml blood sample was drawn from each animal using a 23 gauge needle. Before the animal regained consciousness and was released back into its habitat, all pertinent data such as species, sex and reproductive stage were recorded. All necessary survey site information was also recorded before leaving the area.

All collected blood samples were brought to the laboratory where they were centrifuged for 20 minutes at 2000 rpm before the serum from each sample was transferred to labeled 2 ml polypropylene screw cap tubes. All sera and flea samples, along with completed paperwork, were sent on blue ice by overnight mail to the California Department of Health Services-Vector-Borne Disease Section (CDHS-VBDS) in Sacramento for laboratory analysis.

The laboratory at CDHS-VBDS in Sacramento would immediately inform us via telephone of any plague-positive samples. In the event of plague-positive sample confirmation, the standard plague epizootic protocol would be followed. The protocol includes posting the area with "Plague Warning" signs, public education, press releases (if warranted), immediate evacuation (if a campground), pre-treatment flea index evaluation depending on the date of original survey, burrow dusting for fleas followed by rodent control, if necessary, and finally post-treatment evaluation flea index prior to re-opening the area for public use, especially a campground or public park.

RESULTS AND DISCUSSION

During 1994, the SBCVCP carried out routine plague surveys at 23 locations situated in plague enzootic mountain and foothill areas of San Bernardino County (Table 1). Of the 314 live trap collected rodents, 309 (98.4%) were ground squirrels, namely 305 (113♂, 192♀) *Spermophilus beecheyi* and 4 (1♂, 3♀) *Spermophilus lateralis*. The remaining rodents were 1 (♀) chipmunk, *Tamias merriami*, 3 kangaroo rats, 2 (♂) *Dipodomys agilis* and 1 (♀) *Dipodomys merriami*, and 1 (♀) wood rat, *Neotoma fuscipes*.

All 1219 fleas combed from the animals tested negative for the plague bacterium. Of the 1219 fleas collected, 64.4% were *Oropsylla (Dimanus) montanus* (245♂ and 564♀) and 1.4% *Hoplopsyllus anomalus* (7♂ and 10♀) with the remaining 32.2% unidentified.

Blood samples collected from all rodents during the season were found negative except for 10 samples collected May 4 at Hanna Flats Campground and 4 samples collected May 10 and 1 sample collected July 12 at Green Valley Lake Campground which were diagnosed positive, showing titers ranging from 1:16 to 1:2048 for plague antibodies. Generally, an antibody titer of 1:16 or higher in the serum is considered plague positive. Moreover, the flea index in the May 4, May 10 and July 12 survey was 3.8, 0.3 and 7.1 fleas/animal, respectively. A flea index of ≥ 1 is considered high enough to require flea control during plague epizootics.

The survey of May 4, 1994 was a follow up on the 1993 epizootic reported at the Hanna Flat campground (Mian et al. 1994). Of the 18 ground squirrel sera collected during this survey, 10 tested positive for plague

Table 1. Summary of plague surveys carried out in San Bernardino County during 1994.

Survey Date	Site	Altitude (Ft)	Sera Samples Tested (♂,♀)	Sera Samples Positive	Ectoparasites Tested (♂,♀)	Ectoparasites Positive	Flea Index
4-12	Baldy Mesa	4,000	11 (6,5)	0	79 (31,48)	0	7.2
4-29	Applewhite Campground	3,300	10 (2,8)	0	61 (?)	0	6.1
5-4	Hanna Flats Campground	7,000	26 (9,17)	10*	101 (28,73)	0	3.8
5-10	Green Valley Lake Campground	7,000	21 (6,15)	4**	7 (1,6)	0	0.3
5-16	Northshore Campground	5,300	11 (6,5)	0	33 (12,21)	0	3.0
6-14	Yucaipa Creek	3,500	10 (7,3)	0	166 (?)	0	16.6
7-7	Yucaipa Regional Park	3,700	9 (3,6)	0	63 (24,39)	0	7.0
7-8	Barton Flats Campground	7,000	10 (3,7)	0	22 (7,15)	0	2.2
7-12	Green Valley Lake Campground	7,000	16 (6,10)	1***	114 (40,74)	0	7.1
7-21	Yucaipa Creek	3,500	21 (9,12)	0	101 (26,75)	0	4.8
7-27	Lobo-Oso Campground	7,000	8 (2,6)	0	55 (18,37)	0	6.9
8-4	Green Valley Lake Campground	7,000	20 (4,16)	0	66 (12,54)	0	3.3
8-10	Green Valley Lake Campground	7,000	21 (9,12)	0	16 (2,14)	0	0.7
8-22	East Flats Campground	7,000	11 (2,9)	0	29 (10,19)	0	2.6
8-23	Forest Falls Campground	6,000	16 (6,10)	0	32 (10,22)	0	2.0
8-24	San Gorgonio Campground	7,000	7 (0,7)	0	3 (0,3)	0	0.4
9-12	Shady Grove Group Camp	6,800	0 (0,0)	0	0 (0,0)	0	0.0
9-21	Oak Glen	3,500	8 (3,5)	0	18 (5,13)	0	2.2
9-26	Rio Barranca Group Camp	4,200	23 (10,13)	0	166 (?)	0	7.2
10-3	Silverwood Lake Campground	3,800	16 (8,8)	0	87 (26,61)	0	5.4
10-4	Silverwood Lake Campground	3,800	12 (4,8)	0	Not collected	--	--
10-17	Miller Canyon Group Camp	4,200	15 (7,8)	0	Not collected	--	--
10-18	Crab Flats Campground	6,200	12 (4,8)	0	Not collected	--	--
		Totals	314 (116,198)	15	1219	0	

* Positive titer range of 1:16-1:128; **Positive titer range of 1:16-1:64; ***Positive titer of 1:2048

with antibody titers of 1:16 to 1:128. The flea index in this area at the time was 3.8 fleas/animal. These two factors necessitated immediate ectoparasite and rodent control measures prior to reopening the campground for public use. The following week, a combination of both ectoparasite and rodent control measures was undertaken using disposable (18" x 4" x 4") cardboard bait stations. The bait stations were set up at all the 88 campsite tables and 5 restrooms. Each bait station, after being fastened to the ground with a 12" iron spike, was baited with a 4 oz diphacinone paraffin block in the center and about 4 oz of diazinon 2D placed at each end inside the station using a long-handle spatula. A post-treatment survey carried out May 24, revealed only 5 ground squirrels with a flea index down to 0.3, thus allowing the reopening of the campground for public use.

The May 10 survey conducted at the Green Valley Lake Campground yielded four of 21 samples seropositive for plague and a low flea index of 0.3. Upon receipt of these results on July 11, about two months after the survey, and considering the low flea index early in the season, the campsite was immediately posted with "Plague Warning" signs followed by a pre-treatment survey on July 12 and press releases in local newspapers a day later.

Lab results of the July 12 survey showed one of 16 samples plague positive with a high antibody titer of 1:2040. This high titer accompanied by a high flea index of 7.1 resulted in closure of the campground to public use on July 25. Ectoparasite control and rodent baiting were carried out on July 28. A total of 42 bait stations (38 campsite and 4 restrooms) was used in this survey. These bait stations measured 18" in length and were made up of 4" diameter PVC drainage pipe with holes in the center. Each bait station was fastened to the ground with a 12" iron spike through the center hole and baited with a 4 oz peanut butter and rolled oats bait block in the center. Using a long-handle spatula, about 4 oz of diazinon 2D was placed at either end inside the station. Rodent burrows over the campground were dusted with diazinon 2D. A post-treatment survey on August 4 yielded a flea index of 3.3 that necessitated rebaiting and redusting with diazinon on August 9. A second post-treatment survey on August 10 showed a flea index of 0.6, allowing the reopening of the campground to public use on August 11, 1994.

ACKNOWLEDGMENTS

The authors thank the staff members of the USDA-FS Big Bear and Arrowhead Ranger Stations for their collaboration. The authors also thank Pam Felts for typing the manuscript.

REFERENCES CITED

Anonymous. 1983. Plague. pp. 320-326. *In:* Control of Communicable Diseases in California. Calif. State Dept. Health Serv., Sacramento, CA. 594 pp.

Kettle, D.S. 1984. Plague. pp. 499-509. *In:* Medical and Veterinary Entomology. John Wiley and Sons, New York, NY. 658 pp.

Lang, J.D. and W. Wills. 1991. Ecology of sylvatic plague in the San Jacinto mountains of southern California. Bull. Soc. Vector Ecol. 16:183-199.

Mian, L.S., C.N. Nwadike, J.C. Hitchcock and M.B. Madon. 1994. Plague surveillance in San Bernardino County during 1993. Proc. Calif. Mosq. Vector Control Assoc. 62:44-46.

Salmon, T.P. and W.P. Gorenzel. 1981. *In:* Plague, What You Should Know About It. U.S. Dept. Agric. and Univer. Calif. Coop. Exten. Leaflet #21233. 14 pp.

Twiggy, G.I. 1978. The role of rodents in plague transmission: A worldwide review. Mammal Rev. 8:77-110.

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:39-44

COMPARISON OF MOSQUITO ABUNDANCE AND SEASONALITY IN WETLANDS WITH GROUNDWATER AND WASTEWATER

Stan A. Wright, Glenn Yoshimura and Jill Townzen

Sacramento/Yolo Mosquito and Vector Control District
8631 Bond Road
Elk Grove, California 95624

ABSTRACT

In 1993, the Sacramento Regional Wastewater Treatment Plant began construction of a series of experimental wetlands designed to polish secondary effluent. During a two year project to compare the effects of groundwater usage (1993) and wastewater usage (1994) on a 15.4 acre wetland, larval and adult mosquito samples were collected each year for 24 weeks from May to October. During 1993, *Culex tarsalis* represented 43.2% of the population and peaked at 0.4 larva per dip, while *Anopheles freeborni* represented 54.5% of the population and peaked at 0.8 larva per dip. During 1994, *Cx. tarsalis* comprised 37.9%, *Cx. erythrothorax* 30.5%, *Cx. pipiens* 26.3% and *Cx. stigmatosoma* <0.5% of the population. An application of *Bti* at maximum allowable rates reduced larval *Culex* numbers from a peak of 12.7 per dip to 2.0 per dip, but the population rebounded to >12.0 per dip within one week.

Water conservation has become critically important as our human population continues to grow. Closely allied with this is the concern for the environment, especially for wetlands which store water and also provide necessary habitat to essential wildlife.

Considering this, two important facts have been brought together, 1) it has been long observed that water moving through wetlands is cleansed of many of its inorganic and organic impurities through sedimentation, photo-oxidation and through conversion by aquatic organisms such as bacteria, fungi and hydrophytes and 2) enormous volumes of water are involved in the transveyance and treatment of residential, commercial and industrial waste. The creation of wetlands with the goal of treating wastewater has been proposed as a means by which both wetland habitat creation and water conservation goals be attained while reducing the long term expense of wastewater treatment.

The construction of wetlands for the treatment of wastewater has been a concern for California mosquito abatement and vector control districts since the 1970s (Townzen and Wilson 1983). Preliminary efforts have had a measure of success at meeting these goals, however, wastewater wetlands create optimum habitat for mosquito production (Townzen and Wilson 1983, Bogaert et al. 1985, Schaefer and Miura 1986 and Martin and Eldridge 1989). Generally, *Culex* production is significantly increased in any system employing wastewater (Smith and Enns 1967, Schaefer et al. 1982). Municipal wastewater engineers realize that constructed wetlands for wastewater treatment that are not properly designed and maintained create serious mosquito problems (Dill 1989, Hammer and Bastian 1989).

The early use of water hyacinth, *Eichornia crassipes* (Mart.) Solms, as a primary macrophyte in wastewater wetlands led to severe mosquito problems and hence five of six projects using these plants were ended because of their inability to suppress mosquito populations (Eldridge and Martin 1987). The use of cattails (*Typha* spp.) and bulrush (*Scirpus* spp.) have been more successful as two of three wetlands using these plants are still operating. However, research and experience has shown that wastewater wetlands using bulrushes and cattails can produce large numbers of mosquitoes if the growth of these plants is allowed to become extremely dense or if matting occurs (Schaefer and Miura 1986). Despite some short-term success, long term control of mosquitoes in various wetland types constructed for wastewater treatment has not yet been satisfactorily accomplished.

In 1993, the Sacramento Regional Wastewater Treatment Plant (SRWTP) began construction of a series of experimental wetland cells designed to polish (i.e.- remove organics and inorganics) secondary effluent. Their objective is to remove heavy metals and organics by passing the effluent over bulrush and cattail rhizomes to sufficiently cleanse it for release into the natural waterways, Laguna and Morrison Creeks (Nolte and Associates 1992). Water from Laguna and Morrison Creeks will be used in created wetlands currently in the planning stages (Hart 1992).

In order to monitor mosquito production in these new wetlands, the Sacramento/Yolo Mosquito and Vector Control District conducted a two year sampling project to compare the effects of groundwater and wastewater usage. During 1993, all of the cells in the created wetlands were irrigated with water pumped from an on-site well to establish the growth of bulrushes and cattails. Seeds and rhizomes of the aquatic plants were obtained from soil samples collected at Grey Lodge National Wildlife Refuge. Secondary effluent was then used in nine of the eleven cells during 1994. Cell 5 which served as a control was irrigated with well water and Cell 6 which was to be irrigated with secondary effluent was switched over to well water due to mechanical difficulties in the delivery system.

MATERIALS AND METHODS

Site Description.

The demonstration wetlands of the SRWTP is located 0.25 miles west of Franklin Boulevard and is bounded by residential areas to the north and east of the complex. The wetlands consist of eleven full cells, each 1.44 acres in size for a total acreage of 15.4 acres. A two acre habitat wetland is located adjacent to the cells on the western side (Fig. 1). Each bow-shaped cell is then subdivided into two half cells (A and B), each being 1,260 feet x 50 feet. Water is introduced into the north end of every B half cell with the north end of every A half cell serving as the outlet. Half cells A and B are inter-connected by an underground pipe on the south end of each half cell. The outflow from all cells is then discharged into the two acre habitat wetland before returning to the treatment plant for discharge into the Sacramento River.

Larval Sampling.

During 1993 and 1994, the experimental cells were monitored for adult and larval mosquitoes for a 24 week period from May through October. Larval samples were taken each week using a standard 250 ml dipper with 48 dips taken from along the cell margins of each of the eleven experimental cells at a distance of approximately every 50 feet. Each subcell was divided into a north and south half for sampling and after every 12 dips (representing one-quarter of the experimental cell), the samples were transferred to labeled jars by backwashing with 75% isopropyl alcohol.

Larval mosquitoes were sorted from the debris at the laboratory. The immatures were grouped into three developmental stages; first and second instars, third and fourth instars, and pupae. Each individual was counted and identified to species using taxonomic keys (Bohart and Washino 1978, Meyer and Durso 1993). All larval specimens were preserved in a voucher collection.

Adult Sampling.

Adult mosquitoes were collected weekly using standard Encephalitis Virus Surveillance (EVS) style carbon dioxide (CO_2)-baited traps. One trap was placed

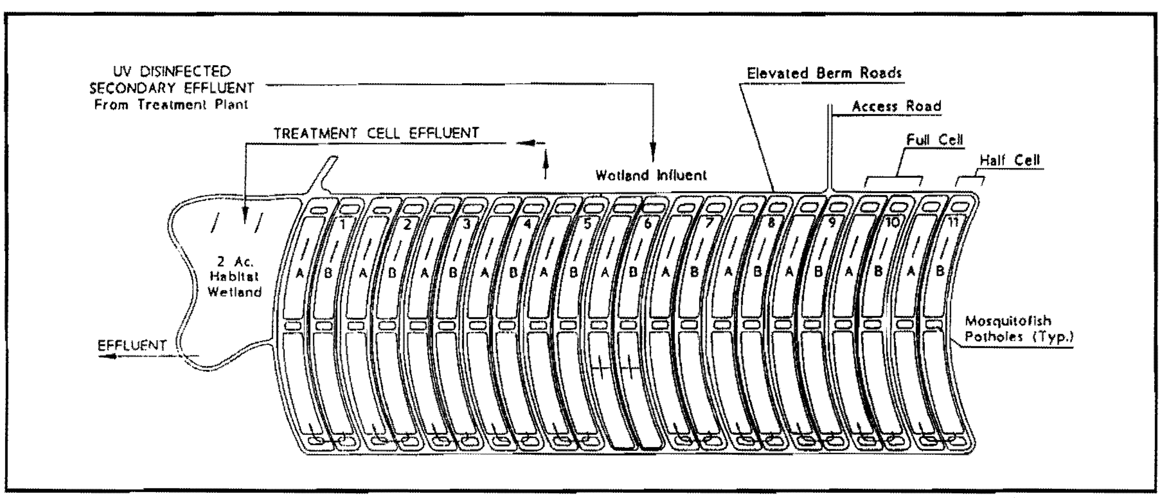


Figure 1. Sacramento Regional Wastewater Treatment Plant (SRWTP) demonstration wetland pond design for polishing secondary effluent.

at each of the four compass directions (north, south, east and west) along the periphery of the wetland site. A single trap was set 100 yards NW of the wetland site at a vegetated harborage location otherwise surrounded by open grassland. Three traps were set 0.25 mile south of the wetland at Lost Lake to function as a control area.

Adults from each trap were brought back to the laboratory, counted, identified to species and discarded.

Water Quality Sampling.

Water temperature and depth were measured at each sample quadrant during the 1993 sampling season when well water was being used. These characteristics fluctuated as water levels in the cells were lowered for maintenance purposes during the year. During 1994, the secondary effluent entered the cells at a constant temperature and remained at relatively constant depths, so water temperature and depth were not monitored on a weekly basis. Water quality data, such as temperature, dissolved oxygen (DO), turbidity, conductivity and pH were collected weekly by SRWTP during 1994.

RESULTS

Larval Populations.

Larval mosquito species composition changed dramatically from the groundwater wetland in 1993 to the secondary effluent wetland in 1994 (Table 1). With the use of well-pumped groundwater, the wetland was dominated by two species of mosquitoes; *Culex tarsalis* Coquillett, representing 43.19% of the total mosquito population sampled and peaking on June 9 at 0.4 larva per dip, and the dominant mosquito, *Anopheles freeborni* Aitken, which represented 54.5% of the total mosquito population sampled and peaked on July 28 at 0.8 larva per dip. Both populations exhibited bimodal curves with peak densities seasonally separated. Also during that season, two other mosquito species were collected in small numbers, *Culex erythrothorax* Dyar represented 0.4% of the sample and *Anopheles franciscanus* McCracken comprised 1.9% of the sample.

With secondary effluent flooding the wetland in 1994, the collected mosquitoes consisted primarily of Culicines, while Anophelines occurred only in the groundwater control cell. As with the groundwater wetland in 1993, *Cx. tarsalis* was one of the dominant species comprising 37.9% of the sample. *Culex erythrothorax* numbers increased in the effluent to 30.5% of the collection and was likely the dominant species since dipping along the margins of the cells selectively favored collection of *Cx. tarsalis*. In support of this assumption, transect sampling of the cells showed that

Table 1. Mosquito species composition for 1993 and 1994 at the SRWTP demonstration wetlands.

Species	1993 %	1994 %
Culex tarsalis	43.2	37.9
Culex erythrothorax	0.4	30.5
Culex pipiens		26.3
Culex stigmatosoma		0.5
Culiseta inornata		4.7
Anopheles freeborni	54.5	0.1
Anopheles franciscanus	1.9	>0.0

Cx. tarsalis were most numerous along the edges of the cells, while *Cx. erythrothorax* were most numerous in the interior of the cells where fewer samples were taken. In addition, *Cx. erythrothorax* larvae are very sensitive to disturbance and standard dipping techniques can easily underestimate their density (Chapman 1962).

During 1994, two other *Culex* species were collected in the effluent water; *Culex pipiens* L. represented 26.3% of the total and *Culex stigmatosoma* Dyar comprised 0.5% of the total sample. Both species are usually associated with organically rich waters typical of wastewater systems. *Culiseta inornata* (Williston) was also collected in the spring and fall samples and represented 4.7% of the total sample. *Anopheles freeborni* and *An. franciscanus* were collected infrequently and only in control cell 5. Both species accounted for 0.1% and 0.01% of the total sample, respectively - a significant change from the *An. freeborni*-dominated groundwater wetland in 1993.

Throughout the 1994 season, there was a gradual increase of Culicine larvae to 0.9 per dip through June and early July. An initial larvicidal treatment of cell 1 on June 13 at the rate of 10 lbs. of Bactimos® granules per acre failed to suppress the mosquito populations adequately, but no follow up treatment was made. A granular *Bti* treatment of cells 1, 3 and 9 at 20 lbs per acre on July 11 reduced the larval numbers in those ponds to ≤0.1 per dip where it remained until September when it began to climb. By September 15, mosquito numbers had risen back to 12.1 larvae per dip. There was a reduction of larval numbers after a granular *Bti* treatment at the rate of 20 lbs. per acre on September 16, but the average number of larvae remained >4 per dip. Mosquito collections peaked at 12.7 per dip on September 29 and then began a seasonal decline through October. During the last sample week on October 20, mosquito collections still averaged 8.4 per dip with *Cx. erythrothorax* being the dominantly collected species, often occurring in clusters where single dips were as high as 40 larvae per dip. An unsurprising fact since this species can overwinter in larval broods where late instar larvae diapause without pupating until spring (Chapman 1962).

Adult Populations.

Mosquito production increased significantly in 1994 as compared to 1993 (Fig. 2). The increase in density and vegetative growth of tules and cattails averaging over two cm/day (C. Williams, personal communication) in the warmer, organic rich effluent during 1994 provided better harborage for the mosquitoes as well as making them difficult to control.

Monitoring of the adult mosquito population at the demonstration wetlands during 1994 resulted in a similar species composition as did the dipping. However, the predominant species collected in the EVS traps at the demonstration wetlands was *Cx. erythrothorax*, whereas the majority of mosquito larvae were *Cx. tarsalis*. This suggests that *Cx. erythrothorax* was, in fact, the dominant mosquito at the wetland during this time period. There was a large disparity between the total number of adults collected at the wetlands (on-site) and the control traps at Lost Lake (Fig. 3). The adult mosquito population showed a seasonal bimodal curve much as the larvae did.

DISCUSSION

During the first year at the demonstration wetlands, mosquito productivity resembled that of a seasonal wetland such as a rice field. Two species dominated at different parts of the season. *Culex tarsalis* was the dominant mosquito species during the spring and early summer with *Anopheles freeborni* dominating in late summer and early fall. Other mosquito species occurred in relatively small numbers throughout the season.

The cattail and bulrush canopy during the first year of sampling was dense enough to effectively shade the water surface. Cattails averaged two to five feet above the water surface toward the end of the season. Applications of Vectobac® 12AS at two pints per acre was effective in controlling mosquitoes. Mosquitofish were stocked in the ponds at two pounds per acre as an added control measure. The bulrushes and cattails thatched after the winter and new shoots grew through this mat as secondary effluent was added during 1994.

