PROCEEDINGS AND PAPERS

of the

Seventieth Annual Conference of the

Mosquito and Vector Control Association of California

January 27 thru January 30, 2002

Held at the Tenaya Lodge at Yosemite
Fish Camp, California

Editor: Minoo B. Madon
Greater L.A. County VCD
12545 Florence Avenue
Santa Fe Springs, CA 90670
Phone: (562) 944-9656
Fax: (562) 944-7976
E-mail: mmadon@glacvcd.org

Reviewers: Steve Schutz, Contra Costa Mosquito Control District; Bruce F. Eldridge, UC Davis; Lal S. Mian, California State University, San Bernardino; William E. Walton, UC Riverside; James P. Webb, Orange County VCD; and Glenn M. Yoshimura, Sacramento-Yolo MVCD
Layout and Editorial Assistance: Emily Young, MVCAC

Mosquito and Vector Control Association of California
660 J Street, Suite 480, Sacramento, California 95814
Phone: (916) 440-0826 • Fax: (916) 442-4182
E-mail: mvcac@mvcac.org

Published January 2003

2002
Mosquito and Vector Control Association of California

Christopher J. Voight, Executive Director
Charlie Dill, Treasurer
Minoo B. Madon, Proceedings Editor

BOARD OF DIRECTORS

President: Major S. Dhillon, Ph.D.
President Elect: Bill Hazeleur
Vice President: Craig Downs
Past President: Steve Mulligan
Chair, Trustee Advisory Council: Gary Risberg

Coastal Rep: John Rusmisel
Sacramento Valley Rep: David Brown
No. San Joaquin Valley Rep: John Stroh
So. San Joaquin Valley Rep: Elizabeth Cline
S. California Rep: Robert Sjogren, Ph.D.

TRUSTEE ADVISORY COUNCIL

Chair: Gary Risberg
Vice Chair: Wilbert Aalto
Secretary: Grant McCombs
So. San Joaquin Valley Rep: Wilbert Aalto

Coastal Rep: Gary Risberg
Sacramento Valley Rep: Bruce Wade
No. San Joaquin Valley Rep: Tom Lauret
S. California Rep: Sydney Gordon

CORPORATE MEMBERS

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

2002 MVCAC SUSTAINING MEMBERS

AVENTIS CHEMICAL CORPORATION

CLARKE MOSQUITO CONTROL PRODUCTS, INC.

ELECTRAMIST, INC.

FENNIMORE CHEMICALS

PIGOTT & ASSOCIATES, INC.

VALENT BIOSCIENCES CORPORATION

VOPAK USA

ZOECON PROFESSIONAL PRODUCTS

Volume 70 .... January 27 - 30, 2002

CONTENTS

Board of Directors, Trustee Advisory Council and Corporate Members iii
Sustaining Members v

Conference Dedication

Conference Dedication to Dr. William Donald Murray 1
Earl W. Mortenson

Proceedings

The "Dark Side" of Stormwater Runoff Management: Vectors Associated with BMPs
Marco E. Metzger, Charles M. Myers, and Vicki L. Kramer 2

Climate Variability and Encephalitis Epidemiology
W.K. Reisen 11

Climate Variability and Encephalitis Epidemiology
Database Structure, Access, and Use
B.F. Eldridge 13

Climate Linkages to Female *Culex Cx. tarsalis* Abundance in California
Dan Cayan, Mary Tyree, and Mike Dettinger 15

Degree-day Models: Relationship to Mosquito Seasonality
and Virus Occurrence

Farida Mahmood 20

Evaluation of the California Encephalitis Epidemic Risk Assessment Scheme Using Conditional
Simulations with Historical Data
Christopher M. Barker, William K. Reisen, and Vicki L. Kramer 31

Effect of Vegetation Management on the Abundance of Mosquitoes at a Constructed Treatment
Wetland in Southern California
J. B. Keiper, J.A. Jiannino, M.R. Sanford, and W.E. Walton 35

Surveillance for Mosquito-Borne Encephalitis Virus Activity and Human Disease in California, 2001
Stan Husted, Ashley B. Houchin, Vicki L. Kramer, Robert E. Chiles, Michele Jay,
William K. Reisen, Bruce F. Eldridge, Carol Glaser, Cindi Cossen, Evelyn Tu, William C.
Reeves, Thomas W. Scott, Martin Castro, Aline Cornelius, and Malcolm Thompson 44

Mosquitofish in the High Desert
Keith White, Karen S. Mellor 53

Factors Affecting the Outcome of *Culex tarsalis* Mark-Release-Recapture Experiments in the Coachella Valley of California
Hugh D. Lothrop and William K. Reisen 55

Description of a Nurse Rig Trailer Used as Support for Larviciding Operations by the San Mateo County Mosquito Abatement District
Stanley Kamiya and Chindi Peavey 58

Description of Equipment Used for Application of Pesticides from an Argo® All-Terrain Vehicle
Christopher Edwards and Chindi A. Peavey 60

Field Evaluation of Aqua-Reslin Against *Culex quinquefasciatus* Using Standard and Optimized Aerial Application Techniques
Allan Inman, David Sykes, Janice Stroud, and Bill Reynolds 62

Notes on the Aerial Application of Trumpet In the Butte Sink
Kenneth Townzen[1], Michael Kimball[2], Ronald McBride[2], and David Whitesell 64

Larval Control in Rice Fields Utilizing the Icy Pearl Method
D.B. Jacks, P. DeChant, G. Yoshimura 67

Aedes albopictus Infestations in California
Kenneth J. Linthicum, Vicki Kramer, and The Surveillance Team 70

Development and Implementation of Greater Los Angeles County Vector Control District's Non-Medical Emergency Response Africanized Honeybee Program
Jack Hazelrigg 73

Surveillance for *Aedes albopictus* to Determine Local Extent of Infestation
Susanne Kluh, Jack E. Hazelrigg, Michael W. Shaw & Minoo B. Madon 79

Evaluation of Mosquito and Arbovirus and Arbovirus Activity in Orange County During 2001
Robert F. Cummings, Stephen G. Bennett, Carrie L. Fogarty, Ralph Havickhorst, Greg Williams, Amber Mills, Shana Lowe and James P. Webb, Jr. 87

Operational Applications of GIS Technology in the Detection and Eradication of Red Imported Fire Ants (*Solenopsis invicta*) in Orange County California
Michael G. Hearst, Shana Lowe, Maria Diuk-Wasser, and Richard P. Meyer 92

Vertical Distribution of Mosquitoes in the Prado Basin, Riverside County, CA
Lal S. Mian 95

Comparison of the Efficacy of Three Pesticides for Control of Ground Nesting Yellowjackets (*Vespula vulgaris* L.)
James P. O'Brien and Chindi A. Peavey 98

Previously Submitted Papers

Geospatial and Statistical Modeling of Mosquito Distribution in an Emerging Focus of La Crosse Virus
Christopher M. Barker, Carlyle C. Brewster, and Sally L. Paulson 103

Vectolex CG® Trial on Ochlerotatus washinoi, Shasta County, CA
John Albright 108

Early Season Factors Affecting *Vespula pensylvanica* Peak Populations, Shasta County, CA
John Albright 110

A Two-year Study of the Application of Altosid® (Methoprene) for Controlling Ground-nesting Yellowjackets (Hymenoptera: Vespidae)
Lucia T. Hui, William W. Pitcher, Ronald Keith, Robert Schoeppner, Charles H. Dill, and Charles P. Hansen 113

Submitted Papers

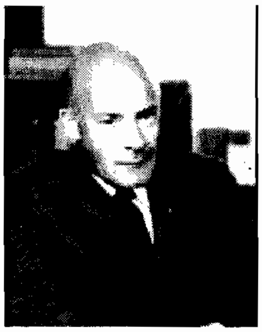
Mosquito Abundance and Arbovirus Surveillance in Northwestern Riverside County in 2001
Lal S. Mian1 and Cecillia Reed 121

Guidelines for Contributors

Guidelines 125

Conference Dedication in Memory of Dr. William Donald Murray

Submitted by Earl Mortenson, Trustee, Contra Costa Mosquito and Vector Control District



The 70th Annual Conference of the MVCAC is dedicated in memory of Dr. Don Murray and in recognition of his 35 years of service to mosquito and vector control.