With the introduction of secondary effluent, the two largest differences to water quality was the temperature and dissolved oxygen levels (Table 2). When the effluent entered the cells, the water temperature was just over 80°F. The temperature slowly dropped as the water

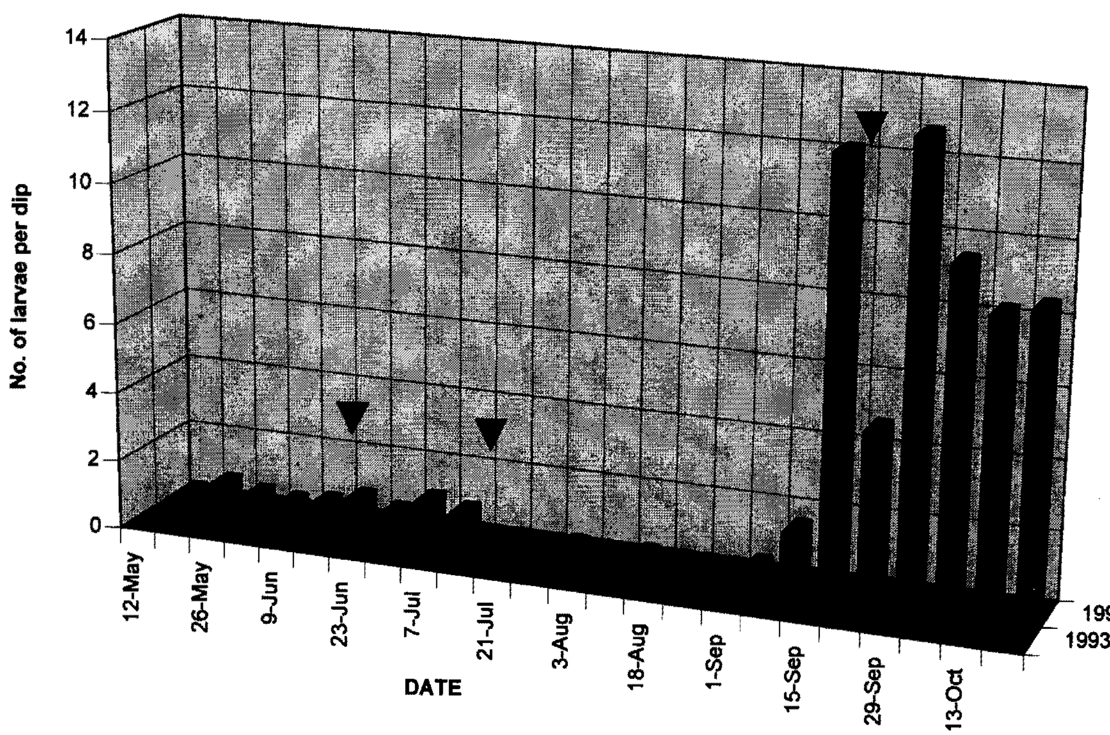


Figure 2. Average number of mosquito larvae per dip from all experimental cells (exclusive of cell 5) at the SRWTP demonstration wetlands during 1993 and 1994. Arrows indicate dates of *Bti* applications during 1994.

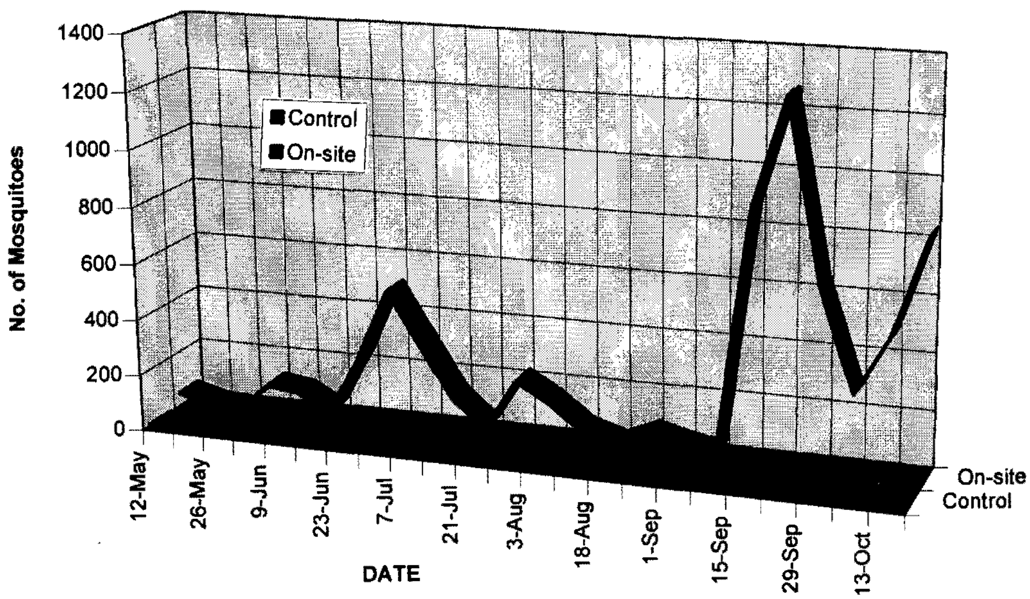


Figure 3. Adult mosquito populations during 1994 as sampled by carbon dioxide-baited traps at the SRWTP demonstration wetlands (on-site) and Lost Lake (control).

progressed through the cells. The difference in change was most notable during the fall months as the temperature differential between the inlet and outlet was as much as 30 degrees. This was in contrast to 1993 where the groundwater temperature remained relatively constant daily and ranged only twenty degrees seasonally.

The second significant difference was the dissolved oxygen level. The dissolved oxygen levels ranged widely between 3.0 and 12.4 mg/l during 1993 with the majority of cells remaining relatively constant between 4 to 6 mg/l. In the effluent wetland, the dissolved oxygen levels were considerably lower, ranging overall from 0.02 to 1.6 mg/l with the majority averaging less than 1.0 mg/l.

These differences in water quality predictably had a significant impact on the animal community structure in the wetland. Thermal stress alone reduces the ability of mosquitofish to control mosquito populations (Castleberry and Cech 1988). At dissolved oxygen levels below 1.0 mg/l, fish are seriously stressed (Mian et al. 1986), and most invertebrates other than atmospheric breathing insects are significantly reduced.

Table 2. Water quality measurements at the SRWTP demonstration wetlands during 1993 (groundwater) and 1994 (secondary effluent).

	1993	1994
Temperature Range (°F)	43.0 - 65.0	48.0 - 81.5
pH Range	6.8 - 7.3	6.1 - 7.5
D.O. Range (mg/l)	3.00 - 12.44	0.02 - 1.6

Culicidae would be greatly advantaged by the changes in conditions that occurred at the demonstration wetland after the effluent was introduced. Introduction of organics increases overall success of *Culex tarsalis*, *Cx. pipiens*, and *Cx. stigmatosoma* immatures and acts as an oviposition attractant for gravid adults (Gerhardt 1959, Mortenson 1982). The increased water temperatures, coupled with increased organic material for food, allowed the greatest development rate for immature mosquitoes. The lower dissolved oxygen levels have little or no effect on mosquito larvae (Smith and Enns 1967), but did reduce the presence or effectiveness of its predators and competitors. These conditions clearly lead to extreme productions of mosquitoes at the site, especially *Cx. tarsalis* which is the vector of western equine encephalomyelitis. Western equine encephalomyelitis virus was isolated from pools of *Cx. tarsalis* collected at the wetland site in 1993. With dense urban conditions surrounding this wastewater wetland, the potential for human encephalitis has significantly increased.

The increase in organics and temperature at the wetlands benefitted the aquatic vegetation. Bulrushes increased in density and extended as high as 10-12 feet above the water surface. Duckweed also was so dense that in most cells it completely covered the water surface.

Dense, floating algal mats were abundant in open water areas at the south ends of the ponds. These conditions further favor mosquito production (Smith and Enns 1967), and made it difficult to effectively apply larvicide through the canopy later in the season.

Mid-September applications of Bactimos® at the maximum rate of 20 lbs per acre had some impact on the mosquito population, but the numbers remained well above 2.0 per dip and quickly rebounded to >9.0 per dip in one week. Control measures were ineffective in reducing mosquito numbers during September due to the elevated macrophyte height and densities.

Our recommendation for the suppression of mosquito populations in these wastewater wetlands would be for the continuous maintenance of the aquatic vegetation and the annual removal of thatch. This will allow for the penetration of larvicidal materials to the water surface during the summer months and reduce harborage for larval and adult mosquitoes.

ACKNOWLEDGMENTS

The authors thank Chuck Williams, Roy Nelson, Jeff Kimble and Kevin Cassady at the Sacramento Regional Wastewater Treatment Plant for their cooperation in the project. We also thank Tom Yu, University of California, Davis, for his help in sampling the demonstration wetland ponds in 1993.

REFERENCES CITED

Bogaert, R., C. Beesley and R. Yescott. 1985. Mosquito occurrence in a manmade wetland habitat maintained by treated wastewater. Unpublished report. Calif. Dept. Health Services, Vector Biology and Control Branch. 18 pp.

Bohart, R.M. and R.K. Washino. 1978. Mosquitoes in California. Agric. Sci. Publ. 4084. Div. Agri. Sciences, Univ. California, Berkeley. 153 pp.

Castleberry, D.T. and J.J. Cech, Jr. 1988. Fish predation on mosquitoes in wastewater: effects of thermal stress and increased fish community complexity. Proc. Calif. Mosq. Vector Control Assoc. 56:180.

Chapman, H.C. 1962. The bio-ecology of *Culex erythrothorax* Dyar. Mosq. News 22(2):130-134.

Dill, C.H. 1989. Wastewater wetlands: user friendly mosquito habitats. pp. 664-667. *In*: D.A. Hammer (ed.) Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural. Lewis Publications, Chelsea, MI.

Eldridge, B.F. and C. Martin. 1987. Mosquito problems in sewage treatment plants using aquatic macrophytes in California. Proc. Calif. Mosq. Vector Control Assoc. 55:87-91.

Gerhardt, R.W. 1959. The influence of soil fermentation on oviposition site selection by mosquitoes. Mosq. News 19(3):151-155.

Hammer, D.A. and R.K. Bastian. 1989. Wetland ecosystems: natural water purifiers? pp. 5-19. *In*: D.A. Hammer (ed.) Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural. Lewis Publications, Chelsea, MI.

Hart, J. 1992. North Stone Lake Restoration and Management Master Plan. Sacramento County Dept. of Parks, Recreation and Open Space, U.S. Fish and Wildlife Service, Ducks Unlimited, Inc., and National Audubon Society. 92 pp.

Meyer, R.P. and S.L. Durso. 1993. Identification of the Mosquitoes of California. CMVCA Publication, Sacramento, CA. 80 pp.

Martin, C.V. and B.F. Eldridge. 1989. California's experience with mosquitoes in aquatic wastewater treatment systems. pp. 393-398. *In*: D.A. Hammer (ed.) Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural. Lewis Publications, Chelsea, MI.

Mian, L.S., M.S. Mulla and B.A. Wilson. 1986. Studies on potential biological control agents of immature mosquitoes in sewage wastewater in southern California. J. Am. Mosq. Control Assoc. 2(3):329-335.

Mortenson, E.W. 1982. Mosquito occurrence in wastewater marshes: A potential new community problem. 50:65-67.

Nolte and Associates. 1992. Sacramento Regional Wastewater Treatment Plant Demonstration Wetlands Project Implementation Plan. 13 pp.

Schaefer, C.H., T. Miura, R.J. Stewart and R.M. Takahashi. 1982. Studies on the relationship of mosquito breeding on rice fields and the use of sewage effluent for irrigation. Proc. Calif. Mosq. Vector Control Assoc. 38:59-65.

Schaefer, C.H. and T. Miura. 1986. Mosquito breeding in a cattail-tule marsh managed for clean-up of secondary sewage effluent. Proc. Calif. Mosq. Vector Control Assoc. 53:119-122.

Smith, W.L., Jr. and W.R. Enns. 1967. Laboratory and field investigations of mosquito populations associated with oxidation lagoons in Missouri. Mosq. News 27(4):462-466.

Townzen, K.R. and B.A. Wilson. 1983. Survey of mosquito production associated with water hyacinth. State of California Water Resources Control Board and Department of Health Services, Vector Biology and Control Branch. 46 pp.

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:45-47

MOSQUITO BREEDING AND CONTROL PROBLEMS ASSOCIATED WITH A *LEMNA* WASTEWATER TREATMENT POND

Mark Westerline

Moorpark Mosquito Abatement District
P.O. Box 62
Moorpark, California 93020

ABSTRACT

Sewage treatment plants are increasing their use of wastewater treatment systems utilizing aquatic plants. Early in 1992, the first wastewater treatment pond in California to use Duckweed (*Lemna* sp.) went into operation at the Moorpark Sewage Treatment Plant. This paper highlights some of the mosquito breeding problems and control measures associated with this pond over the past three years.

Over the period of several months at the end of 1991 and the beginning of 1992, Ventura County Waterworks District #1, which operates Moorpark's sewage treatment plant, constructed and began operation of a wastewater treatment pond that utilizes the aquatic plant Duckweed (*Lemna* sp.) as part of its wastewater treatment process. The sewage treatment plant is located approximately 1.5 miles west of Moorpark's city limits and the nearest residential area, known as Home Acres, lies about one mile east of the treatment plant.

Aquatic plants are being used with increased frequency at wastewater treatment facilities throughout the United States and California. One of the more familiar examples is the city of San Diego's pilot project at the Mission Valley Water Treatment Plant which utilizes the aquatic plant Water Hyacinth (*Eichornia crassipess*) for wastewater treatment. Wastewater treatment ponds utilizing *Lemna* have been built across the U.S. and currently exist in such locations as Devils Lake, North Dakota; Ogema, Wisconsin; Baldwin, Louisiana; Hermitage, Arkansas; Boulder City, Nevada; and San Juan Batista and Moorpark, California. With the exception of Moorpark, it appears that little, if any, monitoring of these ponds for mosquito breeding has been undertaken. All of these ponds were designed and built by the Lemna Corporation, headquartered in St. Paul, Minnesota.

About ten species of *Lemna* are known throughout the world, most are widespread and range in size from 2-12 mm in diameter. This small, floating aquatic plant consists of a leaf-like frond with one to several thin roots dangling into the water. In theory, on the wastewater treatment pond, the *Lemna* plants form a thick mat which helps prevent light penetration into the water, decreasing algal growth. Theoretically, this thick *Lemna* mat also aids in controlling mosquito breeding and controlling odors emanating from the pond surface while the roots absorb such nutrients as nitrogen and phosphorus and other undesirable compounds such as heavy metals.

The *Lemna* species primarily used in the Moorpark pond is *Lemna minor*, sometimes called Common Duckweed or Lesser Duckweed. At times, the sewage treatment plant staff experimented with introducing mixed species of *Lemna* into the pond to help prevent complete die-offs during adverse conditions.

Lemna ponds across the country vary in size depending upon many factors such as land availability, effluent flow rates, manpower and funding. The surface area of the *Lemna* pond in the Moorpark facility measures approximately 3.8 acres in size (Fig. 1). The pond also has a floating plastic grid consisting of individual 100 square foot cells to keep the water still and the *Lemna* equally dispersed across the pond surface.

MOSQUITO CONTROL

It is this plastic grid system that also appears to make an excellent breeding structure for mosquitoes. In February of 1992, mosquito larvae began appearing in the pond and steps were taken to initiate control measures. Application of pesticides was somewhat

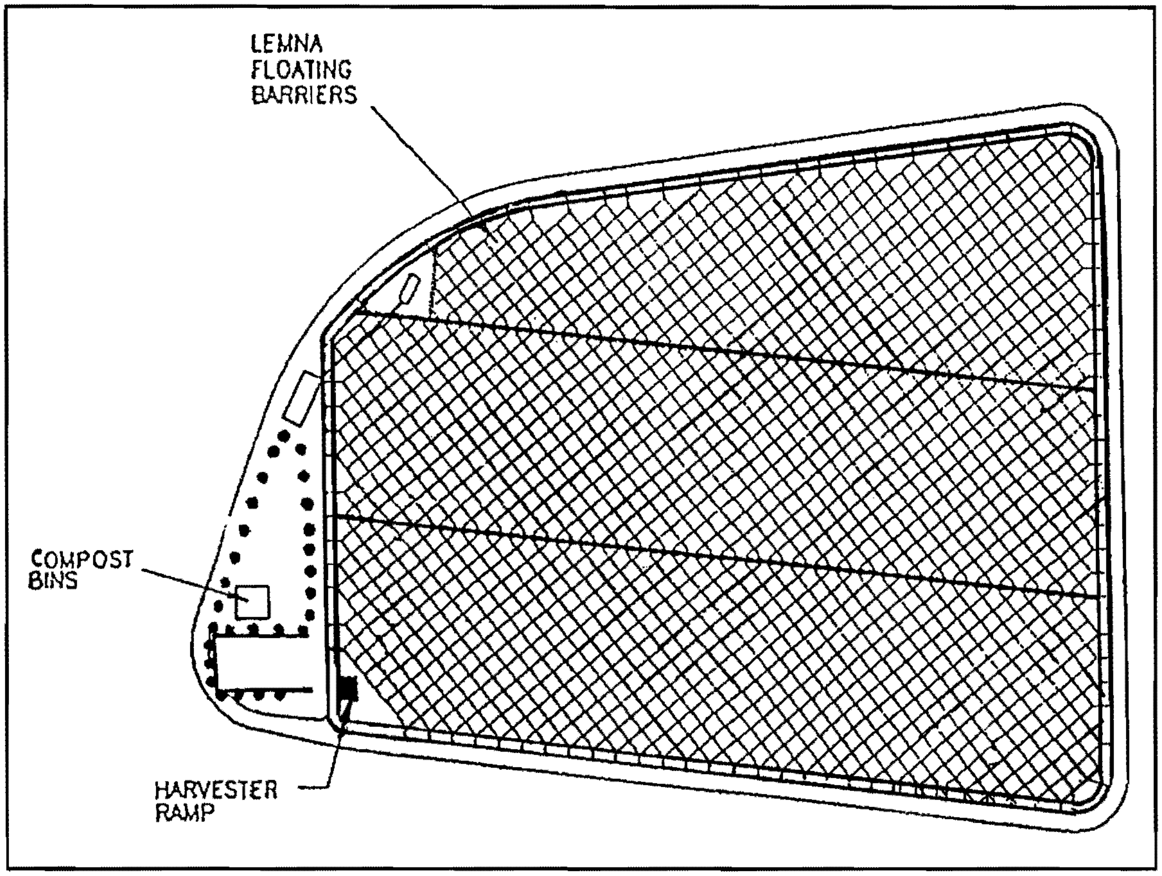


Figure 1. Diagram of the 3.8 acre *Lemna* wastewater treatment pond at the Moorpark Sewage Treatment Plant.

difficult due to the shape of the pond. While the pond perimeter can easily be treated by hand or truck mounted sprayer, the inside of the pond had to be accessed with a specially designed small paddle wheel style boat that can traverse the plastic *Lemna* grid. This small boat was called a harvester and was normally used to collect and move *Lemna* in the pond. Other possible, but more expensive application alternatives would be the use of fixed or rotary winged aircraft or hovercraft. The unique shape of the pond and the constant need for the harvester to routinely traverse its surface precluded the use of a water sprinkler system to deter mosquito oviposition as used in wastewater treatment ponds utilizing Water Hyacinth.

The Moorpark Mosquito Abatement District first attempted to control mosquitoes breeding in the *Lemna* pond using the petroleum-based larviciding oil Golden Bear 1356. While effective at killing mosquito larvae and pupae breeding in the pond, this oil had adverse affects on the *Lemna* plants themselves and caused partial die-offs of the *Lemna* mat. Similar die-offs in the *Lemna* mat during the operation of this pond have been caused by several other substances including Aerosurf MSF larvicide (used after the Golden Bear 1356 Oil) and most recently, in the spring of 1994, a polymer that the sewage treatment facility used in its own water treatment system. Another constant past and future threat to the *Lemna* mat is illegally dumped oils, solvents and pesticides.

The mosquito abatement district also attempted some biological control on this pond by repeatedly stocking it with a total of nearly 10,000 mosquitofish (*Gambusia affinis*) over a period of approximately one month. It was noted that all the mosquitofish died off within a few days after each introduction and that conditions within the pond were too inhospitable for further attempts at biocontrol with *Gambusia*.

Attempts to chemically control mosquitoes in the *Lemna* pond included the application of *Bti* (*Bacillus thruingiensis* var. *israelensis*) in several formulations, including granules, pellets, briquets and liquid. Although the liquid *Bti* formulation yielded the best results, it was sometimes ineffective at totally controlling mosquito breeding in the pond and high concentrations of immatures were occasionally seen after applications. The liquid form of the insect growth regulator methoprene was also used on the pond with mixed control results.

MONITORING FOR MOSQUITOES

Due to the large amounts of time and pesticides needed for mosquito control on the *Lemna* pond, the sewage treatment plant was made responsible for the purchase and application of insecticides for mosquito control since the summer of 1992 with the mosquito abatement district monitoring mosquito breeding in the pond by dipping for larvae and pupae 1-3 times per week, depending on the time of year. Larval and pupal concentrations from each dipping session were recorded on a diagram of the pond with a copy given to sewage treatment plant staff to help evaluate control methods. Larval samples from the pond were identified every few months. To date, all samples from the pond have been identified as *Culex stigmatosoma* Dyar, although the author is of the opinion that *Culex quinquefasciatus* Say can quite possibly breed in this pond as well.

In March of 1992, the mosquito abatement district installed a New Jersey light trap next to the *Lemna* pond to help monitor the number and species of adult mosquitoes emerging from the pond. In 1992, the trap ran for 240 trap-nights and collected 726 mosquitoes (3.0 mosquitoes per trap-night) with 623 (85.8%) of these being *Cx. stigmatosoma*. In 1993, the trap ran for 268 trap-nights and collected 1,636 mosquitoes (6.1 mosquitoes per trap-night) with 1,026 (62.7%) of these being *Cx. stigmatosoma*. In 1994, the trap ran for 341 trap-nights and collected only 137 mosquitoes (0.4 mosquitoes per trap-night) with 61 (44.5%) of these being *Cx. stigmatosoma*. Also, a significant number of *Cx. quinquefasciatus* were collected in this trap over the years including 71 in 1992, 404 in 1993 and 43 in 1994.

CONCLUSION

In the author's opinion, one of the most important lessons learned from this experience is to have good communications with the local water treatment plant(s) within your district. It would be wise to determine if any of them have plans to build a facility like this and let them know of some of the potential problems that may exist.

ACKNOWLEDGMENTS

The author gratefully thanks Mr. Edward Heidig from the city of San Diego's Aquaculture Project for demonstrating their project's use of Water Hyacinths for wastewater treatment and for his advice on mosquito control in aquaculture systems. Similarly, the author thanks Mr. Chuck Myers and Mr. Minoo Madon from the California Department of Health Services for their assistance and advice on this project.

REPORT ON THE OCCURRENCE OF *CULEX ERRATICUS* IN CALIFORNIA[1]

Branka B. Lothrop[2] and Richard P. Meyer[3]

Department of Entomology
University of California
Riverside, California 92207

ABSTRACT

In late summer 1994, *Culex (Melaniconion) erraticus* (Dyar and Knab) was collected near the southeastern shore of the Salton Sea in the Imperial Valley (Imperial County) of California. This is the first record of this species in California. *Culex erraticus* adults are distinctively small with a blue-metallic iridescence on the abdomen and dark-scaled legs with narrow pale knee bands. Considering the known *Culex* in California, they are unlikely to be overlooked, but could be confused with small melanistic *Culex quinquefasciatus* Say. The closest distribution records are to the east in southern Arizona and to the south in Baja California Sur, Mexico on the Gulf of California. The species range in the United States is north to Michigan and west to South Dakota and New Mexico.

The Imperial Waterfowl Management Area was established by the State of California Department of Fish and Game in the early 1960s at the southeast shore of the Salton Sea. Currently, it occupies 2,122 ha, of which 1,820 ha were flooded during the summer of 1994. Irrigation during the fall and winter provides barley, wild millet and alkali bulrush to wintering ducks and geese during their migratory flights through the area. Mean monthly temperatures range from 10-35°C, with late summer and winter storms yielding less than 12.7 cm average annual rainfall.

On August 29, 1994, a CO_2-baited trap on the east side of Davis Road at the Wister Wildlife Refuge collected four female and one male *Cx. erraticus* with additional specimens collected on October 24, 1994.

On October 31, 1994 *Cx. erraticus* breeding sites were located in the same area. Two types of habitats were sampled for larvae; small, shallow pools formed by an overflowed canal along Davis Road and commercial fish ponds. *Culex erraticus* immatures were collected first from a pool (2.5m x 3.5m x 0.30m) adjacent to the trap site. Similar sites along Davis Road to the north were unsuccessfully sampled. The second site where *Cx. erraticus* larvae were collected was the corner of a nearby fish production pond (ca. 2.0 ha). Overall, the small numbers of *Cx. erraticus* collected indicates a restricted distribution and low numbers of the species in the Imperial Valley.

Although the Colorado River Delta provides a natural corridor for the introduction of *Cx. erraticus* into California from the south, the dry climate of Baja California Norte decreases the probability of this passage. The introduction of *Cx. erraticus* into California from the east seems equally improbable since the high plains of Arizona and New Mexico lack suitable habitats and impose a natural barrier to westward expansion.

Absence of suitable *Cx. erraticus* habitats in the areas surrounding its southern California distribution suggests transportation by man as an alternative method in the introduction of this species although the monsoon storm fronts traveling northward from the Gulf of Mexico could be another alternative.

For the time being, *Cx. erraticus* in southern California is probably best considered an isolated population, until a more definite link is found

---

[1]A manuscript summarizing these findings will be submitted for publication in the Journal of the American Mosquito Control Association.

[2]Address correspondence to: Dr. Branka B. Lothrop, Coachella Valley Mosquito Abatement District, 83-733 Avenue 55, Thermal, California 92274.

[3]Orange County Vector Control District, P.O. Box 87, Santa Ana, California 92702.

PRELIMINARY NOTES ABOUT OVIPOSITION OF *CHIRONOMUS ANONYMUS* (DIPTERA: CHIRONOMIDAE)[1]

Branka B. Lothrop[2] and Mir S. Mulla

Department of Entomology
University of California
Riverside, California 92521

ABSTRACT

Chironomus anonymus Williston is one of many nuisance aquatic midge species posing a problem to residents near man-made lakes in country clubs or resorts throughout southern California. Although currently registered larval midge control insecticides have good levels of activity and low toxicity to non-target organisms, their use has certain limitations. Therefore, other strategies need to be developed for an effective integrated control of nuisance aquatic midges. One of these strategies is the possibility of interrupting oviposition.

To efficiently collect *C. anonymus* egg masses and gather more information about the oviposition habits of *C. anonymus*, a series of experiments was conducted at Portola Country Club in Palm Desert, California, from September 22 to October 12, 1994. The seven lakes within this location had concrete edges and bottoms sealed with plastic liners. All lakes, except Lake #18, were supplied with tertiary-effluent water from the nearby Palm Desert Sewage Treatment Plant. Studies were carried out in Lake #10, which had a surface area of approximately 0.5 ha, a maximum depth of 1.5 m and two aeration fountains in one end of the lake.

To collect *C. anonymus* egg masses, 0.75 cm diameter polypropylene ropes in different colors and lengths were tied with twine to 2 m wooden stakes and randomly placed in the lake so that the ropes floated at or just below the water surface. To study the effect of water turbulence on interruption of *C. anonymus* oviposition, the area around the aeration fountains was used as the agitated portion of the lake while the still area of the lake served as the control. During two separate 24-hr experiments yellow 30 cm long polypropylene ropes were placed in the lake with 5 ropes placed in the agitated section and 5 in the still portion. The diel oviposition rhythms of *C. anonymus* were study on two separate dates. On each date, 5 yellow 30 cm long polypropylene ropes were placed in the still portion of the lake and changed every 2 hours.

Data on oviposition interruption indicated a significant difference in the number of egg masses deposited on the ropes placed in the still and agitated sections of the lake. An applied T-test (df=8) showed a significant difference in both study periods ($t_{obs(1)} = 2.14$; $t_{obs(2)} = 2.03$ and $t_{0.05} = 1.86$). During the first period, approximately 19 times more egg masses were deposited on the ropes placed in still water, while during the second period there was a difference of about 3 times.

During the first diel oviposition cycle experiment, when sunset was at 6:35 p.m. and sunrise at 6:39 a.m., peak oviposition occurred between 7 p.m. and 9 p.m. (2,206 egg masses collected) with a smaller peak observed between 5 a.m. and 7 a.m. (320 egg masses collected). During the second experiment, when sunset was at 6:16 p.m. and sunrise at 6:49 a.m., maximum oviposition occurred between 7 a.m. and 9 a.m. (34 egg masses collected).

Preliminary data indicated that *C. anonymus* oviposition can be interrupted by disturbing the water surface. This non-chemical control measure targets the midge population at the egg level, reserving chemical control for emergencies. However, before oviposition interruption becomes a standard in integrated aquatic midge control, more information on oviposition rhythms of other problematic midge species must be obtained.

[1] A manuscript summarizing this study will be submitted for publication in the Journal of Environmental Entomology.

[2] Address correspondence to: Dr. Branka B. Lothrop, Coachella Valley Mosquito Abatement District, 83-733 Avenue 55, Thermal, California 92274.

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:50-51

A GEOGRAPHICAL INFORMATION SYSTEM TO DESCRIBE MOSQUITO ABUNDANCE AND ARBOVIRUS TRANSMISSION IN THE COACHELLA VALLEY

Hugh D. Lothrop[1] and William K. Reisen

Arbovirus Research Program
School of Public Health
University of California
Berkeley, California 94720

ABSTRACT

The development and application of a geographical information system is described to monitor and analyze mosquito abundance and encephalitis activity in the Coachella Valley. The components and progress of the system are discussed.

Geographical information systems (GIS) have gained wide acceptance in fields outside of biology because of their ability to present data relationships in a spatial format. However, since biological systems often have important spatial components which are difficult to represent through linear mathematical models or tables of numbers, biologists have, likewise, begun to access GIS technology, especially in ecological studies.

Since 1991 we have intensively investigated encephalitis ecology in the Coachella Valley in an effort to find relationships among virus transmission rates and other factors, such as habitat type and vector abundance. In the past we have used "best estimate" sampling design that positioned sampling stations to discover differences related to adjacent spatial and environmental factors. Although no consistent differences were found among habitat types, we did document that transmission rates decreased as a function of distance from the Salton Sea. However, the diversity of landscapes created by various land uses confounded these results and left many unanswered questions.

In 1994, we turned to GIS technology to provide a multi-variate approach to factors related to mosquito and virus ecology, especially mosquito abundance. The latter is particularly valuable to the Coachella Valley Mosquito Abatement District in assessing their mosquito control program.

The term GIS has different definitions depending upon the needs of the user. Many applications can be described more accurately as geographical databases, because they store and present data through a pictorial interface. However, a full geographical information system also should have the capability to analyze spatial relationships among data elements. The development of a GIS begins by creating a geographical database. This is the current state of our system.

BUILDING THE SYSTEM

Our studies began with an assessment of the computer hardware requirements based upon the projected size of the database and the needs of the software used in producing the system. The Coachella Valley Mosquito Abatement District has provided this equipment in the form of a modest 80486 33 MHz IBM clone computer with 8 megabytes (MB) of RAM memory and a 340 MB hard drive. In addition, a 12" x 12" digitizing tablet with a 4 button puck and a 600 dpi color scanner are used to enter data.

The Microsoft Windows operating environment was selected because of its ease of use, memory management

[1] Address correspondence to: Hugh D. Lothrop, Coachella Valley Mosquito Abatement District, 83-733 Avenue 55, Thermal, California 92274.

capabilities and wide acceptance. Because we have chosen to develop the software application, we are using a C++ language compiler with support libraries to write the code and a dBASE support library for producing dBASE compatible file structures for non-graphical elements of the data set. A graphics editor was used to scan and edit the base map images. These tools provide flexibility of design and extendibility for the final system.

The basic structure of the program is comprised of three units. The first unit is the interface, which is composed of graphical and text dialogs. Graphical dialogs input and output spatial data such as sample site position or delineation of boundaries of abiotic characters such as soil type. Text dialogs input data on characters such as adult and larval abundance, mosquito control activities, and site names. The second unit is the database manager which sorts and indexes the non-graphical data. The third unit, the analytical engine, correlates the data elements.

DATA ENTRY

To date, our data fall into eight categories, but the final version is not limited to these:

1. Surface features and base maps were derived from scanned aerial photographs of 1":4000' scale. Larger scales were judged to be inappropriate for images defining mosquito breeding sites.
2. Soil types and distribution were found in a 1980 soil survey published by the United States Department of Agriculture (USDA) Soil Conservation Service and were entered using the digitizing tablet.
3. Elevations came from 7.5' series topographical maps of the United States Geological Survey (USGS) and also were entered using the tablet.
4. Water usage was gathered by ground survey, plotted on printouts of the section base maps and entered with the tablet.
5. Ground vegetation was observed from aerial photos and confirmed through ground survey before being traced over the base map images.
6. Adult abundance was determined bi-weekly using an uniform sampling scheme with 63 trap sites located at section corners. CDC style traps baited with CO_2 were operated from April through November.
7. Larval abundance was sampled by taking multiple dips from each breeding site using a standard dipper. Larval samples were returned for counting and identification from May to November.
8. Virus transmission activity was monitored using 10 flocks of 10 sentinel chickens each that were bled bi-weekly from April to November.

The first interface presented to the user is a map of the entire study site in a scale which allows viewing on four scrolled pages. This image was a fusion of 32 blocks of four sections each, from five aerial photos covering our study area around the northern margin of the Salton Sea. The scale of this map image was a compromise between memory size, resolution and scrollable page size. Sections are the second and smaller scale interface and are accessed by locating the cursor over the desired section on the main map and double clicking the mouse. The section interface was set at a resolution of one pixel to ten feet, seen in VGA mode. This resolution was sufficient for fixing locations and areas of larval habitat as well as other spatial data such as elevation and soil type.

Our plan for 1995 is to continue field sampling, expand our database, and develop the analytical engine to produce output using geographical statistics. Additional functionality for the system will address the operational needs of the vector district such as accurate logging of treatment sites and rates of insecticide application.

Expansion of the the system to encompass the entire district is in progress, using additional aerial photos and data gathered from the new areas.

ACKNOWLEDGEMENTS

We especially thank Stan Husted, Manager of the Coachella Valley Mosquito Abatement District, and his field staff for their continued support of and assistance with this project. Special thanks to Arturo Gutierrez for technical assistance. This research was funded entirely by the Coachella Valley Mosquito Abatement District.

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:52-56

WETLANDS AND MOSQUITO BREEDING: CHALLENGE FOR THE 1990s

Bruce F. Eldridge

Department of Entomology
University of California
Davis, California 95616

Those of us who are involved with the biology and control of arthropod vectors of human diseases in California find ourselves in an unusually difficult position. On the one hand, there is in this state a strong movement to rectify partially the results of nearly 150 years of alteration and exploitation of our natural resources, and on the other hand there is a continued disregard for the public health consequences of the environmental manipulations needed to achieve this goal. We ourselves are partially to blame. We have a relatively passive and ineffective public education program, and the public generally associates vector control with the narrowly focused programs of 50 years ago.

I would like to provide an overview of our current problems with mosquitoes in wetlands. This overview will touch briefly on the history of wetlands reduction in the state, the recent attempts to reverse this reduction and the legislation which provides the authority for these attempts, the nature of problems with mosquitoes, and some possible general solutions to the problems. In my view, reconstructed wetlands will be the dominant issue of the next 20 years or so. Some people believe that mosquito problems in wetlands can be solved through cooperation between vector abatement agencies and fish and wildlife agencies. That is true only up to a point. We still need answers to a number of difficult questions.

HISTORICAL WETLANDS IN CALIFORNIA

The Great Valley in California before the time of the Gold Rush was a very wet place (Kahrl 1979). In addition to the Sacramento and San Joaquin Rivers and their many tributaries, there were a number of Pleistocene lakes, especially in the western portion of the San Joaquin Valley. These lakes included Lake Corcoran (Stockton to Firebaugh), Lake Tulare (terminus of Kings River) and Buena Vista Lake (terminus of Kern River). All of these lakes are now extinct. Furthermore, because of many years of water diversion and deposition of soil into the river systems, there has been considerable subsidence in the Sacramento-San Joaquin Delta, which in connection with levee construction, has drastically altered that landscape (Norris and Webb 1990).

There has been a tendency to blame the alteration of the California landscape and the accompanying loss of wetlands on events which took place after the creation of the Central Valley Project in the 1930s. Actually, the process began more than 200 years ago with the first irrigation and reclamation projects, but was accelerated with the discovery of gold on the American River, and the eventual expansion of hydraulic mining in the foothills of the Sacramento-San Joaquin River drainages. Before this activity was banned in 1884, enormous silting of the river system occurred, leading to recurring floods and irreparable damage to the entire system (Watkins 1973). Wholesale removal of timber contributed as well.

During the period 1910-1930 there was a significant expansion of the hard-surfaced roads in the Central Valley, with the construction of many levees and diversion canals, and the reclamation of poorly drained land for farming. In the Sacramento Valley, projects such as the Sutter Basin Land Company and the Holland Land Company involved thousands of acres. In 1933, the Central Valley Project was authorized by Congress. This led to the construction of Shasta Dam, Friant Dam, the Delta-Mendota Canal and the Friant-Kern Canal. Then in 1957, the California Water Plan came into being, which led to construction of Oroville Dam, the California Aqueduct and San Luis Reservoir.

With the beginning of the second half of the 20th Century, the realization that wetlands had real value began to grow. Environmental scientists began to emphasize that wetlands could ameliorate flooding, provide biodiversity, and thus environmental homeostasis, provide habitat for many species of plants and animals, and provide educational and aesthetic values to people (Salvesen 1990). Then in the 1970s, this realization began to find voice in the legislature.

LAWS AND REGULATIONS AFFECTING WETLANDS

Anything which is legislated is bound to be far from simple in its implementation. This is certainly true in the case of wetlands. Even definitions of wetlands vary in many practical and politically loaded ways (Salvesen 1990, Leitch et al. 1994). For our purposes we can divide wetlands into two subdivisions: Tidal and non-tidal.

The real struggle involved in wetlands restoration appears to me to be between property owners who desire to profit from their ownership of property by development or other use, and state and federal agencies who desire to accomplish wetlands restoration goals through use of laws and regulations. The need for vector control appears to be caught in the crossfire.

The primary laws which affect the restoration, protection and management of wetlands are:

1. The National Environmental Policy Act of 1969 (NEPA) created the Council on Environmental Quality, and stipulated that all federal agencies must consider the environmental consequences of any "major action". It may be best remembered as the act which created the requirement for environmental impact statements (EIS).
2. The Clean Water Act of 1977 (CWA) is the most important piece of legislation dealing with wetlands. It expanded the role of the Army Corps of Engineers in wetlands regulations (Salvesen 1990). It also created the requirement, under Section 404 of the Act, for a permit from the Corps before engaging in a variety of activities in various types of waters defined under the act, including bodies of waters now considered "wetlands".
3. The Endangered Species Act of 1973 (ESA) states that no federal agency will authorize any action which will jeopardize any endangered or threatened plant or animal species. This is interpreted to mean that any federal legislation must be implemented in a way conforming to the ESA. That is why one hears the expression "Implementation of the ESA under the provisions of the Federal Insecticide, Rodenticide and Fungicide Act (FIFRA)" when referring to the restriction of pesticide use within the range of endangered species.
4. The Fish and Wildlife Coordination Act of 1934 (amended 1977) stipulates that the Army Corps must consider comments of state and federal fish and wildlife agencies before issuing a Section 404 permit.
5. State laws. There are several coordinating bodies at the state level in California which have responsibility for wetlands uses. There is a statewide coastal commission for tidal wetlands in general. Wetlands issues involving the San Francisco Bay come under the jurisdiction of the San Francisco Bay Conservation and Development Commission (BCDC).

MOSQUITO BREEDING IN WETLANDS

There are basically two reasons why one does or does not find mosquitoes in a given body of water: (1) Female mosquitoes do not lay eggs in the habitat, or (2) mosquito larvae do not survive in the habitat. In both instances, there are various characteristics of water which can affect presence or absence of mosquitoes:

Physical Characteristics.

Mosquitoes are rarely found in water which is deeper than a foot or so. This is also the limit for the depth of emergent vegetation, and mosquitoes will avoid open water which does not provide protection from predation. Also, mosquitoes are rarely found in water which has any degree of movement. Female mosquitoes do not oviposit in such habitats, and water movement flushes any mosquito larvae which are present.

Chemical Characteristics.

Water chemistry is an involved science, but in terms of effects on mosquitoes, the most important considerations are the concentration and composition of various inorganic ions, and organic matter present which can serve as nutrients for mosquito larvae and other organisms. Although mosquito biologists have taken pH measurements for years, this seems to have little effect on presence or absence of mosquito larvae. The ionic composition (which cations and anions are present) has been little studied in relation to mosquito breeding. On the other hand, the effect of ionic concentration is much better known. Mosquito species generally can be divided into three groups: Fresh water breeders, brackish water breeders, and saline water breeders (Bradley 1987). All three groups are represented in both tidal and non-tidal wetlands. The nutrient level present has an effect on mosquito breeding at many levels. Nutrient level in water affects the abundance of plankton (the mixed group of tiny plants and animals floating, drifting, or feebly swimming in the water mass), and thus mosquitoes which feed on them, as well as mosquito predators, and on submerged and emergent vegetation which can provide protection from predators for mosquitoes.

Biological Characteristics.

The relationship between mosquitoes and other biological components of aquatic habitats (invertebrate plankton, prey, and predators; aquatic vertebrates) is very complex and not fully understood. It involves many levels of effects and feeding relationships (trophic levels). The relationship may not be constant, but may vary over the course of a season. Further complicating matters is the fact that manipulating a habitat for minimal mosquito production may not be the optimal situation for other organisms such as ducks or other waterfowl. This applies to nearly all the factors discussed: physical, chemical and biological.

MOSQUITOES AS VECTORS OF HUMAN DISEASE PATHOGENS

There are two aspects of mosquito-borne disease ecology which should be kept in mind:

1. Generally, the higher the density of mosquitoes, the greater the chance of mosquito-host contact, and the greater the chance of transmission of disease pathogens to people.
2. Given species of mosquitoes can be very important in the ecology of human diseases besides their role as primary vectors. They may also serve as vectors between other vertebrate hosts, and thus be factors in the amplification of pathogens, and their availability to primary vectors.

In general, the relationship between human infections of an arthropod-borne pathogen and human cases of a disease caused by the pathogen are pyramidal in shape (Fig. 1), highlighting the fact that the number of cases of a human mosquito-borne disease such as western equine encephalomyelitis (WEE) which are reported as confirmed represent only a small fraction of the number of human infections which occur. Figure 2 illustrates the relationship between mosquito density and all components of the disease pyramid. It stresses that mosquito density is the most important factor in the occurrence of human disease cases (Olson et al. 1979).

Arbovirus transmission cycles can be complex and interrelated, involving several species of invertebrate and vertebrate hosts. The significance of this fact to mosquito planning is that control of secondary vectors is also important, and may influence the possibility of human

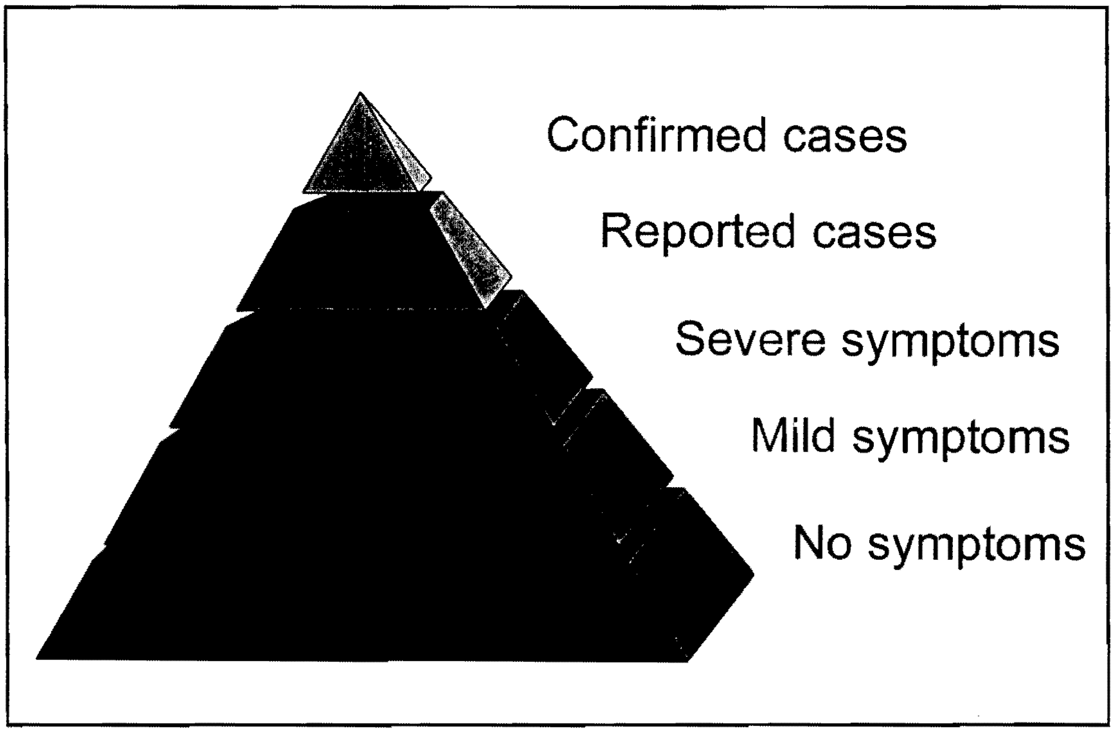


Figure 1. Hypothetical pyramid illustrating the relationship between human infections of a vector-borne pathogen and the number of confirmed human cases of disease caused by the pathogen.