Dr. Murray passed away on March 18, 1998 at his home in Visalia, California. He was born on August 9, 1913 in Shadyside, Ohio. His early interest in natural history was spawned by his experience in summer YMCA camp sessions. There he met Dr. Donald Borror and Dr. D. M. Delong, both professors of entomology at Ohio State University, who influenced him to attend Ohio State and major in entomology, where he received his B. S. degree. Don then received a scholarship to attend the University of Minnesota. While there he was awarded a Master's degree and later a Ph.D. in Medical Entomology. He married his wife Frances during the time he was in graduate school.

After he received his Ph.D., he taught zoology at Eveleth College and then Bemidji State Teachers' College in Minnesota from 1942-1943.

In November of 1943, Don received an Officer's commission in the U.S. Navy and was sent to the South Pacific to head up a malaria control unit, then later transferred to Samoa to conduct a mosquito-filaria control program.

He worked for the California State Health Department, Bureau of Vector Control in 1946.

He was hired in 1947 as Manager-Entomologist for the Delta Mosquito Abatement District. In his 31 years (1947-1978) as manager of the District, Don developed many important innovative approaches to mosquito surveillance, source reduction, and community fly control.

Dr. Murray was Past-President (1956), elected Honorary Member in 1979, and served as Secretary-Treasurer for the California Mosquito and Vector Control Association. He was awarded the "Medal of Honor" for his service as Treasurer (8 years) by the American Mosquito Control Association. He was a founding member for the Society for Vector Ecology. He continued to be active as an officer and consultant to the U.S. Navy Medical Service Corps., obtaining the rank of Captain before his retirement from the U.S. Naval Reserve.

He is survived by his wife Frances Murray of Visalia, son David Murray and daughter Lois Feleay.

The "Dark Side" of Stormwater Runoff Management: Vectors Associated with BMPs

Marco E. Metzger, Charles M. Myers, and Vicki L. Kramer

Vector-Borne Disease Section, Department of Health Services, 2151 Convention Center Way, Suite 218B, Ontario, CA 91764-5429, (909) 937-3440, mmetzger@dhs.ca.gov

ABSTRACT: New federal, state, and local clean water regulations are forcing tremendous changes with regards to how stormwater and urban runoff must be treated and managed. Implementation of structural devices known as Best Management Practices (BMPs) is required under the new laws in an effort to improve the quality of urban stormwater runoff. Unfortunately, many of these devices can create significant vector habitats when not properly designed and maintained. Since 1998, the California Department of Health Services, Vector-Borne Disease Section (VBDS), has been involved in a study funded by the California Department of Transportation (Caltrans) designed to investigate the association between vectors and stormwater BMPs in collaboration with four southern California vector control agencies and stormwater consultants. A total of 37 Caltrans BMPs in San Diego and Los Angeles Counties were monitored for the presence of vectors and/or vector habitat between May 1999 and April 2001. Eight mosquito species were collected and identified, including *Culex tarsalis*, *Cx. stigmatosoma*, *Cx. quinquefasciatus*, and *Anopheles hermsi*. Of the different BMP devices monitored, those that maintained permanent sources of standing water (by design) in sumps or basins frequently supported large populations of mosquitoes. In contrast, those BMPs designed to drain rapidly and completely rarely contained suitable mosquito breeding habitats. Information gathered during this study provided an initial assessment of the factors within BMPs conducive to mosquito production and which species utilize these structures. Based on these findings, appropriate BMP alterations and design modifications were recommended to reduce or eliminate standing water.

INTRODUCTION AND BACKGROUND

Water pollution caused by urban stormwater runoff is viewed by some as the "last frontier" in a half-century effort to clean and restore the nation's waters. In recent years, new legislation and aggressive litigation by environmental groups has put increasing pressure on government and industry to reduce or eliminate runoff pollution (Copeland 1999, 2000). Unfortunately, an unintentional result of the measures being implemented to mitigate this pollution is the potential to significantly increase vector breeding habitats, particularly in and around urban landscapes (Amalfi et al. 1999, Dorothy and Staker 1990, Florida Coordinating Council on Mosquito Control 1998, Kluh et al. 2002, McLean 2000, Metzger et al. 2002, Santana et al. 1994). To better understand the current situation and how it may influence public health, it is necessary to briefly summarize the history of water pollution control (Copeland 1999).

The 1940's. In 1948, the Federal Water Pollution Control Act was born as the principal law governing pollution in the nation's surface waters (e.g., streams, lakes, and estuaries) and represented the first comprehensive statement of federal interest in clean water programs. The original statute specifically provided state and local governments with technical assistance and research funds to address water pollution problems. The Surgeon General of the Public Health Service, in cooperation with other Federal, state, and local entities, was authorized to prepare comprehensive programs for eliminating or reducing the pollution of interstate waters and tributaries and improving the sanitary condition of surface and underground waters. Water pollution was viewed primarily as a state and local problem, thus there were no federally required goals, objectives, limits, or guidelines. Since 1948, the original statute has been amended extensively to authorize additional water quality programs, standards and procedures to govern allowable discharges, and funding for construction grants or general programs.

The 1960's. In the 1960's the growing environmental movement found ready examples of the vulnerability of America's waters. The Cuyahoga River, located in northeast Ohio, and Lake Erie played important roles in the birth of the environmental movement. Fires erupted on the surface of the Cuyahoga beginning in 1936, fueled by floating chemicals, industrial wastes, debris and oils. In 1969, Time Magazine captured national attention in an article that described the Cuyahoga as a river that "oozes rather than flows" and in which a person "does not drown but decays" (Anon 1969). This event helped set the stage for an avalanche of pollution control activities, including the Great Lakes Water Quality Agreement, the creation of the federal and state Environmental Protection Agencies, and significant amendments to the Federal Water Pollution Control Act.

The 1970's. In 1972, the Federal Water Pollution Control Act was totally revised by amendments that gave the Act its current form and spelled out ambitious programs for water quality improvements to be adopted by government and industry. The objectives of the new legislation were the restoration and maintenance of the chemical, physical, and biological integrity of the nation's waters. The new amendments prohibited the discharge of any pollutant to waters of the United States (U.S.) from a "point source" unless the discharge was authorized by a

National Pollutant Discharge Elimination System (NPDES) Permit.

The 1972 amendments mandated that the United States Environmental Protection Agency (EPA) design an NPDES program to track point sources, monitor the discharge of pollutants from specific sources to surface waters, and require the implementation of the controls necessary to minimize the discharge of pollutants. Point sources were defined by the EPA as discrete and identifiable sources such as pipes or man-made ditches and culverts. Initial efforts focused primarily on reducing pollutants in industrial process wastewater and discharges from municipal sewage treatment plants. Target pollutants included human wastes, ground up food from trash disposals, laundry and bath waters, toxic chemicals, oils and grease, metals, and pesticides. Congress made certain fine-tuning amendments in 1977 and 1981, which included a name change to the Clean Water Act (CWA) as we know it today.