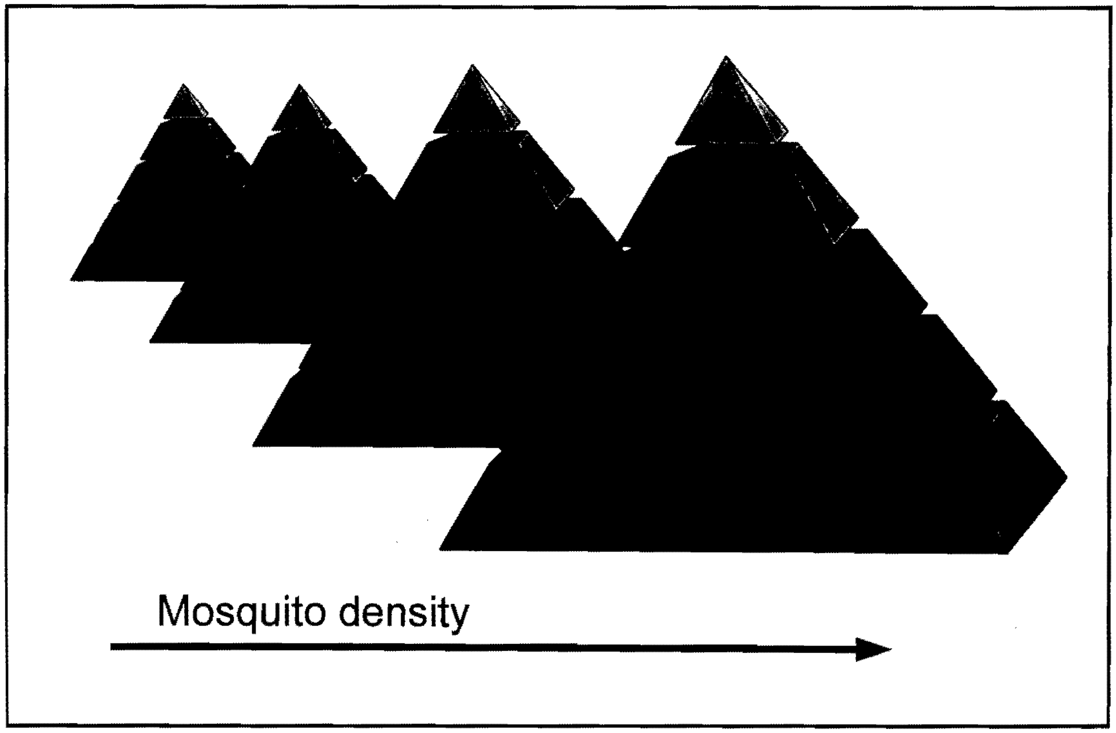


Figure 2. Hypothetical pyramid illustrating the relationship between density of vectors and numbers of confirmed human cases of a vector-borne disease.

infections of a disease such as WEE (Hardy 1987).

PUBLIC HEALTH AND WETLANDS PLANNING

Mosquito and vector control agencies can not effectively protect human health by responding to problems only after they have developed to the point where cases of human diseases are occurring. This requires intervention in the form of broad scale applications of chemical pesticides and other strategies which are not favored by anyone. The alternative to this is to involve public health agencies in the planning and operational phases of wetlands projects, something rarely done. For example, The San Joaquin River Plan was drafted without input from public health agencies.

But even if public health agencies are involved in the wetlands planning process, there will still be some problems with mosquitoes, because there is still much that we do not know about the breeding of mosquitoes.

CONCLUSIONS

The creation of new wetlands will continue. Wetlands restoration is not only inevitable, but it is also an endeavor we should all support. At the same time, we should continue to support our vector-borne disease surveillance system. It is an effective tool for protecting people from vector-borne diseases.

For wetlands creation, restoration and management to take place in a way which will not create public health problems,

1. Public health agencies must be full partners in the planning and operation of wetlands.
2. Public awareness of the positive and negative potentials for wetlands must be raised.
3. Allowance must be made for public funding of the total costs for wetlands, not just for their creation.
4. Public health agencies need to educate the public in a way that distances them from the public perception of them as exploiters of the environment.

REFERENCES CITED

Bradley, T.J. 1987. Physiology of osmoregulation in mosquitoes. Ann. Rev. Entomol. 32:439-462.

Hardy, J.L. 1987. The ecology of western equine encephalomyelitis in the Central Valley of California, 1945-1985. Am. J. Trop. Med. Hyg. 37:185-325.

Kahrl, W.L. 1979. California Water Atlas. Governor's Office of Planning and Research, Sacramento, CA.

Leitch, J.A., W.B. Clifford, J.L. Richardson and J.H. Sather. 1994. Wetland Policy Issues. Council for Agricultural Science and Technology. 47 pp.

Norris, R.M. and R.W. Webb. 1990. Geology of California, 2nd edition. John Wiley & Sons, Inc., New York, NY. 541 pp.

Olson, J.G., W.C. Reeves, R.W. Emmons and M.M. Milby. 1979. Correlation of *Culex tarsalis* population indices with the incidence of St. Louis encephalitis and western equine encephalomyelitis in California. Am. J. Trop. Med. Hyg. 28:335-343.

Salvesen, D. 1990. Wetlands: Mitigating and Regulating Development Impacts. The Urban Land Institute, Washington, DC. 117 pp.

Watkins, T.H. 1973. California, an Illustrated History. American West Publishing Co., Palo Alto, CA. 543 pp.

ACTIVITIES OF THE STATEWIDE AD HOC AFRICANIZED HONEY BEE STEERING COMMITTEE[1]

Bill Routhier and Franklin Ennik[2]

California Department of Food and Agriculture
7845 Lemon Grove Way, Suite A
Lemon Grove, California 91945

In anticipation of the arrival of the Africanized Honey Bee (*Apis mellifera scutellata*), both houses of the California State Legislature passed resolutions in 1989 asking the Governor to appoint an Africanized Honey Bee (AHB) Task Force. The Africanized Honey Bee Action Plan was completed by the Task Force in late 1989. Task Force participants included the California State Departments of Food and Agriculture (CDFA), Health Services (CDHS), Emergency Services, Education, Tourism and Justice as well as the University of California (UC), the California State Beekeepers' Association, the California Farm Bureau Federation and the California Agricultural Commissioners' and Sealers' Association.

The Task Force's report was submitted to the Governor's Office in December of 1989. The report recommended a three-phase plan to prepare for the bees' arrival and to mitigate harmful effects once the bees are established. There were many actions outlined in the AHB Action Plan which could be implemented without the need for new resources. They included the following activities:

1. Establish an AHB information network among state agencies.
2. Develop mailing lists for groups targeted for information and training.
3. Set-up and maintain a data bank on AHB biology, behavior, control measures and identification procedures for multi-agency use.
4. Develop information brochures for the general public and targeted groups.
5. Identify and prioritize AHB research and disseminate research findings.
6. Draft AHB quarantines.
7. Develop guidelines for first aid and treatment of multiple bee sting victims and reporting protocols.
8. Keep all agencies apprised of AHB migration.
9. Address statutory changes relative to AHB threat.
10. Suggest a model bee keeping ordinance for counties and cities.
11. First aid training in response to human and animal stinging incidents.

The Governor's Office approved release of the AHB Task Force Report and implementation of the included budget neutral action items in October, 1991.

Late in 1992, all former members of the AHB Task Force were contacted and the CDFA, CDHS, UC Division of Agriculture and Natural Resources and the California Agricultural Commissioners' and Sealers' Association agreed to work cooperatively as an Ad Hoc Steering Committee to provide overall leadership for state and local agencies. A memorandum of understanding (MOU) was signed by representatives of the four above agencies in February of 1993 and thirty-five different state, federal and local agencies were invited to participate as members of either a technical working group or a coordinating council.

Since there seemed to be no restrictions on the northward migration of the bees, preparing for their eventual arrival was viewed with some urgency. A wealth of information had already been developed by the United States Department of Agriculture (USDA) and the states of Texas and Arizona based on their experience of the impacts caused by the AHB. All of these materials,

[1] This paper was presented for the authors at the CMVCA annual conference in Sacramento by Charles M. Myers, California Department of Health Services, 2151 East D Street, Suite 218B, Ontario, California 91764.

[2] California Department of Health Services, 2151 Berkeley Way, Room 506, Berkeley, California 94704.

information, and ideas were incorporated into the response plans developed by the State of California and its counties.

The Ad Hoc Steering Committee has met bi-monthly at various locations since February, 1993, and its chair has rotated routinely as outlined in the MOU. Five sub-committees were formed to better address the ultimate establishment of AHB and their activities are summarized below.

Research Sub-Committee.

This sub-committee was charged with providing information and direction on AHB identification and learning to live with the bees; adapting the apiary industry to accommodate their presence and studying their pollination practices and habits. Procedures used by federal, state and county personnel to identify AHB are based upon three levels of testing: observations on habits and behavior, multi-variate morphometric analysis and mitochondrial DNA analysis.

Local counties have received initial identification training and are cooperating with CDFA. An information network has been established between the UC, the state of Arizona and members of the sub-committee. The CDFA Bee Swarm Trapping Manual has been approved. The sub-committee has channeled the research needs of California growers and local governments to principal researchers. Research in progress includes the development of rapid inexpensive diagnostic techniques such as ELISA kits, the effects of hybridization on colony defense and a search for additional nuclear markers. Guidelines were developed to define areas declared as "africanized". The sub-committee continues to stress the importance of continued funding to the UC system for AHB research.

Public Relations Sub-Committee.

This sub-committee was charged with establishing a state and local network to disseminate information on the arrival and migration of the bees. A media conference plan was adopted with designated participants, protocols and notification procedures. The committee established contacts with local agencies in each county and a listing of available PR resources was sent to each local task force. Pamphlets were prepared in English, Spanish and several Asian languages with masters available for distribution. Press kits were sent to newspapers and selected radio and television stations. School-age curricula (K-12) were developed and were made available, particularly in Southern California. A presentation consisting of forty photographic slides and accompanying script was prepared with one hundred copies produced for distribution. A general information AHB video was prepared for public use. Prior to the formation of this sub-committee, 500 copies of CDFA's AHB reference manual were distributed to all city managers and county boards of supervisors in Southern California, all agricultural commissioners, CDFA Plant Industry offices and UC Extension offices statewide, and to numerous private organizations.

Regulatory Sub-Committee.

This sub-committee was charged with investigating and proposing changes in existing state regulations and laws relating to AHB. It was the sub-committee's findings that regulations designed to limit the natural spread of AHB would be ineffective, costly and counterproductive. At best, by regulating commercial beekeepers, the public, agricultural users and local governments could be assured that commercial beekeepers and agriculture were allies and not enemies. Quarantine measures, as a means of restricting AHB movement, were rejected by CDFA. Much of the sub-committee's work went into encouraging a California self-certification program. The recommendation, however, was rejected by the California State Beekeepers' Association but was pursued by the San Diego/Imperial Counties Beekeeper's Association.

Control and Prevention Sub-Committee.

This sub-committee was charged with developing practical methods of prevention and abatement, mitigating the effects of multiple sting episodes, preparing informational brochures and training materials, and preparing emergency response protocols.

A major accomplishment was a Section 24(c) registration of the insecticide formulation M-PEDE® for use against AHB swarms by governmental agencies and commercial Pest Control Operators (PCO's.)

Swarm control will ultimately be a regional issue handled by local government, local emergency response personnel, local vector control agencies and private pest control operators. Training and certification of vector control technicians and private pest control operators was provided by the UC, California Mosquito and Vector Control Association (CMVCA) and Pest Control Operators of California (PCOC). Emergency rescue personnel have been trained in the control of attacking bees and victim rescue operations. Legislation allowing for the abatement of abandoned hives and equipment, certification of PCO's, and training of beekeepers has been signed into law. Health advisories informing physicians, EMS responders, hospitals and clinics about the effects of multiple stings have also been distributed. A variety of brochures and educational materials have been developed and distributed. A model bee keeping ordinance was developed and made available to cities and counties.

Funding Sub-Committee.
No new funding sources were identified.

CONCLUSIONS

There seems little doubt that if AHB become established in the highly urbanized and agricultural areas of California it will negatively impact our economy and public health. Intelligent planning and preparation will prove useful in alleviating the public panic and hysteria associated with these insects. Public information and education will be of tremendous importance in preventing injury and in preparing our medical systems to handle this threat.

We wish to thank the individuals from the many agencies who have made significant contributions to the Ad Hoc Steering Committee. Efforts by the Committee have done much to achieve the budget neutral objectives outlined above. However, these objectives represent only the tip of the iceberg, and much more needs to be done to mitigate the impacts on agriculture, tourism, public health, housing and the economy in general.

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:60-61

THE IMPORTANCE OF LEGISLATIVE NETWORKING

Elizabeth Ann Cline

Fresno Westside Mosquito Abatement District
P.O. Box 125
Firebaugh, California 93622

The need for developing relationships with your legislators has never been greater. Prior to passage of Proposition 13, it was not a pressing issue because we were insulated from politics by the health and safety code. Our funding was independent from state control. Prop. 13 changed all of that, however, by allowing the State to distribute all property taxes remaining after the Prop. 13-imposed rollbacks and caps. The money which your constituents voted as a tax upon themselves to support you, no longer exists. All property taxes are now pooled and distributed by politicians. A whole new ball game!

We have been very successful in our mosquito and vector control efforts in the past. Citizens look around and don't see mosquitoes or disease to threaten them. They then look at us and say, "We don't have these problems. Why do we need you?" To them, I say, "Thank you very much! We appreciate the fact you recognize the good job we are doing. The problems are not here because we are here."

The problems which created the original mosquito control agencies in the early 1900s are still with us. They are under control to a certain degree, but they still exist and, without us, would eventually return with a vengeance. People must understand the public health importance of what we do!

Another problem, is the uneducated politician and staff. With term limits in Sacramento, we now have a turnover of politicians and staff members that makes it difficult for us to develop long term relationships and educate them as to what we do and why. This is the reason our efforts at legislative networking must be continuous. We cannot assume our message is known in Sacramento. We must continue to tell our story to anyone and everyone that might have an impact on us. We must be known to all of them.

The present fiscal crisis in California has created most of our problems. When the State had money, it did not need to look for more. The legislators were content to leave us alone, to some degree. My, how times have changed! Unfortunately, we are easy pickings! According to the politicians, we have no constituency. They have no one coming into their office to complain about us. However, they also do not have anyone praising or defending us. That is because the public, in general, does not give us much thought, at all. They only complain about or defend those programs that are on their minds or have an immediate affect on their lives. If the playground shuts down and their child is used to going there after school, that is an immediate inconvenience to them and they will complain. If the politicians take money from us, the general public does not notice an immediate affect on their lives. By the time we have to cut services enough for them to notice and begin to complain, they have forgotten why we had to cut services.

Developing successful political relationships is a difficult and time consuming job. We must begin by remembering the golden rule, ALL POLITICS IS LOCAL. You better believe it. It all comes back to local politics one way or another. So, our first approach should be through the managers of our districts and their staff. They must have relationships with the city councils, city managers, counties, other local government staff people, etc. They must have these relationships in order to run the district. These same relationships can be used to develop a legislative network. You expand it by using the people you know to get to know the people that you don't yet know. You should encourage your district manager to get to know all of the local politicians and then to graduate to the state politicians and their staffs.

Much of this can be accomplished regionally. One successful method of accomplishing this used in our region, is group meetings. We got together, made a list of politicians and staff people we needed to get to know, made appointments with them and went to see them, as a group. A group of four or five district managers can then have a round table discussion with that politician or staff person and more information is developed because there is more input. The person with whom you are meeting remembers the meeting better because it was a group and he does not have to remember each individual in detail.

Then, you stop in their office whenever you are in the neighborhood. Another helpful tool is your regional meeting. Politicians enjoy addressing groups of the citizens in their local districts. The next time you have a regional meeting, invite a local or state politician to address your group. They will get to know you faster and better. You can begin educating them on the importance of mosquito and vector control and public health. Attend social or political functions where politicians are present whenever you can. You may not get to talk to them much, if at all, but they will see you there. Politicians are very good at remembering faces. Soon they recognize your face when you walk in the door. You are comfortable with them and they are comfortable with you. This is very important. Politicians view strangers who walk into their offices with suspicion. They will always be defensive with someone they do not know. Make sure they know you before you have to walk in and ask for a favor or make a complaint.

Now that you are getting to know your politicians and vice versa, we must also foster our other relationships. The fight for survival must utilize all resources. Public Education is one area where we can increase the awareness of the people as to what we do, why we do it and how we protect them and their families, we must educate the public and they then become an ally when an issue involving us becomes public. Public relations are also very important. Good service to the public in our districts is vital to developing their good will and loyalty. We need them to support us if we have to tight for our funding or perhaps our existence.

Finally, we must become a resource to the people, other agencies, the media, the politicians and their staff. If an issue comes up involving mosquito control, I want that person to come to me with any questions. Only then can I be sure that he has accurate information while we are portrayed in the proper light. At the same time, I am becoming a resource that makes his job easier. We both benefit. Our relationship is strengthened.

Trustee involvement in the process cannot be overemphasized. Politicians do not see our districts as having any constituents, but they look at trustees as politicians who may have constituents who can help or hurt them. Many times, I have been told that the politician would rather talk to a trustee than to me. Why? Because when they look at me, they see staff. When they look at you, they see another politician. Trustees must encourage the efforts of the manager of your district. Whenever possible, you should try to join in those efforts. Try to be present at meetings your manager sets up when a politician will be there. Join your regional group in visits to politicians' offices. Attend social and political events so you too can become known to that politician.

You, as a trustee, are already active in your community. There are many people with whom you already have a relationship that can help you in your efforts to become more involved politically. Talk to people about mosquito and vector control, about the situation with our funding, and about the State and the actions they have taken. Who are your constituents? Everyone in your community and in your district. Everyone who does business with your community and in your district. Everyone who does business with the district itself. If the district suffers, all of these people will be affected. All of these people can be asked to help educate politicians, if they know the situation.

Encourage communication between the district and your constituents, your constituents and the politicians, your constituents and the media, the media and the politicians, etc. Make sure that all of these people are educated and talking to one another. Promote the benefits of mosquito and vector control. Make sure everyone understands the consequences if mosquito and vector control should be lost or even just diminished.

When dealing with legislators, it also helps to know who their constituents are. If you cannot get through to your politician, rest assured that there is someone who can. Find out who their constituents are and you will know who to talk to when you need that politician to really listen. Who are the politician's constituents? They are the contributors to his campaign. Large contributors usually have even more influence, despite assurances to the contrary. You can easily obtain this information from the elections office or his campaign committee. The politician's other constituents may be his advisors, the people in his district (especially in groups), the media, his family, his friends, etc. These are the people to whom a politician listens. ALL POLITICS IS LOCAL. Again, you want to encourage communication between all of these people and the politician. Make sure that they are all well versed in the message and let them deliver it. They will back you up and strengthen your position.

When you talk to any of the people we have discussed, make sure you offer yourself as a resource. Most of them will be very happy to know someone they can call on for information or assistance on the subject of mosquito and vector control and public health. If you cannot answer their questions, either get someone else to or get the information yourself and report back to them, quickly. You have helped them and they have helped you. You both benefit and they won't forget it.

We may not win all battles, but if we fight valiantly and continuously, we will win some of them and future engagements will be easier for our past experiences. Get to know your local and state politicians. Above all, let them get to know you.

WHY MOSQUITO ABATEMENT DISTRICTS SHOULD BECOME VECTOR CONTROL DISTRICTS

Gilbert L. Challet

Orange County Vector Control District
P.O. Box 87
Santa Ana, California 92702

There is a need for a community based speciality unit of government to respond to community-wide vector, vector-borne disease and nuisance insect problems. Fortunately, there are vector control districts provided for in the California Health and Safety Code. These are special district forms of government designed to respond to these very problems in the community in a timely and responsive manner. These vector control districts are not held back from addressing problems by an unresponsive bureaucracy, unconcerned politicians or excessive rules.

In California at this time is a movement to reinvent government. Part of that movement targets the elimination of special districts, saying we are shadow government, unresponsive to the public and have unknown boards. It is interesting now that a certain county has declared bankruptcy, these same charges are being levied against that county. In fact, the cities are saying they do not need county government any more. Part of this reinvention process is the thought of moving special districts into county or city government.

In my opinion, this is a mistake. In almost every case in California where vector control is within county government (a district governed by the Board of Supervisors), the administrators of those programs say they would be more effective as special districts governed by a Board of Trustees. Also, it is my understanding that all of the money collected to do vector control in those county programs is not spent on vector control, but used in other closely related county programs. In fact, in many situations where a Board of Supervisors establishes a district, assumes the powers of a district and collects fees for vector control, I believe they are using this process to be a revenue source for other programs. There should be an audit of these programs.

The state of New Jersey is in the midst of a situation where a number of their mosquito control commissions (similar to our districts) are being absorbed into county departments. The experience for those independent commissions being put into a county department has not been good. First, the mosquito control program has been buried in a larger county department, usually the road department. The program is then considered just another program and has to compete for funds. Since there are no mosquitoes in the winter, typical winter activities such as source reduction are eliminated. It is difficult to imagine that a vector control program as part of larger county programs would have fewer barriers for purchasing and personnel hiring, fewer barriers to the media, fewer barriers to offering public education, fewer bureaucratic problems and less paperwork. Vector control units in large multipurpose units of government do not survive.

The local mosquito abatement districts have to be more responsive to the growing political trends of consolidating special districts, increased public concerns over new and/or introduced pests and diseases and peculiar vector problems within each district. In my opinion, our districts should become medical entomological resources for the community. This would include surveillance programs for all vector-borne diseases, controlling additional vectors such as flies and rats and providing medical entomological consultation (including forensic entomological consultation) for city and county police forces.

Boards of trustees should review what they are currently doing and look into controlling rats, flies and Africanized honey bees (AHB) as well as any new additional programs. Why should mosquito abatement districts consider controlling other vectors and surveying for other vector-borne diseases? The answer is - "Political Survival!" With dwindling state, county and city budgets it makes sense for a specialized labor intensive activity to be done by a special district which is funded specifically for this activity.

Board members, if you need to know what your district should be doing, there are two articles that can help you. The first was published in 1973 by Hatch and others in Mosquito News (Vol. 33:278-233) and the second in 1991 by Challet in the Journal of the American Mosquito Control Association (Vol. 7:103-106).

If you are in an urbanized area you should be especially aware of a single purpose (mosquito control) agency being an insignificant part of the political landscape and being put into a larger governmental organization.

You should look at what you are doing, at what other problems are in your community that would be in your purview and how you should address them. Many communities are experiencing rat problems - wouldn't it make sense for a vector control district to at least give information to a homeowner on how to deal with a rat problem. There are a number of related vector problems which districts should evaluate for potential inclusion into their programs. They are:

1. Africanized honey bees (AHB)
2. Chironomid midges
3. Flies
4. Biting midges
5. Ticks
6. Stinging hymenoptera
7. Rodents other than rats

Others of concern are:

1. House dust mites
2. Hantavirus rodent hosts
3. Forensic entomology
4. Delusory parasitosis

And how about disease surveillance programs for the diseases vectored by these organisms? How many of your districts have detection programs for Lyme disease, plague, Hantavirus or erhlichiosis? How many of you encourage specimen identification for the public or other governmental agencies? The more you are part of the fabric of local government by offering the highest quality community service, having effective operational programs and providing excellent public relations, the greater your chances of survival.

Boards of mosquito abatement districts should consider becoming a vector control district. Go back and ask your manager what other vector related problems are out there. Do not take "it's not a problem" for an answer. Get the facts. Look at your local situation. Review the number of calls the district gets on various vector-related subjects. Also, get some data from health departments.

In the 1994 CMVCA Yearbook, there are 50 member agencies listed and 24 of them have the word vector in their name. In the 1984 Yearbook, there were only two agencies with vector in their name. So, in California, the tide is shifting to include vector in the name of the agency and hopefully in providing vector control services.

The United States picture would seem to be changing as well. There is movement in districts nationwide to add the control of additional vectors other than mosquitoes into their programs, but not to add the word "vector" to the agency's name. In the 1988 Directory of Mosquito Control Agencies published by the American Mosquito Control Association, there were 156 agencies listed (22 in California) that performed control of other vectors. This was down from 214 listed in the 1982 Directory. I do not think these numbers are reflective of what is happening in the United States. I think the number of agencies doing vector control across the nation are increasing.

In conclusion, I think that single purpose units of government, such as mosquito abatement districts, especially in urban areas, will not remain in tact in the future. These single purpose districts should review their present situation, determine if there are other vector related problems in their district and pursue the control of those problems for public good and for political survival. After all, what better agency in the community to do this related work. We have the trained technical staff, the operational capability and excellent managers.

SHOULD BOARDS OF TRUSTEES PROMOTE MANAGERS FROM WITHIN?

Gilbert L. Challet

Orange County Vector Control District
P.O. Box 87
Santa Ana, California 92702

Look around this room. Many of the younger people here will be managers of a vector control district in the near future. In most cases, I look forward to an excellent future for these districts.

But what are we doing as agencies to prepare these people for these managerial positions? Should we be doing anything about it? The district board of trustees has a responsibility to prepare for the eventual replacement of a manager by having an assistant manager or preparing another position to take over these responsibilities. There should be ongoing training for these people. This training is available through a wide variety of community sources.

In two recent managerial replacements, the trustees on the interview board were amazed at the lack of candidates with managerial experience at a vector control district. Because of the boards' lack of experience at replacing a manager, these boards thought there should be a cadre of trained, experienced people to take these jobs; very similar to the city manager situation where they work up from smaller to larger cities. We, as part of our business, do not prepare for our eventual replacements. Part of the problem is the provincial nature of our business. Many of us tend to hire local people and they tend to want to stay in the local area. This, at times, restricts the choices a local board has in getting a new manager either by not having someone on staff capable of management or a good managerial candidate not wanting to move to a new location. There are exceptions to this, however.

Life is change. As agencies, we are constantly living with change. Many retirements are caused by the inability to adapt to change or the attitude that they have had enough change. In our business the science changes and the rules by which we operate change and if we do not have managers and staffs that can adapt to these changes, we have serious problems.

We need managers that can adapt to change and use change to benefit the district. I believe they should be college graduates with a biological background. The only downside to this is the only people I know who have been fired from these jobs are college graduates with those qualifications. But changes can he dealt with through education on the problem and how to deal with these problems. In my opinion, managers should be required to have yearly continuing education. This would be very similar to what we require of our technicians, so many hours of specific education per year. This could also help prepare other people in management such as entomologists, assistant managers and superintendents. In Florida, you have to pass an examination before you can be appointed to the job of manager at a District. Maybe as part of the cooperative agreement, management certification should be required.

Probably the biggest headache for all managers, old or new, are personnel problems. We need to train people in those areas before they get managerial jobs. Also, many of today's biggest changes and challenges are in the area of personnel management.

Back to the managerial training. In my opinion, the CMVCA should have a managerial training program. It should be a yearly format separate from our annual conference. Not only would this provide ongoing training for present managers, it would provide training for would-be managers. Also, it would bring trustees up-to-date on new issues.

Providing managerial training is one of the ways we can assure success of the people getting these positions and assure that we have a cadre of people trained to step into these positions. We, as an association, as districts, and as trustees for a district, need to be assured that the person that serves as district manager has had the training needed to do the best job. Some of the trustees in this room are saying, "Why do I have to send my manager to this training? Our district is small. We're out of the main stream and few people in the community know who we are." But when it comes to the time when the grand jury says you should consolidate or be absorbed by the county health department, or you have a wrongful termination suit or you have a pesticide spill, you better have a manager that can deal with those issues.

How does a new manager on the job get this training? That manager has a number of sources available for management training. There is the CSDA that offers a district management institute, as well as training in areas where the law has changed recently. There are a number of management seminar companies that offer training, usually one-day seminars, for management employees. There are university and state college extension courses, as well as community college courses.

Another item that reinforces the management training for both the manager and the board of trustees is to put management training as a line item with an amount of money attached in your yearly budget. Board members, follow up with your oversight duties to see that your manager gets the training needed.

In this respect, it may be advisable for the board of trustees to have a contract with the manager that requires ongoing management training. At several seminars given by the California Special Districts Association, the seminar leaders, Michael Glase and Betty Harrison-Smith, advocated manager contracts on a yearly or longer basis. During the development of the contract with its attendant goals, this management training could be listed as one of the goals.

When I first became manager of a district, I immediately looked for a book to tell me how to do this job. I can assure you that there are no books that do this. But there are classes and courses you can take, as well as an overabundance of books on management. You should do a self-assessment to determine your needs in training and get that specific training. I have found for me that seminars for three days or less suit my needs and schedule. Semester long classes are difficult for timing reasons. Most helpful was a CSDA seminar that was three or four days long that I took with Jim Caton and Bill Hazeleur. We were immersed in different problems daily, followed by sessions on how to solve them.

In conclusion, the points I would like to make are:

1. Managers and boards of trustees should start preparing for management replacement by getting someone else on staff trained in district management.
2. CMVCA should provide management training on a yearly basis to all managers and want-to-be managers.
3. Young people desiring to be managers of districts should start getting training now.

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:66-70

HOW EFFECTIVE IS YOUR MANAGEMENT TRAINING?

Charles Beesley

Contra Costa Mosquito and Vector Control District
155 Mason Circle
Concord, California 94520

Does your mosquito or vector control district have a management training program? Do you want to be a better manager? If you are a trustee, does your district provide on-going education/management skills training for your manager or management trainees? If you answered "no" to any of these questions, it may be that your district is not effectively supporting the manager. In today's increasingly political world, a well-trained manager is essential to program stability, growth and development. The skills, knowledge and abilities of management must keep pace with technical develoments, labor laws, and public sector demands if you expect your district and manager to be effective.

My goal today is to outline the basics of a successful training program for managers. To accomplish this, I will present you with an inventory of essential management skills by which to measure yourself, or your district, and enable you to determine what skills and knowledge you need to develop and reinforce. Although much of what I say may like seem common knowledge, this does not mean management comes naturally.

BACKGROUND

There appears to be little commitment to management training, development and on-going support within our field. The earliest evidence I have found was a seminar at Asilomar, California in 1959, entitled "Management for Mosquito Control Administrators." It took another thirty years before the California Mosquito and Vector Control Association (CMVCA) and the American Mosquito Control Association (AMCA) sponsored similar workshops in 1991. Despite the relevant information at these workshops, there was no direct continuity between them. Since then, the CMVCA Management Committee has conducted intermittent management seminars to address various management topics, but to my knowledge, there is no "master plan" to train managers within our profession. This needs to be addressed because although the job of manager requires fundamental skills that are universal and timeless, it also requires the continued development of new skills and knowledge, especially for those who wish to become an effective manager in local government in the 21st century.

This sporadic approach to management training is unfortunate and is the opposite of one of the fundamental tenets of good management practices: To provide on-going training and development of employees. All mosquito and vector control agencies in California provide on-going training for Certified Technicians, as evidenced by our Continuing Education Program. There should be a similar program for management personnel. Our skills and knowledge need to be developed and updated just like anyone else. I believe this will become more critical for the next generation of managers, because of the increased scrutiny of public agencies and the increased demands by the public for effective local government in times of limited resources. The manager, in our field and others, is the final litmus test of program credibility. It is the manager who most represents the agency, and who ultimately sets the stage for community trust and credibility. The public, board and employees constantly assess your knowledge, skills and leadership abilities, which in turn establishes your credibility and level of trust within these different groups.

NEEDED SKILLS AND KNOWLEDGE

There are many definitions of a manager. Webster's dictionary defines the verb "manage" as: "to handle with a degree of skill or address, to direct or carry on business or affairs." While the type of business will vary, the essential skills, knowledge and other characteristics unique to the job of management can be extended to mosquito and vector control management positions.

I believe that the job of manager can be divided into eight distinct functions, which are discussed below. Two examples of each function are provided, and a distinction is made as to whether or not they require good skills, knowledge, or some other intangible characteristic, for effective management. I recognize that there is often a direct relationship between skills and knowledge, for knowledge is often the prelude to the development of skills. Webster's defines these three characteristics as follows:

Skill -	(n) proficiency, ability or dexterity
Knowledge -	(n) the state or fact of knowing, familiarity, awareness or comprehension acquired by experience or study
Intangible -	(adj) incapable of being defined, especially an asset that cannot be apprehended by the mind or senses

The eight distinct functions of the job of a manager (as listed in Table 1) are:

The Administrator.
The role of an administrator requires good organizational and planning skills, whether it involves board or staff activities, or projects requiring teamwork. Knowledge of administrative skills is imperative in creating an environment that supports teamwork within an organization. A good administrator will know the environment that fosters teamwork among subordinates, and will make it happen.

The Communicator.
An effective manager knows how to communicate. Managers write numerous status reports and policy documents which have a variety of audiences, from other staff, academicians, boards of trustees, to the public we serve. Managers must also be effective public speakers. The ability to make effective presentations must not be overlooked, as this strongly influences public perception and confidence in a district's agenda and program. It is also essential when the audience is staff, and the manager must win their support. An effective, well trained manager must be able to speak to the community they serve, so that the community knows about mosquito and vector control, understands the district's role, and supports its programs.

The Finance Director.
It takes money to provide a good community public health program. How to get that money, how to keep it, and how to properly manage it over the long term is a key component of any organization. A mosquito and vector control district manager must have a fundamental knowledge of accounting principles as they relate to the operation of a public agency and its fiduciary responsibilities. Knowledge of investment practices and procedures is also worthwhile, because effective utilization of reserves can make the difference between district success or failure. Continuous, on-going education in finance is essential, especially given the ever-increasing State budget woes and their impact on special districts.

The Personnel Analyst.
Managers manage people, or human resources as it is now known. This includes all aspects of hiring and training personnel: having a thorough understanding of labor laws, and developing good personnel policies and practices. It may also require being an effective labor negotiator. It is important to understand that the manager plays a key role fostering the growth and development of employees. Managers are also in a key position to motivate employees, to excite them to want to learn and improve themselves, and the organization. If this role is overlooked, the organization can become stagnant and unable to reach its full potential.

The Information Technologist.
We live in an expanding information age which will only continue to change. We see this in all aspects of our lives, including our offices, laboratories and field programs. It is essential that our agencies keep pace with technological changes, if we want to maintain first class control operations and our independent agency status. Managers, as well staff, must have a good understanding of computer software and hardware, and be able to facilitate the design of an effective computer system that can be upgraded when needed. The imminent use of information systems, whether external networks or large databases, will continue to stretch our knowledge and abilities. The manager who has up-to-date training and experience in this area will ensure that his/her district will be among those that can assimilate information and data quickly, and be responsive to changing conditions in public health.

The Leader.
Harold F. Gray, who attended the Asilomar conference in 1959, had an important message regarding leadership qualities: "At least two characteristics are essential for a successful manager: integrity and respect. He must be respected by his employees, associates and the public. He must have integrity, not only the integrity expressed as honesty in money matters, in keeping his given word, but intellectual honesty as well." I would

Table 1. Management skills, knowledge and abilities inventory necessary to be an effective mosquito or vector control agency manager.

FUNCTION	SKILL	KNOWLEDGE	INTANGIBLE
Administrative			
Organization/Planning	X		
Teamwork	X		X
Communication			
Presentations	X		X
Speaking/Writing	X		
Community			X
Finances			
Accounting		X	
Investments	X	X	X
Personnel			
Employment Practices	X	X	
Organizational Culture	X		X
Information Technology			
Office Technology	X	X	
Information Systems	X	X	
Leadership			
Integrity/Respect			X
Developing Subordinates	X		X
Public Agency			
Program/Practices	X	X	
Laws/Regulations		X	
Technical			
Program/Growth		X	
Research and Development		X	

Table 2. Assessment of a hypothetical mosquito or vector control agency manager or management candidate based upon a completed skills, knowledge and abilities inventory.

FUNCTION	EXPERIENCE	RATING	DEVELOPMENT
Administrative			
Organization/Planning	A Lot	Good	N/A
Teamwork	Some	Fair	Needed
Communication			
Presentations	A Few	Poor	Needed
Speaking/Writing	A Lot	Good	N/A
Community	A Little	Fair	Needed
Finances			
Accounting	None	Poor	Needed
Investments	None	Poor	Needed
Personnel			
Employment Practices	Some	Fair	Needed
Organizational Culture	Some	Fair	Needed
Information Technology			
Office Technology	Some	Fair	Needed
Information Systems	A Little	Poor	Needed
Leadership			
Integrity/Respect	Some	Fair	Needed
Developing Subordinates	Uncertain	Good	N/A
Public Agency			
Program/Practices	A Lot	Good	N/A
Laws/Regulations	A Lot	Good	N/A
Technical			
Program/Growth	Some	Fair	Needed
Research and Development	None	Poor	Needed

add that the manager as a leader must also foster growth and development of subordinates for long term effectiveness of their agency.

The Public Agency Representative.

A manager of a public agency has different demands and expectations than that of a manager in the private sector. To be successful, the laws that regulate public agencies must be well understood, and managers must be willing to practice them in an objective, consistent fashion. An effective public agency representative is receptive to the many external factors that influence the way any agency functions. For instance, records are public, business meetings are conducted in compliance with the Brown Act, and employees have a right to be represented by labor unions. All of this requires certain specific managerial skills and abilities. In a sense we work in glass houses, open to scrutiny and criticism. A manager must be well-trained and able to respond effectively to these public pressures.

The Technician.

Mosquito and vector control programs are more technical in nature than many public agencies. Information is constantly changing in response to new research developments. A good manager is also a technician with a solid foundation in all aspects of mosquito and vector control. A manager who maintains his or her technical skills can effectively incorporate a research and development component into the organization, to further enhance both program knowledge and service capabilities.

MANAGEMENT ASSESSMENT

With the above eight functions in mind, it is now possible to develop an individual hypothetical assessment of a manager or management candidate (Table 2). This assessment is done by conducting an objective review of their level of experience, skills and knowledge.

The management assessment can then be used as the basis for developing an individual training program, to fill in gaps, or to improve existing strengths of the manager or management candidate assessed previously (Table 3). Items can be added or subtracted as needed.

SUMMARY / RECOMMENDATIONS

The purpose of this discussion is to stimulate ideas regarding management training. Eight functions have

Table 3. Personalized training program for a hypothetical mosquito or vector control agency manager or management candidate based upon a completed experience, skills and knowledge assessment.

FUNCTION	ACQUISITION METHOD					
	GOAL	WORK	HOME	TRAINING	EDUCATION	DEGREE
Administrative	Better teamwork	Specific projects				
Communication	Conduct presentations	Speeches	Toastmasters			
Finances	Enhance knowledge	Basic accounting	Read	Yes	Register this year	Maybe
Personnel	Be more interactive with employees	Set aside time to meet with employees		Maybe	Maybe	No
Information Technology	Get a better understanding	Review Systems Goals & Design	Read		Maybe	N/A
Leadership	Broaden overall skills	Schedule activities	Join a service club	Maybe		
Public Agency	Better public image	Reports or projects				
Technical	Program growth	Staff projects				
Other	Better credibility	Get employee feedback				

been presented which can be used as measures to judge effective management. In reality these eight functions can be distilled into to two separate skill categories: technical and human. I want to emphasize that although the bulk of the functions fall into the technical category, the importance of the human resource function should not be overlooked. As most of you know, this is often the most difficult aspect of management. Without the ability to deal effectively with agency personnel, accomplishing district objectives will be difficult, as these are based on motivating others to get the job done in a timely and effective manner.

It is my belief that most good managers are developed, not born. This is achieved through a progressive development of relevant skills, based upon both a fundamental knowledge and understanding of the type of business involved, and a willingness to continue learning how to be an effective manager. My objective has been to illustrate how the many functions or aspects of management may fit together and be utilized by employees, managers or boards.

If your agency does not have a management training program currently in place, consider the following potential benefits before you rule it out.

- A more informed Board
- A better trained manager
- Greater management accountability
- More comprehensive management evaluations
- Higher/improved employee morale
- Established career path guidelines
- An improved public image
- A more productive agency
- A more responsive agency

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:71-75

VECTOR CONTROL SUMMARY TABLES FROM C.M.V.C.A. MEMBER AGENCIES, 1994

Donald A. Eliason

CMVCA Executive Director
8633 Bond Road
Elk Grove, California 95624

For many years, the C.M.V.C.A. has published in its annual yearbook summaries of the vector control resources expended by its member agencies during the previous year. The compilation and tabulation of this information from the member agencies is an arduous and time-consuming task which has occasionally delayed publication of the annual yearbook.

Therefore, at the suggestion of the Executive Committee, presentation of the summary tables has been moved to the annual *Proceedings*. Information contained in the summary tables within each volume of the *Proceedings* will reflect the combined expenditures of vector control resources by the member agencies for the calendar year prior to the annual conference.

AIRCRAFT USE IN CALIFORNIA MOSQUITO CONTROL - 1994

	Agency	Type	Hrs. Flown	Acres Treated	Cost per Acre
Agency Owned	Butte County	(2)Gruman Ag. Cat	Liq. 131.1	34,965	$2.05
	East Side	Cessna Ag.Truck 300 49J & Cessna Ag.Truck 91R	Liq. 72	18,314	$4.88
	Fresno Westside	Piper Brave PA 36-300	Liq. 127:88	10,023	$5.37
	Kern	Thrush Commander	Liq.118:35	7,650	$9.40
	Merced Co.	Cessna 188-B	Liq. 124 Gran. 1	46,633 20	$.77 $3.81
	Tulare	Piper Pawnee	Liq. 36	1,362	$13.10
	West Side	Hovercraft	Liq. 30	500	$4.50
Contracted	Alameda Co. MAD	Hiller Helicopter 12E, N68012	Liq. 6:2	66	$6.98
	Delta			40	$10.00
	Lake County	Weatherly	Gran. 11:75	1,682	$4.50
	Marin-Sonoma	Helicopter	Liq. 16:90	1,435	$6.48
	Napa County	Helicopter	Liq. 4.2	396	$6.50
	No. Salinas Vy	Helicopter	Liq. 16:27	2,441	$850/ hr.
	Sac/Yolo	(5) Gruman Ag. Cat	Liq. 291:2 Gran. 3:4	72,145 382	$1.72 $3.78
	San Joaquin County	Hiller Helicopter 12E	Liq. Gran.	20,000 30	$3.75
	San Mateo Co.	Helicopter	Liq. 2.3	510	$3.29
	Santa Clara County	Helicopter	Liq. 7:6	1,280	$4.24
	Santa Cruz	Helicopter	Liq: 4.2	250	$15.12
	Solano Co.	Ayres S 2R Thrush Hiller Helicopter	Liq. 3/3.5	435/1050	$300/hr $550/hr
	Sutter-Yuba	Cessna Ag Husky	Liq. 156	78,115	$.45
	West Side	Ag. Cat	Liq. 8.9	320	$10.00

Insecticides used for Control of Mosquito Larvae in 1994*

Agency	GB 1111 gal	GB 1356 gal	Bti Liquid gal	Bti Granule lbs	Bti Briquet lbs	Bti Tech. P. lbs	Malathion lbs	Methoprene lbs	Baytex lbs	Dimilin lbs	Pyrethrin lbs
Coastal Region											
Alameda County MAD	3,372		136	7,227				16			
Alameda County VCSD		<1									
Contra Costa MVCD	4,687		17	29				46		<1	
Marin-Sonoma MAD	441		524	4,901		246		33			
Napa County MAD	424		86			171		3			
N Salinas Valley MAD	1,994		173		46			1	75		<1
San MAteo County MAD	3,958		11					46			1
Santa Clara County VCD		62	239		192			27			
Santa Cruz MVCD	939		<1	24				<1			
Solano County MAD	591	96	45	2,320		26		30			
Subtotal	**16,406**	**158**	**1,230**	**14,501**	**238**	**444**	**0**	**205**	**75**	**0**	**1**
Sacramento Valley Region											
Burney Basin MAD		35	9	4,560	<1			<1			
Butte County MVCD	1,025		1,107				4,746	<1	132		
Colusa MAD	125							<1			
Durham MAD											
El Dorado Co. V.C.-CSA3		11						<.1			
Glenn County MVCD											
Lake County MAD	5			12,285				<1			5
Pine Grove MAD			13								
Sacramento-Yolo MVCD	15,795		7,106	1,043			1,564	86			
Shasta MVCD	1,868		114	8,106				7	<1		
Sutter-Yuba MVCD	1,157		2,445					<1		2	
Tehama County MVCD	478		23	24	<1			<1	2		
Subtotal	**20,453**	**46**	**10,817**	**24,018**	**0**	**0**	**6,310**	**93**	**134**	**2**	**5**
No. San Joaquin Valley Region											
East Side MAD	14,034		16				1,112	14			
Merced County MAD	11,462		668					424			
San Joaquin MVCD	39,853		4,725	1,722			184	44			
Turlock MAD	15,520		8	200				37		4	
Subtotal	**80,869**	**0**	**5,417**	**1,922**	**0**	**0**	**1,296**	**519**	**0**	**4**	**0**
So. San Joaquin Valley Region											
Coalinga-Huron MAD											
Consolidated MAD	16,244		4			66		100			
Delano MAD											
Delta VCD	5,575		79	110	<1	54		38			
Fresno MVCD	4,536		26	1,240		2		31			
Fresno Westside MAD	475		663					29			
Kern MVCD	34,986		1,353		4	49		97			
Kings MAD	35,037							4			
Madera County MVCD	12,428		398					30			
Tulare MAD	29,906							20			
West Side MVCD	5,013		73	218				<1			<1
Subtotal	**144,200**	**0**	**2,593**	**1,568**	**4**	**171**	**0**	**350**	**0**	**0**	**0**
Southern California Region											
Antelope Valley MVCD	86		<1					<1		18	17
Carpinteria MAD-Goleta VY VCD	110			40	6	1		<1			
Coachella Valley MAD	3,473		4,800	23,602				22			
Compton Creek MAD	32				23					<1	
Greater L.A. County VCD	4,759		342	115				49	<1	65	<1
Los Angeles Co. W. VCD											
Moorpark MAD	37		<1	78	4			<1			
Northwest MVCD	4,397		23	872				<1		<1	<1
Orange County VCD	5,860			18,860	4,160			9			2
Owens Valley MAP	99					18,586		<1			
San Bernardino Co. VCP											
San Gabriel Valley MAD	555		7					2			
West Valley VCD	5,894			111	15		51	4			<1
Subtotal	**25,323**	**0**	**5,171**	**43,478**	**4,208**	**18,587**	**51**	**86**	**0**	**83**	**19**
Total	**287,251**	**204**	**25,227**	**85,487**	**4,449**	**19,202**	**7,658**	**1,252**	**210**	**89**	**25**

*120 lbs of methyl parathion was used by Fresno Westside MAD and small amounts of several other insecticides were used for larval mosquito control including: statewide totals of less than 20 pounds each of Dursban G, Abate, Tempo 20, Baytex G and Baygon; and, less than 20 gallons each of Aerosurf and Flit MLO.