The 1980's. As point source pollution control measures were implemented and refined, studies showed that water runoff over land carried a variety of pollutants that could impact the quality of receiving waters. These pollutants were estimated to represent over 50% of the nation's remaining water pollution problems. As a result, in 1987 the CWA was again amended, this time prohibiting the discharge of any pollutant to waters of the U.S. from a "non-point source" (except for agricultural runoff, which remained exempt) unless the discharge was authorized by an NPDES Permit. According to the EPA's broad definition of a non-point source, these could include areas with snowmelt, irrigation, and/or stormwater runoff originating from diverse sources such as agricultural lands, forests, construction sites, and urban areas. Target pollutants included sediments, heavy metals, oils and greases, and nutrients (i.e. nitrogen and phosphorous).

The 1987 amendments established a framework for regulating municipal, industrial and construction stormwater discharges under the various state implemented NPDES programs. (e.g., California NPDES permits are issued through the State Water Resources Control Board and the nine Regional Water Quality Control Boards). The EPA was mandated to develop a 2-phase implementation strategy for the NPDES Stormwater Program. Regulated entities had to develop and implement Stormwater Pollution Prevention Plans (SWPPPs) and Stormwater Management Programs (SWMP). These programs required the implementation of Best Management Practices (BMPs) to effectively reduce or prevent the discharge of pollutants into receiving waters. A BMP could involve the use of structural, nonstructural, and/or managerial techniques recognized to be the most effective and practical means of reducing surface and ground-water contamination while still allowing for the productive use of resources. BMPs for stormwater management might include modifying activity schedules, prohibitions or modifications of practices, and maintenance procedures as well as the use of structures such as retention and detention ponds, swales, ditches, channels, vaults, infiltration basins, filtration systems and others.

The 1990's. The first phase of the EPA's NPDES Stormwater Program was developed and implemented in 1990, targeting the most likely sources of wet weather pollution including: 1) "medium" and "large" municipal separate storm sewer systems (MS4s) generally serving, or located in incorporated places or counties with populations of 100,000 or more people, and 2) eleven categories of industrial activity, including any construction that disturbed ≥5 acres and discharged stormwater runoff into waters of the U.S. or into an MS4. Phase I required operators of MS4's and industrial activities to obtain NPDES permits for stormwater runoff and develop and implement SWPPPs designed to prevent target pollutants from being washed into local bodies of water. Issuing permits allowed the EPA to monitor and control the nature and quantity of pollutants reaching surface waters. Permit coverage could be either under an individually-tailored NPDES permit (used by MS4s and some industrial facilities) or a general NPDES permit (used by most industrial facilities and construction sites). In addition, under Phase I, MS4's serving less than 100,000 people and non-regulated industrial categories or individual facilities noted as significant contributors of pollutants to waters of the U.S., could be brought under the NPDES Stormwater Program by the NPDES permitting authority. The Phase I Rule established 1994 as a deadline for these agencies to be permitted.

Y2K. The Phase II Rule, implemented in 2000, expanded NPDES permit requirements (mostly under general permits) to include stormwater discharges from: 1) "small" MS4s not previously covered under Phase I regulations located in "urbanized areas" as defined by the Bureau of the Census (termed a "regulated" MS4s), and 2) any construction activity that disturbed ≥1 acre. The Phase II Rule established March 10, 2003 as the deadline for permit applications. Operators of small MS4s are required to fully implement their SWMPs by the end of their first permit term (typically 5 years). Under Phase II, additional small MS4s (outside urbanized areas) and construction sites disturbing < 1 acre of land, as well as other sources identified as significant contributors of pollutants to waters of the U.S., can be brought into the NPDES Stormwater Program by the NPDES permitting authority.

STRUCTURAL BEST MANAGEMENT PRACTICES

Stormwater is a relatively new field of work, with unprecedented growth in government, business, and private industry. As noted earlier, a BMP may refer to a variety of different things. In fact, the term "Best Management Practice" is essentially open to interpretation based on an individual's idea of what is "best". Currently, a major focus in the stormwater field is the development of "treatment" BMPs designed to remove target pollutants from stormwater runoff. A multitude of different devices have been designed and constructed throughout the U.S., with new technologies being created at a furious pace to keep up with the requirements of Phase I and Phase II of the NPDES Stormwater Program. Many of these are still under close scrutiny in trying to determine their true potential for improving water quality and at what cost. Treatment BMPs are typically tailored to capture an estimated volume of water runoff from a given piece of land. Depending on the land allotted for the BMP, these devices are built in all conceivable shapes and sizes (Debo and Reese 1995, Water Environment Federation and American Society of Civil Engineers 1998). Figures 1-5 illustrate a few examples.

Figure 1.

Name: Extended Detention Basin

Purpose: Water volume management and treatment

Function: Capture runoff and release it slowly via small orifices. Pollutants settle out of the water table and remain on the basin floor

Target: Sediment, metals, debris, trash

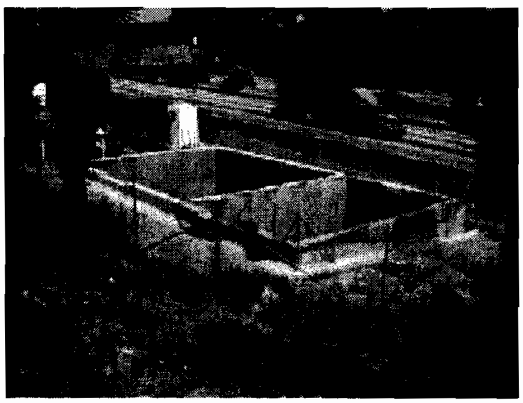


Figure 2.

Name: Sand Media Filter

Purpose: Water volume management and treatment

Function: Two-step process: 1) Capture runoff in a sedimentation basin and release it slowly via small orifices to a sand media basin. Larger pollutants settle out onto the floor of the sedimentation basin. 2) Water is "polished" when it is filtered through sand media

Target: Sediment, metals, debris, trash

Figure 3.

Name: Biological Filtration Swale

Purpose: Water quality treatment

Function: Runoff is directed through a vegetated channel. Pollutants are "trapped" by vegetation as water passes over it

Target: Sediment, nutrients, metals

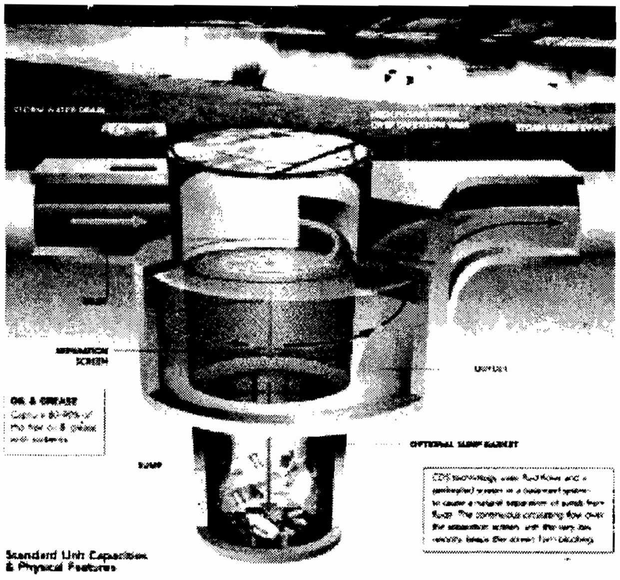


Figure 4.

Name: Continuous Deflective Separator (CDS™)
Purpose: Water quality treatment
Function: Runoff is directed at an angle into an upright cylindrical sump. A vortex is created that helps direct large pollutants into a removable net bag at the bottom of the sump. Water then passes through a fine-mesh screen and exits the device

Target: Heavy sediment, debris, trash

Figure 5.

Name: Retention Pond

Purpose: Water volume management and treatment

Function: Incoming runoff displaces old water, which slowly flows out of the pond until the water level returns to "normal." Long detention promotes pollutant settling and nutrient uptake by aquatic vegetation

Target: Sediment, metals, nutrients, debris, trash

BMPS AND HUMAN HEALTH

From a human health standpoint, BMP structures designed for water volume management and/or treatment are of concern because of their tendency to hold standing water. Several of the above described devices (Figures 1-5) illustrate the potential vector breeding habitats, many of which can be very large.

THE CALTRANS BMP RETROFIT PILOT STUDY

The California Department of Transportation (Caltrans) is the agency responsible for managing California's state highway system and is one of the many agencies affected by the requirements of the CWA. In response to the 1987 amendments to the CWA, Caltrans developed its own stormwater program. The Caltrans Storm Water Program has two primary goals: 1) to comply with requirements of the federal CWA and resulting NPDES permit and other state requirements, and 2) to provide the most cost-effective solutions for mitigating the harmful effects of stormwater runoff.

Litigation between Caltrans and a consortium of plantiffs, including the EPA, National Resources Defense Council (NRDC), Santa Monica Baykeeper, and San Diego BayKeeper, resulted in a stipulation requiring Caltrans to develop a study focused on improving the quality of stormwater runoff by installing treatment BMPs. The primary objectives of the resultant BMP Retrofit Pilot Program were to assess the feasibility of retrofitting various treatment BMPs into existing Caltrans facilities, including freeways, interchanges, park and ride facilities, and maintenance stations, evaluate any water quality benefits (i.e., pollutant removal efficiency) resulting from BMP installations, and determine their cost effectiveness. Results would identify which BMPs were most appropriate for "approval" and eventual widespread deployment.

In 1997, Caltrans initiated an extensive program plan to retrofit 33 selected facilities with 39 treatment BMPs in Caltrans District 7 (Los Angeles County) and Caltrans District 11 (San Diego County). These BMPs included biofiltration strips and swales, various media filtration devices, extended detention basins, infiltration basins and trenches, trash separating devices, drain inlet inserts, an oil/water separator, and a constructed wetland. Construction began in September 1998 and all but two BMPs were completed over the following six months; these included 24 in Los Angeles Co. at 19 sites and 13 in San Diego Co. at 12 sites (Table 2).

It has long been known that construction of stormwater management structures such as treatment BMPs can have direct impacts on the operations of vector control agencies and on public health by increasing habitat availability for disease vectors (Amalfi et al. 1999, Dorothy and Staker 1990, Florida Coordinating Council on Mosquito Control 1998, McLean 2000, Santana et al. 1994). As a result, in 1998 the California Department of Health Services, Vector-Borne Disease Section (VBDS) entered into an agreement with Caltrans to provide technical expertise regarding vector production and the potential of vector-borne diseases within its stormwater BMP Retrofit Pilot Study. The intent of the Caltrans-VBDS project was to protect public health by documenting and, where possible, mitigating vector production and harborage at the BMP study sites. The agreement required VBDS to establish a comprehensive vector surveillance and monitoring study designed to identify which BMPs were least conducive to vector production and, based on results, recommend appropriate engineering modifications that would reduce their potential to produce or harbor vectors.

The study plan for the 37 operational BMPs outlined various activities to be conducted in collaboration with Greater Los Angeles County Vector Control District (GLACVCD), San Gabriel Valley Mosquito and Vector Control District (SGVMVCD), Los Angeles County West Vector Control District (LACWVCD), and San Diego County Vector Surveillance and Control (SDCVSC) in their respective jurisdictions. BMPs were monitored weekly to document and evaluate vector production, particularly of mosquitoes. In addition, observations of vegetative cover, predators of mosquito immatures, and evidence of rodent and other vector populations were recorded. BMPs breeding mosquitoes were treated weekly with a liquid formulation of methoprene (Altosid EC®). Methoprene was selected because it did not impact ongoing pollutant removal efficiency monitoring of BMPs and because it would allow immature mosquitoes to survive, in theory providing a better long-term view of the suitability of the created habitat to support mosquitoes.

PURPOSE AND OBJECTIVES

The primary purpose of this study was to evaluate vector production associated with different treatment BMPs implemented by Caltrans as part of their BMP Retrofit Pilot Study and subsequently make recommendations to mitigate vector production in these devices if needed. Two years of larval mosquito data obtained through weekly monitoring beginning in early May 1999 and running through April 2001 are summarized. These data were used to identify vector sources within BMP types or within individual designs and were used to evaluate the success of efforts to mitigate these problem areas. This study provides an initial assessment of the potential public health risks associated with the construction of treatment BMPs in southern California and addresses the factors that encouraged vector production within these structures.

BMPS AND VECTORS

Eight different species of larval mosquitoes, in four different genera, were collected and identified (Table 1). Each BMP type provided unique challenges in preventing vector production and all but 3 types were found to harbor mosquito larvae at some point during the two-year study (Table 2). BMPs that maintained permanent sources of standing water (i.e., MCTT, CDS™, wet basin) frequently supported large populations of mosquitoes relative to other structural designs. In contrast, BMPs designed to drain rapidly (i.e., biofiltration swales and strips, sand media filters, infiltration basins and trenches, drain inlet inserts, extended detention basins, oil/water separator) provided few habitats for vectors (Figure 6). An overall summary of immature mosquitoes detected from different BMP technology types is presented in Figure 6, and site-specific information is provided in Table 2.

Vector production at the Caltrans BMP structures was influenced not only by design, but also by factors such as weather, location, non-stormwater discharges (e.g., irrigation), site maintenance, and various other unexpected events, making direct comparisons between structures of similar design difficult, if not impossible. The following recommendations were made to Caltrans and their stormwater consultants based on existing knowledge (Amalfi et al. 1999, Dorothy and Staker 1990, Florida Coordinating Council on Mosquito Control 1998, McLean 2000, Santana et al. 1994), as well as on data and observations acquired during this study. If followed, these recommendations should help reduce the probability of vector breeding and allow for routine vector surveillance (and abatement if necessary) and site maintenance in future installations (Figure 7).