Insecticides for Control of Mosquito Adults in 1994*

Agency	Malathion	Pyrethrin	Permethrin	Resmethrin	Dursban	Tempo20	Baygon
Coastal Region							
Alameda County MAD							
Alameda County VCSD							
Contra Costa MVCD		2		1			
Marin-Sonoma MAD		24		4			
Napa County MAD				4			
N Salinas Valley MAD							
San MAteo County MAD		<1					
Santa Clara County VCD							
Santa Cruz MVCD							
Solano County MAD		37	<1				
Subtotal	**0**	**62**	**0**	**9**	**0**	**0**	**0**
Sacramento Valley Region							
Burney Basin MAD			9				
Butte County MVCD	1,438	107	26	1	14		13
Colusa MAD	9,125	90	56	5			
Durham MAD							
El Dorado Co. V.C.-CSA3							
Glenn County MVCD	749	17	38	8			
Lake County MAD	206	15	9		53		
Pine Grove MAD		20	13	4			
Sacramento-Yolo MVCD		174		<1			
Shasta MVCD	97	5	15	11			
Sutter-Yuba MVCD	6,060	110	210			37	
Tehama County MVCD	228	2	18		8		
Subtotal	**17,902**	**539**	**392**	**29**	**75**	**37**	**13**
No. San Joaquin Valley Region							
East Side MAD	9,968	30					
Merced County MAD	327	110		6			
San Joaquin MVCD	3,329	176		44			
Turlock MAD		21		3			
Subtotal	**13,624**	**337**	**0**	**53**	**0**	**0**	**0**
So. San Joaquin Valley Region							
Coalinga-Huron MAD							
Consolidated MAD	228			2			
Delano MAD							
Delta VCD		<1					
Fresno MVCD				<1			
Fresno Westside MAD				5			
Kern MVCD		11		6			
Kings MAD	525						
Madera County MVCD		51		23			
Tulare MAD		5					
West Side MVCD							
Subtotal	**753**	**67**	**0**	**35**	**0**	**0**	**0**
Southern California Region							
Antelope Valley MVCD							
Carpinteria MAD-Goleta VY VCD							
Coachella Valley MAD		10					
Compton Creek MAD							
Greater L.A. County VCD							
Los Angeles Co. W. VCD							
Moorpark MAD		<1					
Northwest MVCD				12			
Orange County VCD		1		138			
Owens Valley MAP		10		4			
San Bernardino Co. VCP							
San Gabriel Valley MAD							
West Valley VCD							
Subtotal	**0**	**20**	**0**	**154**	**0**	**0**	**0**
Total	**32,279**	**1,026**	**392**	**279**	**75**	**37**	**13**

*Pounds of active ingredient

BIOLOGICAL CONTROL PROGRAM SUMMARY - 1994

	Number of Sources			Acres Treated				
Agency	Agricultural	Domestic	Natural	Agricultural	Domestic	Natural	Treatment Rat	Other
Coastal Region								
Alameda County MAD		871			45		<1lb/A	
Alameda County VC		4			<1		100/Source	
			6			<1	500/Source	
Contra Costa		464	213			213	1/Source, <1/A	
Marin/Sonoma	20	200	100				<1lb/A	
Napa County	14	84	24	26	10	158	<1lb/A	
No. Salinas Valley	38	93	56		97		135/A	
San Mateo County		100	10		5	1	12/Source	
Santa Clara County		276			3		12/Source	
Santa Cruz		67	1		<1	<1	12/Source	
Solano County	35	55	45	1200	40	930	<1lb/A	
Sub-total	107	2214	455	26	45	372		
Sacramento Valley Region								
Burney Basin	33	12		720			<1lb/A	
Butte County	745	496	1241	1379	919	2298	<1lb/A	
			5			200	1lb/A	Lagenidium
Colusa	80	10	10	800	80	300	<1lb/A	
Glenn County	150	15	20	6000	5	20	<1lb/A	
Lake County	51	22	9	810	10	4	<1lb/A	
Sacramento Co.-Yolo Co.	1018	514	42	20305	232	59		
Shasta	18	15	30	25	10	50	<1/A	
Sutter-Yuba	305	218	21	8311	98	13	<1lb/A	
Tehama County	36	11	23	1800	25	250	varies	
Sub-total	2436	1313	1401	40150	1379	3194		
North San Joaquin Region								
Merced County	10	15	3	400	10	75	100/A	
San Joaquin County	196	164	79	9594	9	505	<1lb/A	
Turlock	20	58	4		185		<1lbs/A	
Sub-total	226	237	86	9994	204	10747		
South San Joaquin Region								
Consolidated	159	183	81	66	76	96	<1lb/A	
Delta	156	194	33	80	13	27	.5/A	
Fresno	126	332	9	50	100	100	<1lb/A	
Fresno Westside	77	2	4	4843	35	51	<1lb/A	
Kern	660	2410	15	630	65	220	1.5/A	
Kings	28	60	25	140	58	90	.2lbs/A	
Madera County	60	42	180	240	168	2160	A,D-300/A, N-100/A	
Tulare	25	8	4	20	5	20	300/A	
West Side	60	15		950	2		<1lb/A	
Sub-total	1351	3246	3597	7019	522	2764		
Southern California Region								
Antelope Valley	1	62		<1	26		1lb/A	
Carpinteria	10	50	25	10	20	70	150 Fish/A	
Coachella Valley	35	80		70	100		<1lb/A	
Compton Creek		7	2		50	150		
Goleta Valley	50	100	100	20	50	150	150 Fish/A	
Greater L.A. County	115	850	20	5	15	55	200/A	
Moorpark	6	29	3	4	6	2	375/A	
Sub-total								
Northwest	22	175	68	3	46	77	40-1000/Sr.	
Orange County	3	1701	45	3	240	28	<1lb/A	
Owens Valley	2		4	3		5	100 Fish/A	
San Gabriel Valley		300	400		50	20	600/A	
West Valley	27	47		<1	<1		500/A	
Sub-total	271	3401	667	118	603	557		
Totals	4391	10411	6206	57307	2753	57307		

AGENCY SOURCE REDUCTION PROGRAM SUMMARY - 1994

	Agency-Owned Heavy Equipment					Number of Projects		New Contstr.	Maint-tained ft.	# of Water Control Structures	Cubic Yards	Written Requests
Agency	1	2	3	4	5	6	7	8	9	10	11	12
Coastal Region												
Alameda Co. MAD	1.5		4		3	1	27		10			9
Contra Costa					4							
Marin-Sonoma	1.5		2		24							
No. Salinas Valley	1		2	1	1	1	1					
San Mateo County					5							
Santa Clara County		2			3		2					21
Solano County	2		2		2	1	1		6,250			38
Sub-total	6	2	10	1	42	3	31	0	6,260	0	0	68
Sacramento Valley Region												
Butte County				1	1							654
Colusa												1
El Dorado County		2	2		5							
Lake County					9							
Pine Grove												
Sacramento-Yolo		3	6	2	3	30		7,220	41,263		4,087	
Shasta	1		1	1	1	18			17,000			
Sutter-Yuba				0.5	5							3
Tehama County							1					
Sub-total	1	5	9	4.5	24	48	1	7,220	58,263	0	4,087	658
No. San Joaquin Vy. Region												
East Side		1	5		14							1
Merced County		1	2		1							
San Joaquin County				1	2							
Turlock						2	70					161
Sub-total	0	2	7	1	17	2	70	0	0	0	0	162
So. San Joaquin Vy. Region												
Coalinga-Huron												20
Consolidated						1						7
Delano												
Kern	1	2	4	2	2							
Kings										382		27
Madera County							18					217
Tulare												70
West Side					1	4	4			2	920	
Sub-total	1	2	4	5	3	5	22	0	0	384	920	341
Southern California Region												
Antelope Valley										160		
Carpinteria/Goleta Vy.							2		5,000			150
Coachella Valley					5							405
Compton Creek			1		3				43,000			
Greater L.A. County					10		1		14,000	20		1,718
Moorpark	1		1		2						<25	147
Northwest							15					37
Orange County												6
Owens Valley							27					27
San Gabriel Valley					4		35					100
West Valley		1	3		1	267	81			986		38
Sub-total	1	1	5	0	25	267	161	0	62,000	1,166	0	2,628
Totals	9	12	35	11.5	111	325	285	7,220	126,523	1,550	5,007	3,857

***Definition of Source Reduction Program Data by Column Number.**

***1. to 5. Equipment used on source reduction project half the time and used on other projects the rest of the time should be shown as one-half unit.**

1. Bare tractor; 2. Rubber tire, self-propelled power units; 3. Bulldozer blade, carryall or drag scraper, ripper, disc., loader, backhoe, ditcher, pull grader, terracing blade, etc.; 4. Dragline, backhoe, shovels, etc.; 5. Dump trucks, transportation and servicing vehicles, trailer.

*6. Direct involvement by district either by use of district equipment or donation of district funds. (# of projects)

*7. Number of projects completed including legal activity terminating in a completed project.

*8. New construction feet.

*9. Maintained feet.

*10. Return systems or dairy drain collecting areas whether concrete or large earth reservoirs, lakes, ponds, etc.

*11. If earth is both excavated and used as fill, count only one or the other. Expressed cubic yards.

*12. Pertaining to breeding sources either of an informational or corrective nature. (includes septic tanks)

WILLIAM C. REEVES
NEW INVESTIGATOR AWARD

The William C. Reeves New Investigator Award is given annually by the California Mosquito and Vector Control Association in honor of the long and productive scientific career of Dr. William C. Reeves, Professor Emeritus, School of Public Health, University of California at Berkeley.

The award is presented to the outstanding research paper delivered by a new investigator based on quality of the study, the written report, and presentation at the annual conference.

Margaret C. Wirth was the recipient of the 1995 award at the 63rd Annual Conference held in Sacramento. The other finalists were John E. Gimnig and Ferenc A. de Szalay. The three finalists' papers are printed on pages 77-90.

Previous William C. Reeves New Investigator Award Winners:

1994 - Merry L. Holliday-Hanson
1993 - Jeffrey W. Beehler
1992 - Darold P. Batzer
1991 - David R. Mercer
1990 - Gary N. Fritz
1989 - Truls Jensen
1988 - Vicki L. Kramer

1995 WINNER
WILLIAM C. REEVES NEW INVESTIGATOR AWARD

1995 Proc. Calif. Mosq. Vector Control Assoc. **63:77-81**

MULTIPLE MECHANISMS CAUSE ORGANOPHOSPHATE RESISTANCE IN *CULEX PIPIENS* FROM CYPRUS

Margaret C. Wirth and George P. Georghiou

Department of Entomology
University of California
Riverside, California 92521

ABSTRACT

Cyprus has had an intensive antimalarial program since the eradication of the disease and its principal vector, *Anopheles sacharovi*, in 1949. Since 1971 the program has relied on organophosphates, primarily temephos, for mosquito control. Eight collections of *Culex pipiens* were obtained from different locations on the island between 1987 and 1993. All populations were found to possess high levels of organophosphate and carbamate resistance. Overproduced esterases (esterases A2B2 and A5B5) and insensitive acetylcholinesterase were identified as the major organophosphate resistance mechanisms in these populations. Esterases A5B5 and the insensitive acetylcholinesterase were isolated into separate substrains and the relative contribution of each mechanism to the observed resistance spectrum was determined. Carbamate resistance was found to be the result of cross-resistance due to the insensitive acetylcholinesterase.

The introduction of exotic pest species is a recurrent problem in California and throughout the world. The repeated trapping of the Mediterranean fruit fly in southern California, the arrival and rapid worldwide spread of *Aedes albopictus* (Skuse) and the inexorable advance of the Africanized honeybee through Central America into the United States have attracted considerable attention. In contrast, the arrival of species that are already indigenous to this country appears to elicit little or no concern. Yet, the possibility exists that these arrivals may contain genes for mechanisms of insecticide resistance that are new to this country and which could disrupt chemical control programs in effect.

The most important mechanisms of organophosphate resistance in *Culex* species are overproduced esterases and insensitive acetylcholinesterase. Ten electrophoretically distinct, overproduced esterases A and B have been identified (Georghiou and Pasteur 1980, Raymond et al. 1987, Poirie et al. 1992, Prabhaker et al. 1987). Further, insensitive acetylcholinesterase, frequently in association with overproduced esterases, has been identified in *Culex* from several countries (Raymond et al. 1986, Villani and Hemingway 1987, Bisset at al. 1990, Bonning et al. 1991, Ben Cheik and Pasteur 1993, Khayrandish and Wood 1993) but not the United States. Of concern to North America is the presence of this mechanism in *Culex quinquefasciatus* Say populations in Cuba (Bisset et al. 1990).

In California, the primary esterase associated with organophosphate resistance prior to 1979 was B1 (Georghiou and Pasteur 1980). In 1984 the esterases A2 and B2 were discovered in California (Raymond et al. 1987). In subsequent surveys they were identified in mosquito populations along the West Coast, Gulf of Mexico and Atlantic Coast, usually near major airports or shipping ports (G.P. Georghiou unpublished data). The recent arrival of esterases A2 and B2 was also observed in Europe (Villani and Hemingway 1987, Bonning et al. 1991, and Rivet et al. 1993). These observations are important evidence of the propensity of resistance genes to migrate from their point of origin and become established in new distant areas.

This project attempts to characterize the mechanisms of organophosphate resistance based on esterases A5 and B5 and insensitive acetylcholinesterase found in *Culex pipiens* (L.) populations from the Mediterranean island of Cyprus, where a preventive antimalarial spray program has been in effect for 45 years.

MATERIALS AND METHODS

Strains.

Collections of *Cx. pipiens* larvae were made between 1987 and 1993, from eight breeding sites throughout Cyprus. These larvae were used to establish laboratory colonies at the University of California, Riverside for the purpose of studying their spectrum of insecticide resistance. Two strains, A5B5 and Ace-R, were derived from the 1987 Mitsero collection by single pair crosses in order to isolate the two resistance mechanisms. The two strains, A5B5 and Ace-R, were maintained under selection pressure with temephos and propoxur, respectively. A susceptible strain of *Cx. quinquefasciatus*, S-Lab (Georghiou et al. 1966), was used as a reference population for bioassays and biochemical testing.

Insecticide Bioassays.

Bioassays were done on early-fourth instar larvae, following the standard method of Georghiou et al. (1966). Eight insecticides of technical grade dissolved in acetone were used: temephos, chlorpyrifos, malathion, pirimiphos methyl, dichlorvos, fenthion, propoxur and permethrin. *Bacillus thuringiensis* var. *israelensis* (*Bti*) solutions (from IPS 80 preparation) were prepared in distilled water.

Twenty early-fourth instar larvae were tested in 100 ml of tap water in waxed paper cups. Five replications of at least five doses giving mortality between 0 and 100% were used. Mortality was recorded after 24 hours. Assays were replicated on different days. The final concentration of acetone in controls and test cups was adjusted to 1%. Data were subjected to probit analysis by the method of Finney (1971) using a program written for the PC (Raymond 1985).

Selections.

One thousand early-fourth instar larvae were exposed to the desired concentration of insecticide in 1,000 ml of tap water in enamel pans. Survivors were recovered after 24 hours to clean water, fed, and pupae were returned to the cage to continue the colony.

Biochemical Analysis.

Esterases were analyzed by starch gel electrophoresis according to the method of Pasteur et al. (1981). Fifty adult mosquitoes were examined for each population. Esterase activity in the Mitsero collection was measured on single adult homogenates using a microtiter plate assay described by Dary et al. (1990). The frequency of insensitive acetylcholinesterase was determined in the Mitsero collection using single mosquito homogenates by the method of Ellman et al. (1961) with some modifications (Ferrari et al. 1993).

RESULTS

Identification of Esterases Involved in Organophosphate Resistance in Cyprus Populations.

Each of the eight collections contained high frequencies of overproduced esterases known to be associated with organophosphate resistance (Fig. 1). Four different esterases were identified: esterases A2, B2, A5 and B5. Esterases A2 and B2 are strongly linked (Wirth et al. 1990). Esterases A5 and B5 are new esterases and have not yet been reported from any other country (Poirie et al. 1992). Esterases B2 and B5 have been shown to be overproduced due to gene amplification (Raymond et al. 1989, Poirie et al. 1992).

All eight collections possessed both pairs of esterases, although at different frequencies (data not shown). Esterases A5 and B5 were more frequent than esterases A2 and B2 in all collections except Milas. The lowest frequency of overproduced esterases was 45%, but many populations ranged between 75 and 95% (Fig. 1).

Distribution of the Different Resistance Mechanisms Within the Mitsero Population.

Using microtiter tests on homogenates of individual adult mosquitoes, we were able to identify mosquitoes which possessed overproduced esterases as well as insensitive acetylcholinesterase. With this test we were not able to determine specifically which esterases were present in an individual mosquito because identification is dependent on migration patterns in starch gel electrophoresis. A sample of adult mosquitoes from the Mitsero collection was analyzed (data not shown) and 33% had overproduced esterases (either A2B2 or A5B5 or both), 15% had insensitive acetylcholinesterase, and 28% had both insensitive acetylcholinesterase as well as one or both pairs of overproduced esterases.

Resistance Spectrum in Field Populations.

The presence of overproduced esterases and

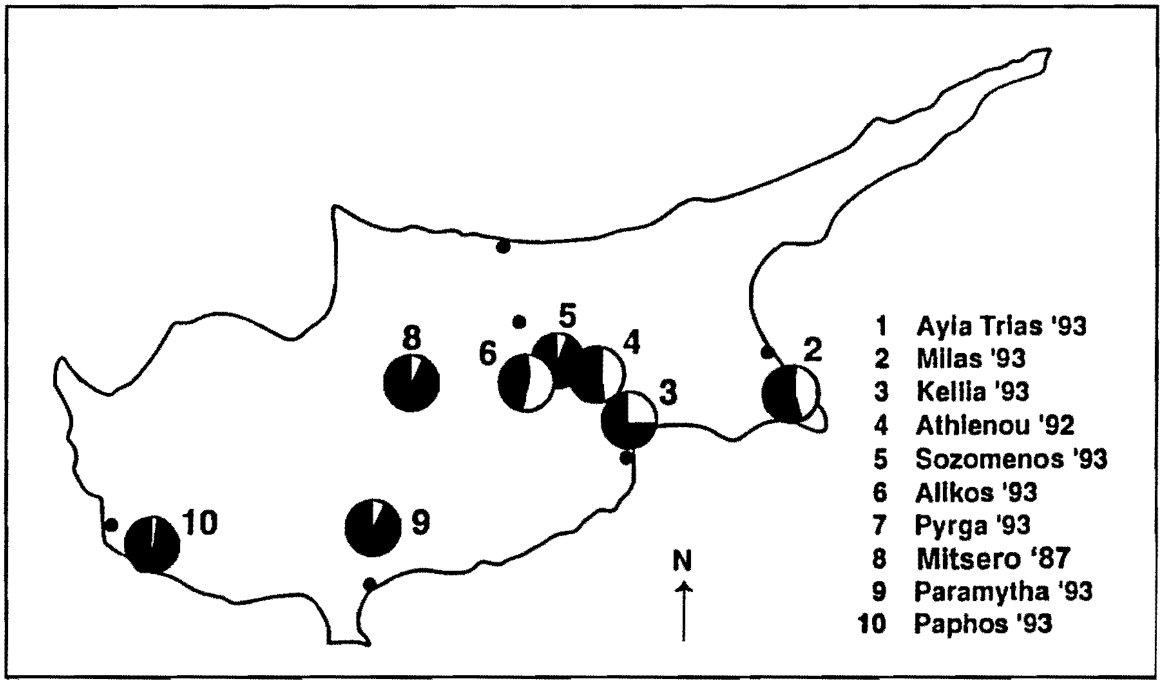


Figure 1. Map of the island of Cyprus showing the eight *Cx. pipiens* collection sites for 1987-1993. The black portion of the circles represents the proportion of mosquitoes from that site possessing elevated esterase activity known to be associated with organophosphate resistance. Black dots indicate principle cities.

insensitive acetylcholinesterase conferred extremely high resistance to organophosphates and to the carbamate propoxur (Fig. 2). Resistance was greatest to malathion and temephos, and, in decreasing order, to chlorpyrifos, pirimiphos methyl, fenthion and dichlorvos. Variation in susceptibility to organophosphates among the populations was smaller than toward the carbamates, pyrethroids or *Bti*. The wider variation in propoxur resistance probably reflects variation in the frequency of insensitive acetylcholinesterase between populations. Whereas both overproduced esterases and insensitive acetylcholinesterase confer resistance to organophosphates, resistance to carbamates is due primarily to the insensitive acetylcholinesterase. Two populations had significant levels of resistance to permethrin. Permethrin is used for some adulticiding as well as in agriculture. There is also a history of DDT use. Despite the lack of any recorded exposure to the bacterial pesticide *Bti*, some variation in sensitivity to these toxins was observed.

Resistance Due to Esterases A5B5 or Insensitive Acetylcholinesterase.

Two strains were derived from the 1987 Mitsero collection by single pair crosses. One strain (A5B5) contains only overproduced esterases A5 and B5 but lacks the insensitive acetylcholinesterase. The other strain (Ace-R) contains only the insensitive acetylcholinesterase. Both strains were mass selected until maximal resistance was obtained.

The selected A5B5 strain had high levels of resistance (Table 1) towards temephos (50-fold), chlorpyrifos (47-fold), and fenthion (53-fold) at the LC95. Only moderate resistance (11-fold) was seen toward malathion whereas no significant resistance was observed toward the carbamate propoxur (3.5-fold).

The pattern of cross-resistance was different in the insensitive acetylcholinesterase strain, involving resistance toward propoxur (72-fold) and surprisingly low resistance (<3-fold) to chlorpyrifos. Moderate levels of resistance were noted toward temephos (44-fold),

Table 1. Relative resistance ratios at the LC_{95} for the Mitsero collection and the substrains derived from it, possessing either esterases A5 and B5 or insensitive acetylcholinesterase.