DISCUSSION

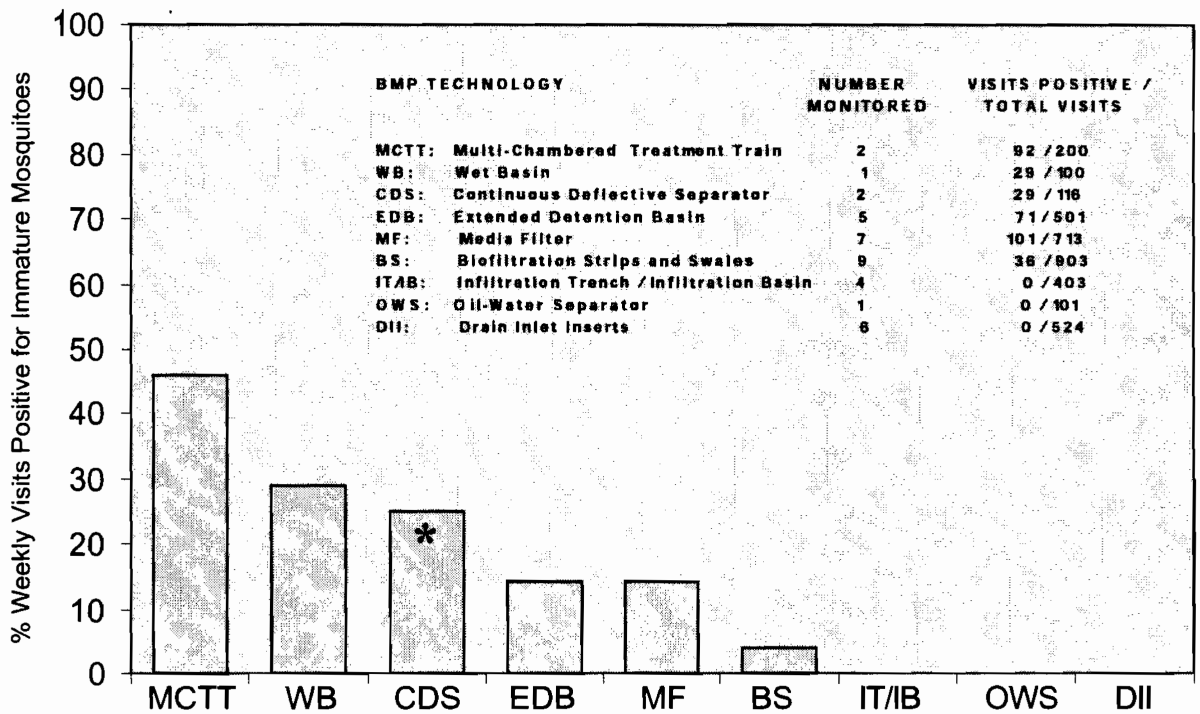
New BMP structures are being designed and installed daily across the country in an effort to comply with regulatory deadlines. Without delay, vector control agencies must get involved with state and local agencies or programs involved with stormwater runoff, and forge relationships with stormwater engineers, planners, regulators, and developers. Ideally, language regarding vectors should be drafted and submitted for incorporation into state and local stormwater laws and regulations. This language should focus on agency collaboration to minimize, and where possible, eliminate mosquito production in proposed and existing construction. Language should also recognize that mosquito production may create a public nuisance and health threat. Ultimately, vector control agencies should consider developing and implementing a formal plan that would include a fee structure (i.e., cost recovery) for project plan review and/or routine vector surveillance and control for all sites. In addition, all stormwater programs should include training in mosquito and vector biology and control. Although several exist at the local level, an "official" and recognized statewide (preferably nationwide) publication on mosquito control in stormwater treatment BMPs is needed to educate designers, builders, and operators.

This study provides an initial assessment of the potential public health risks involved with the construction of various structural BMPs in southern California. Few studies have addressed vector issues in artificial habitats created by BMPs built specifically for reducing non-point source pollution in stormwater runoff. Results indicate that much research remains to be

Table 1. Species of mosquito larvae collected and identified from treatment BMPs constructed for the Caltrans BMP Retrofit Pilot Study, May 1999 - April 2001.

Genus	**species**
Culex	*(pipiens) quinquefasciatus* *tarsalis* *stigmatosoma*
Culiseta	*incidens* *inornata*[a]
Anopheles	*hermsi* *franciscanus*[a]
Ochlerotatus	*squamiger*[a]

[a]Only collected in San Diego County, Caltrans District 11.



*Monitoring from March 2000 to April 2001

Figure 6. Weekly vector monitoring of BMP technology types in Caltrans District 7 and 11, May 1999 - April 2001.

Table 2. Weekly mosquito monitoring of BMPs constructed for the Caltrans BMP Retrofit Pilot Study, May 1999 - April 2001.

		Weekly Mosquito Monitoring			
BMP Type	**Location**	**Monitoring Period**	**Total Visits**	**Pos. Visits**	**% Pos.**
Wet Basin	I-5/La Costa Ave.	6/15/99 - 4/30/01	100	29	29.0%
Extended Detention Basins (EDB)	I-5/I-605	6/2/99 - 4/24/01	103	21	20.4%
	I-605/SR 91	6/2/99 - 4/24/01	103	0	0.0%
	I-5/SR 56	5/5/99 - 4/30/01	103	50	48.5%
	I-15/SR 78	5/5/99 - 4/30/01	93	0	0.0%
	I-5/Manchester Ave.	5/5/99 - 4/30/01	99	0	0.0%
Drain Inlet Inserts (DII): (*Two per site*)	Foothill MS	5/7/99 - 4/26/01	184	0	0.0%
	Rosemead MS	5/7/99 - 4/26/01	198	8	4.0%
	Las Flores MS	12/8/99 - 4/24/01	142	0	0.0%
Infiltration Basins and Trenches	Altadena MS[a]	6/1/99 - 4/24/01	103	0	0.0%
	Carlsbad MS[a]	5/5/99 - 4/30/01	98	0	0.0%
	I-605/SR 91[b]	6/2/99 - 4/24/01	101	0	0.0%
	I-5/La Costa Ave.[b]	5/5/99 - 4/30/01	101	28	27.7%
Oil/Water Separators	Alameda MS	6/1/99 - 4/24/01	101	5	5.0%
Media Filters	Eastern Regional MS[c]	6/1/99 - 4/24/01	103	7	6.8%
	Foothill MS[c]	5/7/99 - 4/26/01	92	3	3.3%
	Termination P&R[c]	5/20/99 - 4/24/01	106	14	13.2%
	SR 78/I-5 P&R[c]	5/5/99 - 4/30/01	103	24	23.3%
	La Costa P&R[c]	5/5/99 - 4/30/01	103	29	28.2%
	Escondido MS[d]	5/5/99 - 4/30/01	102	6	5.9%
	Kearny Mesa MS[e]	5/5/99 - 4/30/01	104	18	17.3%
Multi-Chambered Treatment Trains (MCTT)	Via Verde P&R	5/7/99 - 4/26/01	98	28	28.6%
	Lakewood P&R	5/20/99 - 4/24/01	102	64	62.7%
Continuous Deflective Separators (CDS™)	I-210 (Orcas Ave.)	3/16/00 - 4/24/01	59	22	37.3
	I-210 (Filmore Ave)	3/16/00 - 4/24/01	57	7	12.3%
Biofiltration Swales and Strips	I-5/Palomar Airport[f]	5/5/99 - 4/30/01	97	0	0.0%
	SR 78/Melrose Dr.[f]	5/5/99 - 4/30/01	96	0	0.0%
	I-605/Del Amo Ave.[f]	6/2/99 - 4/24/01	101	4	4.0%
	I-5/I-605[f]	6/2/99 - 4/24/01	102	15	14.7%
	Cerritos MS[f]	6/2/99 - 4/24/01	102	3	2.9%
	I-605/SR 91[f]	6/2/99 - 4/24/01	102	4	3.9%
	I-605/SR 91[g]	6/2/99 - 4/24/01	102	0	0.0%
	Altadena MS[g]	6/1/99 - 4/24/01	103	10	9.7%
	Carlsbad MS[g]	5/5/99 - 4/30/01	98	0	0.0%

[a]Infiltration Trench; [b]Infiltration Basin; [c]Austin-Type Media Filter; [d]Delaware-Type Media Filter; [e]Stormfilter™; [f]Biofiltration Swales; [g]Biofiltration Strip

Dry Systems

1. Structures should be designed such that they do not hold standing water for more than 72 hrs to prevent mosquito development. Provisions to prevent or reduce the possibility of clogged discharge orifices (e.g., debris screens) should be incorporated into the design. The use of weep holes is not recommended due to rapid clogging.

2. The hydraulic grade line of each site should be a primary factor in determining the appropriate BMP that will allow water to flow by gravity through the structure. Pumps are not recommended because they are subject to failure and often require sumps that hold water. Structures that do not require pumping should be favored over those that have this requirement.

3. Designs should avoid the use of loose rip rap or concrete depressions that may hold standing water.

4. Distribution piping and containment basins should be designed with adequate slopes to drain fully and prevent standing water. The design slope should take into consideration buildup of sediment between maintenance periods.

5. The use of barriers or diversions that result in standing water should be avoided.

Systems with Sumps or Basins

1. Structures designed with sumps or basins that retain water permanently or longer than 72 hrs (e.g., CDS™, Stormfilter™, Delaware-type sand media filters) should be sealed completely to prevent entry of adult mosquitoes. Adult female mosquitoes may utilize openings as small as 1/16th of an inch to access water for egg laying. Screening can be used to exclude mosquitoes, but is subject to damage and is not a method of choice.

2. Structures should be designed with the appropriate pumping, piping, valves, or other necessary equipment to allow for easy dewatering of the unit if necessary.

3. If the sump or basin is completely sealed, with the exception of the inlet and outlet, the inlet and outlet should be fully submerged to reduce the available surface area of water for mosquitoes to lay eggs (female mosquitoes can fly through pipes).

Permanent Ponds

1. Permanent ponds should maintain water quality sufficient to support surface feeding fish such as mosquitofish, *Gambusia affinis*, which feed on mosquito larvae.

2. Permanent pond shorelines should be accessible to both maintenance and vector control crews for: 1) periodic maintenance and/or control of emergent and pond-edge vegetation, and 2) for routine monitoring of mosquito immatures and abatement procedures, if necessary. Emergent plant density should be controlled so that mosquito predators are not inhibited or excluded from pond edges (i.e., fish should be able to swim between plant bases).

3. If possible, permanent ponds should be maintained with depths in excess of 4 feet to preclude invasive emergent vegetation such as cattails. Emergent vegetation provides mosquito larvae with refuge from predators and increases nutrient availability. The pond edges below the water surface should be as steep as practicable and uniform to discourage dense plant growth and reduce favorable mosquito habitat.

4. Concrete or liners should be used in areas where vegetation is not necessary to prevent unwanted plant growth.

5. Permanent ponds should be designed to allow for easy dewatering of the basin when needed.

6. Floating vegetation (dead or alive) should be eliminated.

General Access Requirements

1. All BMP structures should be easily and safely accessible, without the need for special requirements (e.g., OSHA requirements for "confined space"). This will allow vector control personnel to effectively monitor and, if necessary, abate vectors.

2. If utilizing covers, the design should include spring-loaded or lightweight access hatches that can be opened easily. Covers should seal completely.

3. All-weather road access (with provisions for turning a full-size work vehicle) should be provided along at least one side of large aboveground BMPs that are less than seven meters wide. Those BMPs that have shoreline-to-shoreline distances in excess of seven meters should have a perimeter road for access to both sides. *Note:* Mosquito larvicides are applied with hand held equipment at small sites and with backpack or truck mounted high-pressure sprayers at large sites. The effective swath width of most backpack or truck mounted larvicide sprayers is approximately seven meters on a windless day.

4. Access roads should be built as close to the shoreline as possible. It is important not to have vegetation and/or other obstacles between the access road and the BMP that might obstruct tbe path of larvicides to the water.

5. Vegetation should be controlled (removal, thinning, or mowing) periodically to prevent access barriers.

Figure 7. Recommended BMP guidelines for mosquito suppression or prevention.

conducted in order to better satisfy water quality and volume reduction goals while preventing vector production. It is critical that the public health impact of BMP design and construction be considered. There is a pressing need for collaboration between stormwater engineers and vector control personnel.

Acknowledgements

This study was accomplished through collaboration with a multitude of people from various agencies including the Greater Los Angeles County Vector Control District, San Gabriel Valley Mosquito and Vector Control District, Los Angeles County West Vector Control District, and San Diego County Vector Surveillance and Control, Larry Walker Associates, California State University, Sacramento, University of California, Riverside, Robert Bein, William Frost and Associates, Kinnetic Laboratories, Inc., LAW Engineering and Environmental Services, Inc., Brown and Caldwell Environmental Engineering and Consulting, Montgomery Watson, and Caltrans staff: thank you for all your efforts. We would like to extend special thanks to J. Wakoli Wekesa, Jeanne-Marie Lane, Susanne Kluh, Minoo Madon, John Davidson, Keith MacBarron, Mike Devine, David Heft, Dean Messer, Cathy Beitia, and Brian Currier for assistance with data collection, reports, and critical project reviews.

REFERENCES

Amalfi, F.A., R. Kadlec, R.L. Knight, G. O'Meara, W.K. Reisen, W.E. Walton, and R. Wass. 1999. A mosquito control strategy for the Tres Rios Demonstration Constructed Wetlands. CH2M Hill, Tempe, AZ, 140 pp.

Anon. 1969. The Cities: the price of optimism. Time Magazine 94(5): 41.

Copeland, C. 2002. Water quality: implementing the Clean Water Act. Congressional Research Service Report IB89102. National Council for Science and the Environment, Washington, D.C., 19 pp.

Copeland, C. 1999. Clean Water Act: a summary of the law. Congressional Research Service Report RL30030. National Council for Science and the Environment, Washington, D.C., 11 pp.

Debo, T.N., and A.J. Reese. 1995. Municipal Storm Water Management. Lewis Publishers, New York, N.Y., 756 pp.

Dorothy, J.M., and K. Staker. 1990. A preliminary survey for mosquito breeding in stormwater retention ponds in three Maryland counties. Mosquito Control, Maryland Department of Agriculture, College Park, MD. 5 pp.

Florida Coordinating Council on Mosquito Control. 1998. Florida Mosquito Control: The state of the mission as defined by mosquito controllers, regulators, and environmental managers. University of Florida. 207 pp.

Kluh, S., M.E. Metzger, D.F. Messer, J.E. Hazelrigg, and M.B. Madon. 2002. Stormwater, BMPs, and vectors: the inevitable impact of new BMP construction on local public health agencies. Stormwater 3(2): 40-46.

McLean, J. 2000. Mosquitoes in constructed wetlands: a management bugaboo? *In* T.R. Schueler and H.K. Holland [eds.], The Practice of Watershed Protection. pp. 29-33. Center for Watershed Protection, Ellicott City, MD.

Metzger, M.E., D.F. Messer, C.L. Beitia, C.M. Myers, and V.L. Kramer. 2002. The dark side of stormwater runoff management: disease vectors associated with structural BMPs. Stormwater 3(2): 24-39.

Metzger, M. E., C. M. Myers, and V. L. Kramer. 2001. Caltrans project: vector production, pp. 47-50. *In* Vector-Borne Diseases in California, 2000 Annual Report. Vector-Borne Disease Section, California Department of Health Services, October 2001.

Santana, F.J., J.R. Wood, R.E. Parsons, and S.K. Chamberlain. 1994. Control of mosquito breeding in permitted stormwater systems. Sarasota County Mosquito Control and Southwest Florida Water Management District, Brooksville, FL., 46 pp.

Water Environment Federation and American Society of Civil Engineers. 1998. Urban Runoff Quality Management. Alexandria, VA., 259 pp.

Climate Variability and Encephalitis Epidemiology

W.K. Reisen[1]

Arbovirus Research Unit, Center for Vector-borne Disease Research, School of Veterinary Medicine, University of California, Davis 95616

INTRODUCTION

The purpose of the California Encephalitis Virus Surveillance program is to measure the level of western equine encephalomyelitis [WEE] and St. Louis encephalitis [SLE] virus activity and from this information forecast the risk of human and equine disease. The current program has monitored 5 factors [Table 1] for over 30 years; however, these measurements have not been carefully archived at a central location, are not in electronic format, and therefore are utilized inadequately by mosquito and vector control agencies and health planners in decision making. The discovery of the global impact of changes in sea surface temperatures in the Pacific Ocean on climate variability and local weather has ushered in a new era of research and has led to the development of predictive circulation models that provide long range forecasts of temperature and rainfall. Forging solid links among climate variability, vector abundance and mosquito-borne encephalitis virus activity may enhance the use of climate predictions in surveillance and provide long lead forecasts of risk, thereby improving and focusing intervention.