Insecticide	Mitsero	A5B5	Ace-R
temephos	63	50	44
chlorpyrifos	80	47	3
malathion	80	11	18
fenthion	28	53	10
propoxur	–	3	72

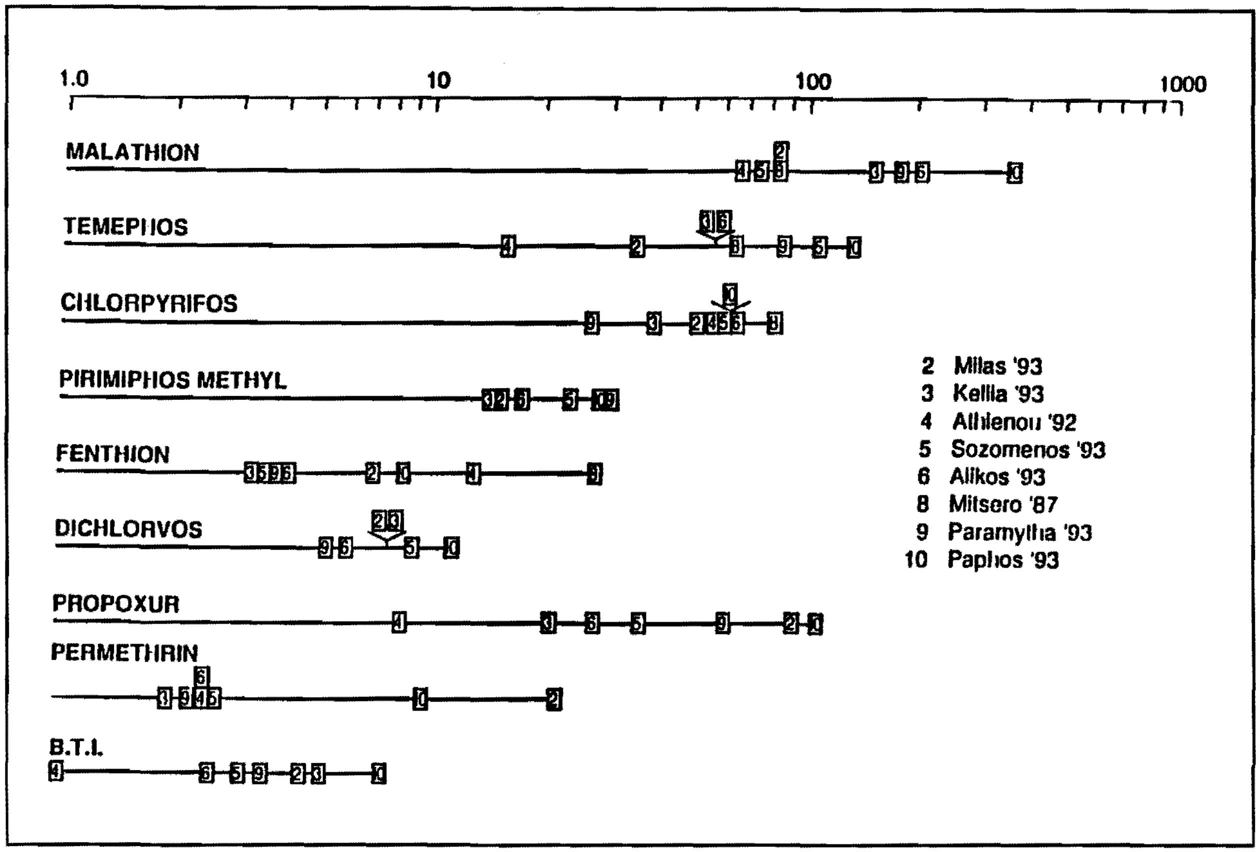


Figure 2. Histogram of the resistance ratios (at the LC_{95}) of the different field collections of *Culex pipiens* from Cyprus, 1987-1993.

malathion (18-fold) and fenthion (10-fold).

CONCLUSIONS

Cyprus was among the first countries in the eastern Mediterranean to have eradicated malaria and its principal vector, *Anopheles sacharovi* Favre. Because of concern over the risk of reintroduction of the vector as well as the disease from neighboring countries, an intensive chemical control program, based mainly on DDT, has been applied at all breeding sites since 1949. DDT was phased out in 1971 and replaced by organophosphates. Temephos is the most commonly used insecticide, but dichlorvos, fenthion, oils, and to a lesser extent, pyrethroids are also being used.

All *Culex* populations sampled for this study (1987-1993) demonstrated high levels of organophosphate and carbamate resistance. Organophosphate resistance was correlated with high frequencies of overproduced esterases. Although carbamates were not used for larval control, significant resistance to propoxur was also observed. The available evidence indicates that this resistance is due to the presence of insensitive acetylcholinesterase. The existence of this mechanism was investigated by microtiter tests on the Mitsero population. Synergism tests indicated that mixed function oxidase, a known mechanism of carbamate resistance, was absent (data not shown). Therefore, organophosphate resistance is due to the presence of both overproduced esterases and insensitive acetylcholinesterase, whereas carbamate resistance is due to cross-resistance conferred by the altered acetylcholinesterase.

Individual mosquitoes in these populations possessed all possible combinations of the different mechanisms of organophosphate resistance, as demonstrated by the Mitsero collection. Individuals were identified as possessing one or both overproduced esterases (A2B2 and/or A5B5), and the biochemical tests demonstrated that individuals with elevated esterases could also possess the insensitive acetylcholinesterase. The presence of such a variety of mechanisms is undoubtedly maintained in the populations in linkage disequilibrium due to intensive insecticide pressure.

Similar cases of resistance have been reported in other areas with intensive mosquito control activities. They include southern France and Italy (Raymond et al. 1986, Villani and Hemingway 1987, Bonning et al. 1991), Cuba (Bisset at al. 1990) and Tunisia (Ben Cheik and Pasteur 1993), and others. It is important that we be aware of the mosquito resistance situation in the world in order to be prepared for any problems which may occur as a result of the inadvertent introduction of new insecticide resistance genes from abroad.

REFERENCES CITED

Ben Cheik, H. and N. Pasteur. 1993. Resistance to temephos, an organophosphorous insecticide, in *Culex pipiens* from Tunisia, North Africa. J. Amer. Mosq. Control Assoc. 9(3):335-337.

Bisset, J., M. Rodriguez, C. Diaz, E. Ortiz, M. Marquetti and J. Hemingway. 1990. The mechanism of organophosphate and carbamate resistance in *Culex quinquefasciatus* (Diptera: Culicidae) from Cuba. Bull. Entomol. Res. 80:245-250.

Bonning, B., J. Hemingway, R. Romi and G. Majori. 1991. Interaction of insecticide resistance genes in field populations of *Culex pipiens* from Italy in response to changing insecticide selection pressure. Bull. Entomol. Res. 81:5-10.

Dary, O., G.P. Georghiou, E. Parsons and N. Pasteur. 1990. Microplate adaptation of Gomori's assay for quantitative determination of general esterase activity in single insects. J. Econ. Entomol. 83(6):2187-2192.

Ellman, G., K. Courtney, V. Andres and R. Featherstone. 1961. A new, rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.

Ferrari, J.A., J. Morse, G.P. Georghiou and Y. Sun. 1993. Elevated esterase activity and acetylcholinesterase insensitivity in Citrus Thrips (Thysanoptera: Thripidae) populations from the San Joaquin Valley in California. J. Econ. Entomol. 86(6):1645-1650.

Finney, D. 1971. Probit Analysis. Cambridge University Press. Cambridge, England. 333 pp.

Georghiou, G.P., R.L. Metcalf and F.E. Gidden. 1966. Carbamate resistance in mosquitoes. Selection of *Culex pipiens fatigans* Wied. for resistance to Baygon. Bull. W.H.O. 35:691-708.

Georghiou, G.P. and N. Pasteur. 1980. Organophosphate resistance and esterase pattern in a natural population of the Southern House mosquito from California. J. Econ. Entomol. 73:489-492.

Khayrandish, A. and R. Wood. 1993. A multiple basis for insecticide resistance in a strain of *Culex quinquefasciatus* (Diptera: Culicidae) from Muheza, Tanzania, studied as resistance declined. Bull. Entomol. Res. 83:75-86.

Pasteur, N., A. Iseki and G.P. Georghiou. 1981. Genetic and biochemical studies on the highly active esterases A' and B associated with organophosphate resistance in the *Culex pipiens* complex. Biochem. Genet. 19:909-919.

Poirie, M., M. Raymond and N. Pasteur. 1992. Identification of two distinct amplifications of the esterase B locus in *Culex pipiens* (L.) from Mediterranean countries. Biochem. Genet. 30(1/2):13-26.

Prabhaker, N., G.P. Georghiou and N. Pasteur. 1987. Genetic association between highly active esterases and organophosphate resistance in *Culex tarsalis*. J. Amer. Mosq. Control Assoc. 3(3):473-475.

Raymond, M. 1985. Presentation d'un programme Basic d'analyse log-probit pour micro-ordinateur. Cah. O.R.S.T.R.O.M. Ser. Entomol. Med. & Parasitol. 23:117-121.

Raymond, M., D. Fournier, J. Bride, A. Cuany, J. Berge, M. Magnin and N. Pasteur. 1986. Identification of resistance mechanisms in *Culex pipiens* (Diptera: Culicidae) from southern France: Insensitive acetylcholinesterase and detoxifying oxidases. J. Econ. Entomol. 79:1452-1458.

Raymond, M., N. Pasteur, G.P. Georghiou, R.B. Mellon, M.C. Wirth and M.K. Hawley. 1987. Detoxification esterases new to California, U.S.A., in organophosphate-resistant *Culex quinquefasciatus* (Diptera: Culicidae). J. Med. Entomol. 24(1):24-27.

Raymond, M., V. Beyssat-Arnaouty, N. Sivasubramanian, C. Mouches, G.P. Georghiou and N. Pasteur. 1989. Amplification of various esterase B's responsible for organophosphate resistance in *Culex* mosquitoes. Biochem. Genet. 27(7/8):417-423.

Rivet, Y., M. Marquine and M. Raymond. 1993. French mosquito populations now invaded by insecticide resistant A2-B2 esterases. Biol. J. Linn. Soc. 49:249-255.

Villani, F. and J. Hemingway. 1987. The detection and interaction of multiple organophosphorous and carbamate insecticide resistance genes in field populations of *Culex pipiens* from Italy. Pestic. Biochem. & Physiol. 27:218-228.

Wirth, M.C., M. Marquine, G.P. Georghiou and N. Pasteur. 1990. Esterases A2 and B2 in *Culex quinquefasciatus* (Diptera: Culicidae): Role in organophosphate resistance and linkage. J. Med. Entomol. 27(2):202-206.

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:82-85

GENETIC VARIATION WITHIN AND AMONG THREE SPECIES OF THE *AEDES* (*OCHLEROTATUS*) *PUNCTOR* SUBGROUP

John E. Gimnig and Bruce F. Eldridge

Department of Entomology
University of California
Davis, California 95616

The *Aedes* (*Ochlerotatus*) *punctor* subgroup of mosquitoes is a distinct subdivision of the *Aedes communis* group (Group G) of Edwards' (1932) classification. It was first described by Dyar (1922) and later revised by Knight (1951) based upon the morphology of the male genitalia. The subgroup consists of *Aedes punctor* (Kirby), *Aedes hexodontus* Dyar, *Aedes aboriginis* Dyar, *Aedes abserratus* (Felt and Young) and *Aedes punctodes* Dyar. *Aedes punctodes* and *Ae. aboriginis* are the two least studied members of this complex. *Aedes punctodes* is found along the Pacific coast of Alaska and *Ae. aboriginis* is found in the coastal ranges of British Columbia and the northwestern United States. *Aedes abserratus* is found in eastern Canada and the northeastern United States extending as far west as Minnesota. *Aedes punctor* and *Ae. hexodontus* are both holarctic mosquitoes with widespread distributions in North America. *Aedes punctor* inhabits the boreal zone of Canada and parts of the northern United States whereas *Ae. hexodontus* is primarily a tundra mosquito, although it is found at high elevations in the mountains of the western United States (Darsie and Ward 1981).

While little is known about these mosquitoes, Jamestown Canyon virus and snowshoe hare virus frequently have been isolated from *Ae. abserratus*, *Ae. hexodontus* and *Ae. punctor* (Belloncik et al. 1983, Campbell et al. 1991, McLean et al. 1977, Wagner et al. 1975). In addition, vector competency studies confirm that these mosquitoes may be potential vectors of one or both of these viruses (Heard et al. 1991).

In any ecological study of mosquitoes, proper identification of species is essential. While several authors have examined the taxonomy and systematics of the *punctor* subgroup, many questions remain about the true status of these mosquitoes. Knight (1951) and Wood (1977) have noted that both *Ae. punctor* and *Ae. hexodontus* exhibit considerable morphological variation over their ranges. Dyar (1922) considered several of these variants to be separate species although they were later synonymized with *Ae. hexodontus*. Widespread mosquitoes such as *Ae. punctor* and *Ae. hexodontus* are often found to be species complexes consisting of several closely related but distinct species (Brust and Munstermann 1992). Keys based upon traditional morphological characters have proven to be unreliable in separating these mosquitoes.

An excellent tool for studying the systematics of an organism is allozyme electrophoresis. Electrophoresis can be used to (1) aid in identification and determine geographic distribution, (2) determine the amount of gene flow between populations, and (3) aid in the reconstruction of phylogenies and evolutionary histories. Therefore, the purpose of this study was to supplement traditional taxonomic studies of the *punctor* subgroup with an analysis of allozyme variation within and among three of the five members of the *punctor* subgroup. The specific objective of this study was to identify species diagnostic loci in three species of the *Ae. punctor* subgroup. In addition, *Ae. hexodontus* was examined in greater detail to provide a comparison of genetic variation within species versus among species. These observations will serve as an aid to identification in areas where species overlap and will provide a better understanding of the phylogenetic relationships of these mosquitoes.

MATERIALS AND METHODS

Mosquitoes were collected by various individuals as fourth instars or pupae using dippers or aquatic nets. *Aedes hexodontus* populations used in this study were from eleven collection localities within five western states (Fig. 1). *Aedes abserratus* and *Ae. punctor* were collected from Gogebic County in the upper peninsula of Michigan. The specimens were brought back to the laboratory and reared to adulthood. Upon emergence,

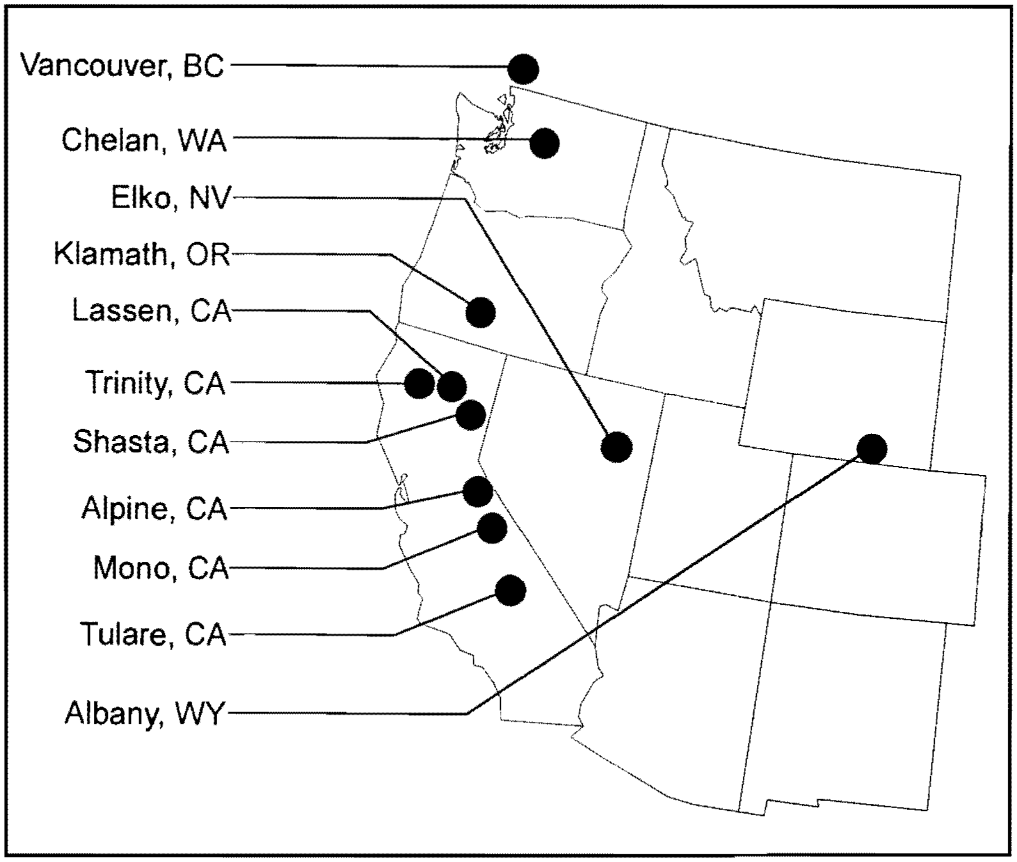


Figure 1. Locations of *Aedes hexodontus* populations examined in present study.

adults were identified and stored in Bio-Freeze Vials (Costar Corporation, Cambridge, MA) at -70°C until the specimens are used for electrophoresis. Several specimens with associated larval skins from each collection were set aside as vouchers. Mosquitoes were then homogenized and proteins separated by polyacrylamide electrophoresis on a 6% slab gel matrix (Munstermann 1980). The specimens were assayed for sixteen loci using standard histochemical staining techniques (Steiner and Joslyn 1979). Genetic distance coefficients were calculated and phenograms were constructed using the computer program BIOSYS-1 (Swofford and Selander 1981). *Aedes tahoensis* Dyar was used as an outgroup to root all trees.

RESULTS AND DISUSSION

Of the sixteen loci examined in this study, eight showed little or no variation within the *Ae. punctor* subgroup. The remaining eight loci exhibited higher levels of variation in one or more populations. The enzymes 6-phosphogluconate dehydrogenase and aconitase-1 had high levels of variation within *Ae. hexodontus* and strongly affected branching order of *Ae. hexodontus* in the phenogram. Differences in the frequencies of alleles could be seen in *Ae. punctor* in aminoaspartate transferase-1 and in *Ae. abserratus* in peptidase. All three species possessed unique alleles in malic enzyme. This was the only diagnostic locus found to differentiate the three species.

A phenogram based upon Nei's genetic distance (Fig. 2) confirms that the three members of the *Ae. punctor* subgroup show a relatively low level of differentiation between species. *Aedes abserratus* clustered with *Ae. hexodontus* populations at genetic distances of approximately 0.2, while *Ae. punctor* clustered with *Ae. abserratus* and *Ae. hexodontus* at a genetic distance of 0.3. In comparison, members of the *Ae. communis* complex clustered at genetic distances of as high as 0.5 (Brust & Munstermann 1992). All three members of the *punctor* subgroup clustered with the outgroup, *Ae. tahoensis*, at a genetic distance of approximately 1.0, suggesting that the *punctor* group is a distinct subdivision of the *Aedes communis* group.

Within *Ae. hexodontus*, the Elko, NV and Albany, WY populations clustered together as did the three

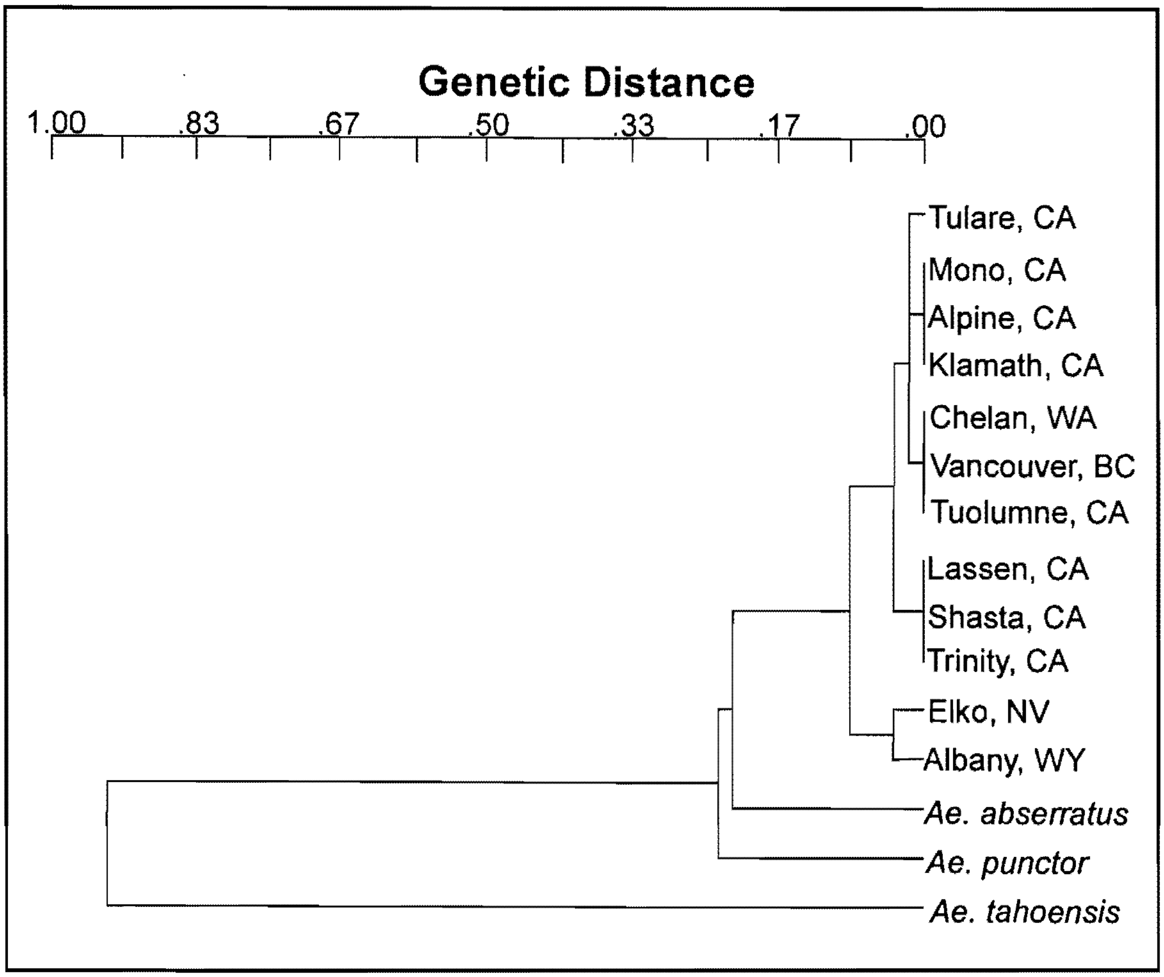


Figure 2. Phenogram for the *punctor* subgroup based on 16 enzyme loci. Geographic localities refer to *Aedes hexodontus* populations.

populations from the northern California (Lassen, Shasta and Trinity). The other populations did not show distinct clustering and were often grouped closer to populations that were geographically very distant. For example, the Tuolumne population clustered with populations from northern Washington and southern British Columbia rather than with the Mono and Tulare populations that are geographically very close. These anomalies can be attributed to the effects of the aconitase-1 locus. Removal of this locus from the analysis results in a tree that better corresponds to the geographic localities, but it also collapses the tree obscuring those clusters that are evident in the original tree. *Aedes hexodontus* is the only member of the *punctor* subgroup found in California.

The low level of variation observed between species is indicative of a very close relationship among these three mosquitoes. This finding is in agreement with morphological studies that have found the separation of the members of the *punctor* group to be difficult or impossible in certain life stages. The relatively low genetic distance calculated for these mosquitoes suggests a relatively recent divergence compared to the *Aedes communis* complex. In comparison, *Ae. hexodontus* exhibited relatively high within species variation. This could be attributed to the fragmented range of this mosquito as it is found only at elevations above 5,000 feet in the southern portions of its range.

In conclusion, the *Ae. punctor* subgroup is a distinct subdivision of the *Ae. communis* Group. The level of differentiation between members of the *punctor* subgroup is higher than that seen within *Ae. hexodontus*. This variation is due primarily to the effects of one locus. Since *Ae. punctor* and *Ae. abserratus* were collected from the same locality and they do not share the same alleles for malic enzyme, they represent two species. However, it cannot be determined conclusively that *Ae. hexodontus* would not be able to interbreed with either *Aedes punctor* or *Aedes abserratus* until sympatric populations are examined.