Our symposium today is entitled "Climate Variability and Encephalitis Epidemiology, A symposium on the use of climate and weather data to forecast risk." This symposium has grown out of collaboration on a grant entitled "Use of climate models to forecast the risk of mosquito-borne encephalitis activity in California" funded by the Office of Global Programs, National Oceanographic and Atmospheric Administration. This project is a collaborative effort among the University of California at Davis and San Diego, the US Geological Survey, the Department of Health Services, and the Mosquito and Vector Control Association of California and has 4 principal objectives:

1. Develop normalized indices of mosquito abundance and virus activity
2. Quantify long- and short-term relationships with climate
3. Assess utility of long-term forecasts
4. Develop interactive data systems for input, visualization and analysis

The long-term goal is to enhance surveillance data collection, storage and analysis and to relate these data to climate variability to provide long-range forecasts of risk.

Table 1. Surveillance factors and their measurement in California [modified from (Eldridge 1987)].

Factor	Measurement
Climate variability	Snow pack, run-off, weather stations
Vector abundance	Mosquito trap counts
Enzootic activity	
Vector infection	Mosquito pools
Host infection	Sentinel chicken seroconversions
Clinical disease	
Horse cases	Veterinarian reported cases
Human cases	Health provider reported cases

[1] Correspondence: Arbovirus Field Station, 4705 Allen Rd., Bakersfield, CA 93312

Our symposium will provide an introduction to this project and will have 5 speakers who will detail different aspects of our preliminary findings:

1. Introduction. W.K. Reisen
2. Data base structure, access and use. B.F. Eldridge
3. Relationships between climate variability and mosquito abundance. D. Cayan
4. Degree-day models: relationships to mosquito seasonality and virus occurrence. F. Mahmood
5. Epidemiological applications. C.M. Barker

By way of background, I would like to introduce some principles and underlying assumptions concerning relationships among these abiotic and biotic factors. Our project focuses on California, its primary vector mosquito *Culex tarsalis*, and the endemic encephalitides, WEE and SLE. Although very different antigenically, both encephalitis viruses utilize the same basic transmission cycle during summer involving *Cx. tarsalis* and perching birds, especially house finches (Milby and Reeves 1990; Reisen et al. 2000). Other birds such as doves and quail also become involved and a secondary cycle among *Ochlerotatus* mosquitoes and rabbits has been described for WEE in the Central Valley (Hardy 1987). Transmission to equines and humans is tangential to these enzootic cycles, and equines typically develop clinical symptoms only after infection with WEE. There are several important similarities among these transmission cycles relating to today's symposium:

1. Overwintering mechanisms are unknown. Detection of virus during the amplification cycle is necessary to provide an assessment of risk, because there is no other known method at present to determine if virus is present or to what level it will amplify in the future.
2. Virus exists mostly at ambient temperature. Most of the virus life history is spent within the adult mosquito host, because the viremia period in birds is of short duration, usually lasting 2-3 days. Mosquitoes, on the other hand, become infected for life, but require one or more gonotrophic cycles to complete viral dissemination to the salivary glands to afford transmission. The duration of this virus extrinsic incubation period and the rapidity of virus amplification therefore are dependent upon mosquito body temperature that parallels ambient conditions.
3. Diseases caused by WEE and SLE are zoonoses. Infection of humans and equines occurs tangential to the basic enzootic transmission cycle among birds and *Culex* mosquitoes, and therefore cases provide a poor indication of virus activity. Relationships to climate variability are best established through data on mosquito abundance and infection rates and transmission rates to sentinel chickens, a surrogate for transmission to wild birds.

Climate is the average long-term temperature and rainfall conditions over large geographical areas, whereas weather is the short-term conditions experienced at various spatial scales. Climate variability also results in changes in weather over varying time scales. Warming temperature tends to accelerate biological processes, especially in poikilotherms such as mosquitoes. Warming temperatures tend to affect mosquito populations by speeding immature development, shortening generation times and increasing population growth rates. Conversely, warming temperatures decrease mosquito daily survival and the duration of infective life. However, warming temperatures also shorten the duration of the extrinsic incubation period of the virus in the mosquito host and therefore increase the rate of virus transmission. Rainfall and snow at high elevations increase water availability and, in turn, increase both vector and vertebrate host population size in rural areas. Increased surface pooling increases the amount of mosquito habitat available for oviposition and immature development and therefore leads to overall increases in population size. Increased water availability also increases plant and insect production, food for various avian host populations, and thereby enhances nesting success. Mosquitoes in urban environments frequently breed in drainage systems and increased rainfall frequently increases discharge actually reducing population size. The symposium today will address data management, relationships among climate factors and mosquito abundance, use of temperature to forecast vector abundance patterns and vector infection rates, and new approaches to assimilate surveillance data into a general risk assessment model.

REFERENCES

Eldridge, B. F. 1987. Strategies for surveillance, prevention and control of arbovirus diseases in western North America. Am. J. Trop. Med. Hyg. 37: 77s-86s.

Hardy, J. L. 1987. The ecology of western equine encephalomyelitis virus in the Central Valley of California, 1945-1985. Am. J. Trop. Med. Hyg. 37: 18s-32s.

Milby, M. M. and W.C.Reeves. 1990. Natural infection in vertebrate hosts other than man. Pp. 26-65.*In:* Reeves,W.C., editor. Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987. Sacramento, Calif.: Calif. Mosq. Vector Control Assoc.

Reisen, W. K., J.O.Lundstrom, T.W.Scott, B.F.Eldridge, R.E.Chiles, R.Cusack, V.M.Martinez, H.D.Lothrop, D.Gutierrez, S.Wright, K.Boyce and B.R.Hill. 2000. Patterns of avian seroprevalence to western equine encephalomyelitis and St. Louis encephalitis viruses in California, USA. J. Med. Entomol. 37: 507-527.

Climate Variability and Encephalitis Epidemiology Database Structure, Access, and Use

B.F. Eldridge

Department of Entomology, University of California, Davis, CA 95616

Databases may be considered electronic stores of data. The data they contain are contained in tables that are arranged in columns and rows. Modern commercial database programs include a rich assortment of tools to search and edit data, and the capability to produced forms, graphs, tables, and reports. In many regards, database tables and spreadsheets are similar, and they share many characteristics in underlying structure. However, spreadsheets are better suited for small to medium amounts of data, and for various statistical analyses. On the other hand, databases are better suited for storing large amounts of data, and for complex data storage systems.

Common commercial databases include Oracle, Adapter Server Anywhere (Sybase), Microsoft Access, FilePro, Paradox, Microsoft SQL Server, and Dbase IV. There are also several open source databases (i.e., available at low or no cost) such as Interbase.

Some problems with modern databases

Nearly all commercial database products are powerful and well-tested products. However, problems can arise when it is necessary to move data among different types of databases. This is because database software programs use proprietary schemes to store data in tables. In computer jargon, this is known as "hosting" a database. Although all databases now provide for import and export of data to a different brand of database, various problems of interoperability of systems may still result. These problems have been minimized in recent years with the development of a standard scheme for connecting to databases known as Open Database Connectivity (ODBC). This scheme permits access to other databases from a variety of other programs, such as Microsoft Visual Basic though ODBC drivers. These drivers are available for all common database programs and are usually downloaded automatically when the database program is installed.