REFERENCES CITED

Belloncik, S., A. Aubin, A. Maire, J. Boisvert, R. Gagnon, C. Th'ng, C. Trudel, C. and H. Artsob. 1983. Arbovirus studies in the Trois-Rivieres area, province of Quebec, Canada. Mosq. News 43:426-431.

Brust, R.A. and L.E. Munstermann. 1992. Morphological and genetic characterization of the *Aedes (Ochlerotatus) communis* complex (Diptera: Culicidae) in North America. Ann. Entomol. Soc. Am. 85:1-10.

Campbell, G.L., B.F. Eldridge, W.C. Reeves and J.L. Hardy. 1991. Isolation of Jamestown Canyon virus from boreal *Aedes* mosquitoes from the Sierra Nevada of California. Am. J. Trop. Med. Hyg. 44:244-249.

Darsie, R.F. and R.A. Ward. 1981. Identification and Geographical Distribution of Mosquitoes of North America, North of Mexico. Am. Mosq. Control Assoc., Fresno, CA. 313 pp.

Dyar, H.G. 1922. The mosquitoes of the United States. Proc. U.S. Nat. Mus. 62:1-119.

Edwards, F.W. 1932. Diptera. Fam. Culicidae. Fasc. 194. pp 258. *In*: Wyman, P. (ed.) Genera Insectorum. Desmet Verteneuil, Brussels, BG.

Heard, P.B., M. Zhang and P.R. Grimstad. 1991. Laboratory transmission of Jamestown Canyon and snowshoe hare viruses (Bunyaviridae: California Serogroup) by several species of mosquitoes. J. Am. Mosq. Control Assoc. 7:94-102.

Knight, K.L. 1951. The *Aedes (Ochlerotatus) punctor* subgroup in North America. Ann. Entomol. Soc. Am. 44:87-99.

MacLean, D.M., P. Grass, D. Judd, D. Cmiralova and D.M. Stuart. 1977. Natural foci of California encephalitis virus activity in the Yukon Territory. Can. J. Publ. Hlth 68:69-73.

Munstermann, L.E. 1980. Distinguishing geographic strains of the *Aedes atropalpus* group (Diptera: Culicidae) by analysis of enzyme variation. Ann. Entomol. Soc. Am. 73:699-704.

Steiner, W.W.M. and D.J. Joslyn. 1979. Electrophoretic techniques for the genetic study of mosquitoes. Mosq. News 39: 35-54.

Swofford, D. L. and R.B. Selander. 1981. BIOSYS-1, A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281-283.

Wagner, R.J., C. DeJong, M.K. Leung, J. McLintock and J.O. Iversen. 1975. Isolations of California encephalitis virus from tundra mosquitoes. Canadian J. Microbiol. 21:574-576.

Wood, D.M. 1977. Notes on the identities of some common Nearctic *Aedes* mosquitoes. Mosq. News 37:71-81.

1995 Proc. Calif. Mosq. Vector Control Assoc. 63:86-90

A COMPARISON OF SMALL AND LARGE SCALE EXPERIMENTS EXAMINING THE EFFECTS OF WETLAND MANAGEMENT PRACTICES ON MOSQUITO DENSITIES

Ferenc A. de Szalay, Darold P. Batzer[1], Eric B. Schlossberg and Vincent H. Resh

Department of Environmental Science, Policy and Management
University of California
Berkeley, California 94720

ABSTRACT

Mesocosm and macrocosm experiments were conducted in seasonal marshes at Grizzly Island Wildlife Area (Solano County, CA) to examine effects of mowing, discing and burning emergent vegetation on the dominant mosquito taxa. Densities of *Culex tarsalis* and *Culiseta inornata* were usually lower in the mowed treatment areas than control areas and densities of *Cx. tarsalis, Cs. inornata* and *Aedes melanimon* were usually lower in disced and burned treatment areas than control areas. Similar results in mesocosm and macrocosm experiments demonstrate that mesocosm studies of mosquito control can be used as alternatives to larger-scale experiments.

Many types of experimental designs are used to study mosquito ecology and control, ranging from small-scale laboratory microcosms to large-scale field experiments. Researchers, however, are well aware that mosquito control methods developed in laboratory microcosms must be field tested to demonstrate their applicability in natural ecosystems. Field trials can be conducted using small-sized experimental enclosures (mesocosms), typically 1-10^4 m^3 in size (Cooper and Barmuta 1993), or larger experimental plots (macrocosms) up to several acres in size can be used.

Mesocosms have become popular because they are less expensive to construct and easier to replicate than macrocosms (Odum 1984). However, some studies have found that mesocosm scale can affect experimental results (Solomon et al. 1989, Stephenson et al. 1984). Therefore, it is important to test whether mesocosm experiments with mosquitoes agree with results of large-scale experiments, especially if the treatments tested are proposed as future management policy.

Emergent marsh habitats can produce high numbers of pestiferous mosquitoes, leading to potential public health problems (Batzer and Resh 1992a, Provost 1977). However, wildlife managers resist using chemical control measures that negatively impact the natural invertebrate communities because these are an important component of waterfowl diets (Euliss and Grodhaus 1987).

Our research examines three cultural practices (mowing, discing and burning) currently used to manage wetland vegetation and enhance waterfowl habitats (Heitmeyer et al. 1989) to determine whether they can be adapted for concurrent control of mosquito populations. This paper compares results from two pairs of mesocosm and macrocosm experiments conducted in a seasonal wetland in California. First, we used mesocosm and macrocosm experiments to examine the response of mosquitoes to mowing in dense stands of pickleweed (*Salicornia virginica* L.). Second, we used mesocosm and macrocosm experiments to examine the response of mosquitoes to discing and burning in stands of saltgrass (*Distichlis spicata* (L.)).

METHODS

Site Description.

All studies were conducted at the California Department of Fish and Game's Grizzly Island Wildlife

[1] Present Address: Canisius College, Department of Biology, 2001 Main Street, Buffalo, New York 14208.

Area (GIWA) in Suisun Marsh (Solano County, CA). This seasonally flooded brackish-water wetland is subdivided into diked cells that are flooded in late summer and drawn down in early spring. *Salicornia virginica* is the dominant vegetation in most wetland cells with extensive stands of *D. spicata* also occurring.

GIWA is managed as overwintering habitat for migratory waterfowl with the peak numbers of dabbling ducks (Anatinae) reaching approximately 98,000-148,000 birds during the winter months (California Department of Fish and Game, personal communication).

Mowing experiments (mesocosm design): In 1987, twelve adjoining mesocosms (11 m x 22 m) were constructed with earthen berms as described in Batzer and Resh (1988) in one of the wetland cells (Pond 12G). The vegetation in these mesocosms is predominantly *S. virginica*.

Prior to flooding, each mesocosm was subdivided into two treatment areas (11 m x 11 m) using fiberglass mesh barriers (1 mm mesh size). In the first area, 50% of the vegetation was hand-mowed, and the slash was not removed. The second area remained unmanipulated (non-mowed) and served as the control. The mesocosms were flooded from an adjacent channel using portable pumps in September 1988 and remained flooded until March 1989.

Mowing experiments (macrocosm design): In 1990, 12 macrocosms were constructed in three wetland cells (Ponds 12A, 12B and 12D) in dense stands of *S. virginica*. Each macrocosm consisted of a mowed treatment area (16 m x 60 m) paired with a control area (16 m x 60 m). Other than a vegetated 4 m wide buffer zone, no barriers separated the treatment and control areas. Prior to flooding, tractor-pulled mowers were used to remove 50% of the vegetation of the mowed treatment area, and the slash was not removed. The control area remained unmanipulated. Flooding began in September 1990, and the macrocosms remained flooded until the wetland cells were drawn down in March 1991.

Discing and burning experiments (mesocosm design): In 1992, six mesocosms were constructed in two wetland cells (Ponds 17B and 21) in stands of *D. spicata*. Each mesocosm consisted of two adjoining treatment areas (10 m x 10 m) and an unmanipulated control area (10 m x 10 m). In one treatment area, 50% of the vegetation was burned. In the other treatment area, 50% of the vegetation was hand-mowed and then roto-tilled (to simulate the effects of discing). Nylon fabric barriers were constructed around the perimeter of each of these areas. Flooding began in September 1992, which was 1-7 weeks after the vegetation had been treated. Saltgrass resprouted quickly after roto-tilling, and dense, emergent saltgrass was observed in many of our disced treatment areas where the vegetation had regenerated. The mesocosms remained flooded until the wetland cells were drawn down in February 1993.

Discing and burning experiments (macrocosm design): In 1994, four macrocosms were constructed in a wetland cell (Pond 18B) in stands of *D. spicata*. Each macrocosm consisted of two adjoining treatment areas (20 m x 30 m) and an unmanipulated control area (20 m x 30 m). In one treatment area, all of the vegetation was burned. In the second, all of the vegetation was disced using tractor-pulled machinery. No barriers separated these three areas. Flooding began in October 1994, which was 1-3 weeks after the vegetation had been treated. Because the macrocosms were flooded too quickly for the vegetation to regenerate, little emergent saltgrass was observed in the treatment areas. Only preliminary results are presented for this experiment.

Sampling Methods.

Mowing experiments: In the mesocosms and macrocosms, mosquitoes were sampled with D-frame aquatic sweep nets (1 mm mesh size). Each epiphytic sample consisted of the combined contents of four sweeps (1 m long) that were taken in the top 20 cm of the water column. The mouth of the sweep net measured 30 cm wide; therefore, each sample represents approximately 240 l of water column.

Sweep samples were collected in the control areas and in the mowed treatment areas. In the mesocosms, samples were collected monthly from September 1988 through March 1989, and in the macrocosm experiment during November 1990, January 1991, and March 1991.

Discing and burning experiments: In both the mesocosms and macrocosms, mosquitoes were sampled using modified mosquito dippers. Smaller dippers (147 ml capacity) were used because the shallow water and dense vegetation could not be sampled effectively with standard mosquito dippers (473 ml capacity).

In the mesocosms, samples were collected 2-5 times in each plot during the flooding season with 25-50 dips collected in each treatment area on each sample date. In the macrocosms, samples were collected in each plot every 1-3 weeks after flooding with 25-50 dips collected in each treatment area on each sample date.

Statistical Analysis.

Portions the data set from the 1988-89 mesocosm mowing experiment were previously presented in other publications (Batzer and Resh 1991, 1992b). We re-analyzed this data set and compared the results to the data from the 1990-91 macrocosm mowing experiment.

Furthermore, only October-December 1994 data were available for the macrocosm discing and burning

Table 1. Effects of vegetation mowing on the mean mosquito densities (± S.E.) in the unmanipulated areas (CONTROL) and the mowed treatment areas (MOW) in both the mesocosm and macrocosm experiments.

Species	Mesocosm Experiment		Macrocosm Experiment	
	CONTROL	MOW	CONTROL	MOW
Culex tarsalis	41.3 (±18.9)	11.3 (±4.2)	10.7 (±4.2)	8.0 (±4.5)
Culiseta inornata	1.5 (±1.0)	2.4 (±1.9)	63.0 (±17.4) *	34.0 (±13.7)

* Indicates a significant difference (P<0.005) for that species and experiment between CONTROL and MOW.

experiment. Because we could not compare spring mosquito densities in our mesocosms and macrocosms, we only used September-December 1992 data from our mesocosm discing and burning experiment.

Data collected in each plot were pooled over the entire year. We compared mosquito densities in the treatment and control areas using non-parametric Wilcoxon's paired sample tests for the mowing experiments and Friedman's tests for the discing and burning experiments. We also examined the effects of mowing, discing and burning on other aquatic invertebrates (results to be published elsewhere).

RESULTS

Three species comprised the majority of mosquitoes collected in these experiments: *Culex tarsalis* Coquillett and *Culiseta inornata* (Williston) were common in all experiments while *Aedes melanimon* Dyar were rarely collected in the mowing experiments, but were common in the discing and burning experiments.

Mowing Experiments.

Culex tarsalis densities were lower in the mowed treatment areas in both the mesocosms and macrocosms (Table 1), although these differences were not statistically significant (P>0.05). *Culiseta inornata* densities were marginally higher in the mowed treatment areas of mesocosms and lower in mowed treatment areas in macrocosms. These differences were only statistically significant in the macrocosm experiment (P<0.005).

Discing and Burning Experiments.

Culex tarsalis densities were highest in the disced treatment areas in mesocosms (Table 2), although these differences were not statistically significant. However, *Cx. tarsalis* densities were significantly lower in disced and burned treatment areas than control areas in the macrocosm experiment (P<0.025). *Culiseta inornata* densities were lower in disced and burned treatment areas than control areas, although these differences were only statistically significant in the macrocosm experiment (P<0.025). *Aedes melanimon* densities were significantly lower in disced and burned treatment areas than control areas in mesocosm and macrocosm experiments (P<0.025, for both).

DISCUSSION

In this study, results in mesocosms and macrocosms were similar in the mowing, and the discing and burning experiments, indicating that mesocosm experiments of

Table 2. Effects of vegetation discing and burning on the mean mosquito densities (±S.E.) in the unmanipulated areas (CONTROL) and the disced (DISC) and burned (BURN) treatment areas in both the mesocosm and macrocosm experiments.

Species	Mesocosm Experiment			Macrocosm Experiment		
	CONTROL	DISC	BURN	CONTROL	DISC	BURN
Culex tarsalis	14.0 (±2.0)	128.3 (±59.1)	11.7 (±3.2)	9.8 (±2.7) *	0.5 (±0.5)	0
Culiseta inornata	9.2 (±5.0)	4.0 (±1.8)	1.5 (±0.8)	23.8 (±8.7) *	0	0
Aedes melanimon	177.0 (±74.2) *	36.2 (±14.4)	96.7 (±52.6)	28.0 (±14.7) *	4.5 (±2.0)	0

* Indicates significant differences (P<0.025) for that species and experiment. For all comparisons, CONTROL is significantly different from DISC and BURN, but DISC and BURN are not significantly different.

mosquito control can be effective alternatives to larger-scale experiments. In the mowing experiments, most mosquito densities were lower in the mowed treatment areas than control areas. In the discing and burning experiments, most mosquito densities were lower in disced and burned treatment areas than control areas.

However, some differences between the results of mesocosm and macrocosm experiments were found. In the discing and burning experiments, *Cx. tarsalis* densities in mesocosms were considerably higher in disced treatment areas than in control areas (although these differences were not statistically significant), but densities in macrocosms were significantly lower in disced treatment areas than in control areas. These differences probably occurred because the vegetation had regenerated in the disced treatment areas in the mesocosms but not in the macrocosms. Ovipositing *Cx. tarsalis* may have chosen areas with dense saltgrass and avoided areas without emergent vegetation. High numbers of *Cx. tarsalis* were observed in the disced treatments areas of mesocosms where the saltgrass had regenerated, and low numbers were in areas without saltgrass (F.A. de Szalay, unpublished data).

Management Implications.

High mosquito populations can be an important public health problem in wetland habitats. However, California marshes are also an important habitat for migratory waterfowl, and wildlife managers resist using insecticidal mosquito control methods. Mowing, discing and burning are already widely used in California wetlands to enhance waterfowl habitats (Heitmeyer et al. 1989, Rollins 1981), and our results show they also reduced the dominant mosquito taxa in these habitats. Therefore, it is likely that these methods could be used to control mosquito populations in other wildlife habitats throughout California as well.

Numbers of larval mosquitoes are greatest in areas of dense emergent vegetation because it provides protection from predators and wind action. Mowing, discing and burning probably decreased mosquito densities by reducing the amount of available mosquito habitat. Furthermore, burning can destroy *Aedes* eggs present in the unflooded vegetation in intermittently flooded wetlands (Wallace et al. 1990, Whittle et al. 1993), but unless all of the vegetation is removed, some mosquito production would remain. However, treating all of the vegetation may decrease densities of some taxa important in the diet of ducks and thus reduce waterfowl food resources (Batzer and Resh 1992b, Batzer et al. 1993). Furthermore, waterfowl use is greatest in areas that provide both open water and emergent vegetation (Kaminski and Prince 1981). Therefore, it may be important to create a mosaic of habitats by leaving areas with intact emergent vegetation to promote the best wildlife habitat. Mosquito control districts working with wildlife management agencies together can identify those areas that produce high numbers of mosquitoes and also can be treated using these methods.

ACKNOWLEDGMENTS

We thank Dennis Becker, Steve Ceniseroz, Dave Feliz, Andre Marechal and Bob Smith at the California Department of Fish and Game for their assistance in constructing the experimental plots. The assistance of Dennis Beebe and Carol Evkhanian at the Solano County Mosquito Abatement District is greatly appreciated. This research has been funded by the University-wide Mosquito Research Program, University-wide Mosquito Research Program Student Mini-grants, Solano M.A.D. and the Switzer Foundation.

REFERENCES CITED

Batzer, D.P. and V.H. Resh. 1988. Waterfowl management and mosquito production in diked salt marshes: preliminary considerations and mesocosm design. Proc. Calif. Mosq. Vector Control Assoc. 56:153-157.

Batzer, D.P. and V.H. Resh. 1991. Trophic interactions among a beetle predator, a chironomid grazer, and periphyton in a seasonal wetland. Oikos 60:251-257.

Batzer, D.P. and V.H. Resh. 1992a. Wetland management strategies that enhance waterfowl habitats can also control mosquitoes. J. Amer. Mosq. Control Assoc. 8:117-125.

Batzer, D.P. and V.H. Resh. 1992b. Macroinvertebrates of a California seasonal wetland and responses to experimental habitat manipulation. Wetlands 12:1-7.

Batzer, D.P., M. McGee, V.H. Resh and R.R. Smith. 1993. Characteristics of invertebrates consumed by mallards and prey responses to wetland flooding schedules. Wetlands 13:41-49.

Cooper, S.D. and L.A. Barmuta. 1993. Field experiments in biomonitoring. pp. 399-441. *In:* D.M. Rosenberg and V.H. Resh (eds.). Freshwater Biomonitoring and Benthic Macroinvertebrates. Routledge, Chapman and Hall, Inc., New York, NY. 488 pp.

Euliss, N.H. and G. Grodhaus. 1987. Management of midges and other invertebrates for waterfowl wintering in California. Calif. Fish Game 73:238-243.

Heitmeyer, M.E., D.P. Connelly and R.L. Pederson. 1989. The Central, Imperial, and Coachella Valleys of California. pp. 475-505. *In:* L.M. Smith, R.L. Pederson and R.M. Kaminski (eds.). Habitat Management for Migratory and Wintering Waterfowl in Northern America. Texas Tech Univ. Press, Lubbock, TX. 560 pp.

Kaminski, R.M. and H.H. Prince. 1981. Dabbling duck and aquatic macroinvertebrate responses to manipulated wetland habitat. J. Wildl. Mgmnt. 45:1-15.

Odum, E.P. 1984. The mesocosm. Bioscience 34:558-562.

Provost, M.W. 1977. Source reduction in salt-marsh mosquito control: past and future. Mosquito News 37:689-698.

Rollins, G.L. 1981. A Guide to Waterfowl Habitat Management in Suisun Marsh. Calif. Dept. Fish Game Publ. 109 pp.

Solomon, K.R., G.L. Stephenson and N.K. Kaushik. 1989. Effects of methoxychlor on zooplankton in freshwater enclosures: influences of enclosure size and number of applications. Environ. Toxicol. Chem. 8:659-669.

Stephenson, G.L., P. Hamilton, N.K. Kaushik, J.B. Robinson and K.R. Solomon. 1984. Spatial distribution of plankton in enclosures of three sizes. Can. J. Fish. Aquatic. Sci. 41:1048-1054.

Wallace, F.L., M.A. Tidwell, D.C. Williams and K.A. Jackson. 1990. Effects of controlled burning on *Aedes taeniorhynchus* eggs in an abandoned rice impoundment in South Carolina. J. Amer. Mosq. Control Assoc. 6:528-529.

Whittle, R.K., K.J. Linthicum, P.C. Thande, J.N. Wagati, C.M. Kamau and C.R. Roberts. 1993. Effect of controlled burning on survival of floodwater *Aedes* eggs in Kenya. J. Amer. Mosq. Control Assoc. 9:72-77.

AUTHOR INDEX

- Pages in Bold Print Indicate Principal Authorship -

PUBLICATION POLICIES AND INFORMATION FOR CONTRIBUTORS

The *Proceedings* is the *Proceedings and Papers of the California Mosquito and Vector Control Association, Inc.* One volume is printed each year and includes the papers and presentations of the Association's annual conference, special contributions and meritorious reports submitted for the conference year, and a synopsis of actions and achievements by the Association during the publication year.

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. An excessive number of papers on one subject or by any one author is generally dissuaded. Although preference is given to papers of the conference program, acceptability for publication rests on merit determined on review by the Editor and the Publications Committee. A non-member author wishing to publish in the *Proceedings* is required to pay the registration fee for the conference.

MANUSCRIPTS: The great diversity of interest and fields of endeavor represented by contributors and readership of the *Proceedings* precludes strict conformance as to style. It is expected that the manuscripts will be in English with proper spelling and punctuation. The list, "References Cited", will include only publications cited in the text, and the style of the citations should conform to the format of the recent articles in the *Proceedings*. Authors should refer to recent issues of the *Journal of the American Mosquito Control Association* and the *Proceedings and Papers of the California Mosquito and Vector Control Association, Inc.* for general guidance. Authors of technical papers should follow the basic recommendations as presented in the *Council of Biology Editors Style Manual (Fifth edition).*

The *Proceedings* subscribes to the scientific abbreviations of mosquito generic names used by the American Mosquito Control Association. The usage and a list of these scientific names is discussed in the *Journal of the American Mosquito Control Association* 5:485 (1989). Bi-letter generic abbreviations are used for Culicidae. Common Latin abbreviations (et al., e.g., i.e., etc.) are not italicized. Use of the metric system is encouraged.

All parts of the manuscripts (text, tables, references, and legends) must be typed, double-spaced with ample margins. Avoid footnotes in text. An IBM-compatible diskette with a copy of the article and one copy of your manuscript with two copies of your plates, tables, and/or photographs attached should be sent to the Editor within 60 days following the end of the conference. Articles received after that time may be returned for resubmission for the next *Proceedings*.

The Editor may refuse to publish abstracts or summaries alone; these are integral parts of the articles. If accepted, the journal where the paper is to be published in full should be stated in the expanded abstract.

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

TABLES: Tables are typed on separate sheets placed in correct sequence in the text and should be limited to those strictly necessary. Tables should be prepared with regard to the ultimate printed size of one (3") or two columns (6 1/2").

ILLUSTRATIONS: Illustrative material (including figures, graphs, line drawings, and photographs) must be mailed flat. Figures should be numbered consecutively. Titles, legends, or other headings should be typed double-spaced on a separate sheet of paper. As with tables, illustrative materials must be planned to fit reasonably within one or two columns or on a single page. Figure numbers, in addition to the author's name should be written in blue pencil on the back of each illustration. Figures generated on dot matrix printers are not acceptable as publication quality.

PROOF AND REPRINTS: Authors will receive a galley proof, as well as order forms for reprints with a schedule of charges. Authors should not make major revisions of their work at this stage. Proofs with corrections, if any, and reprint order forms should be returned within ten days to the CMVCA office or directly to the Editor.