Relational databases

One major advantage databases have over spreadsheets for large datasets is that most databases are relational. This means that the data can be stored in more than one table, as long as the tables are related by some common field, called a key. There are many advantages to using related tables, including efficiency, better data accuracy, and ease of modification of key data elements. A database that is not relational is called a flat file database. To use a simple example of mosquito collections in cities in a county, for a flat file database, each species, county, and city name would have to be repeated in each row (record) of the database. In a relational database, one would use a species table and a city table. Then each record need include only the serial number for the species and for the city, and any changes needed for a species name need be entered only once. This concept is shown in Tables 1 and 2.

Table 1. The design of a relational database. Table are lined together by keys.

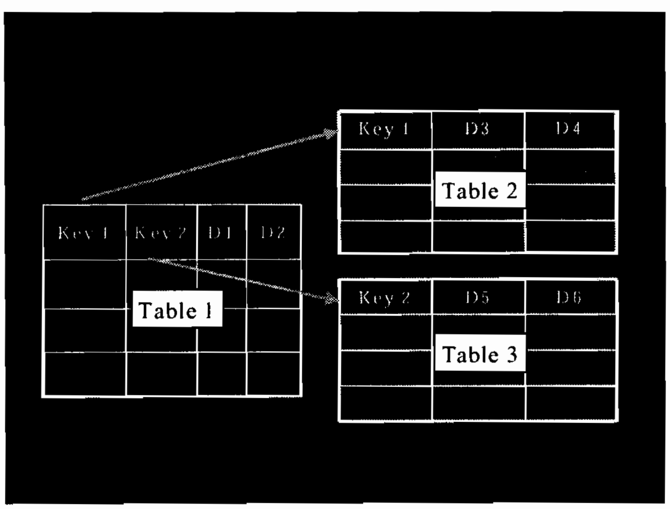


Table 2. A simple example of a relational database.

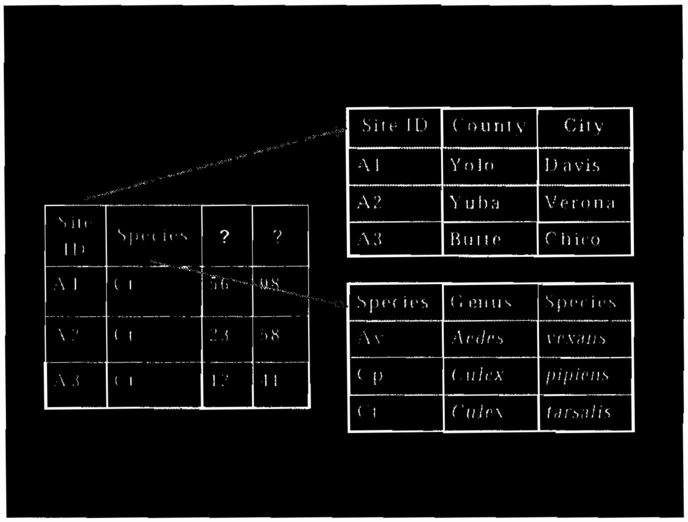
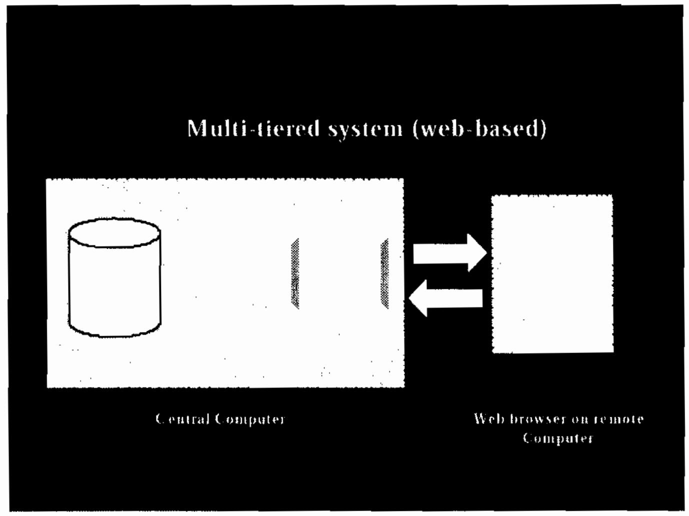


Table 3. The scheme for a multi-tiered database system.



Types of Database Systems

Another way to classify databases is according to how they are used. The traditional approach to database use was to operate data searching, analysis, and storage on a single desktop computer. This represents a single-tiered system. With the advent of networks of various kinds, it became common for data to be stored centrally on a server, and for users to access the data for analysis and reporting from terminals or desktop computers linked by the network. This is a two-tiered system. Now, the growth of the Internet as a means of universal communication has led to three-tiered, or multi-tiered database systems (Table 3). In its simplest form, data are stored centrally on a computer called a server, and hosted by a database server such as Microsoft SQL Server or Sybase Adapter Server Anywhere. It is a confusing fact of life that computer servers (items of hardware) and various kinds of software servers (web servers, database servers, map servers, etc.) are both called servers. Connected directly to the database server is a program such as Zope. Zope is a web server, and represents the second tier. This tier is sometimes called "middleware". The third tier is a program that runs on a user's computer. It can be a browser such as Netscape, or a dedicated program such as VCMS. This type of system has many advantages. The software used by users can be very small and efficient, because the storage and sorting of data is done centrally.

The California Vectorborne Disease Surveillance website

The current website for vectorborne disease surveillance data is operated jointly by the California Department of Health Services (DHS), the Mosquito and Vector Control Association of California (MVCAC), and the University of California (UC). Its URL is: http://vector.ucdavis.edu.

This site was developed in part using funds provided by 10 mosquito and vector control districts to UC as part of its Model Surveillance research program. The site can be reached directly, or through a link from the MVCAC website (http://mvcac.org) or Center for Vectorborne Disease website (http://www.vetmed.ucdavis.edu/cvec/default.html).

For the year 2001, the website is linked to a database hosted in Microsoft Access, and maps showing the results of testing of blood samples from sentinel chicken flocks and virus tests of mosquito pools are produced. Summaries are provided for all vectorborne diseases in California in the form of PDF files.

The Future of Databases in Surveillance Systems

Beginning in 2002, there will be significant changes to the underlying structure of the surveillance website. The architecture of the database system will be changed to adopt a three-tiered system. The mapping software will be changed in anticipation of installation of a mapping server that will permit interactive mapping rather than weekly posting of static maps. Eventually, we hope to provide a system that will permit not only interactive mapping, but also graphing and other analyses based on directly-accessible databases containing both historical and contemporary surveillance data. Interactive risk models based on weather data, vector abundance, sentinel animal serology data, virus isolations from vectors, and human and livestock disease cases will be possible.

An important consideration in the development of complex surveillance datasets is the allowance for efficient and accurate data transfer among local, national, and international health agencies. The US Centers for Disease Control and Prevention is working with collaborating states to develop a standard method for nationwide and worldwide surveillance data exchange. This will undoubtedly involve the adoption of XML (=Extensible Markup Language) as the worldwide "common currency" of data exchange. Adoption of XML should solve most of the problems arising from lack of interoperability among different databases discussed earlier.

Climate Linkages to Female *Culex Cx. tarsalis* Abundance in California

Dan Cayan[1, 2] Mary Tyree[1] and Mike Dettinger[2, 1]

1. Scripps Institution of Oceanography – UCSD, 9500 Gilman Drive – 0224, La Jolla, CA 92093 – 0224
2. U.S. Geological Survey

ABSTRACT: Monthly counts of female *Cx. tarsalis* from scores of Sacramento/Yolo, Sutter/Yuba, and Kern MVCD samples in the Central Valley of California are investigated to determine how they relate to climate variability over the last 2-4 decades. There has been considerable interannual variability in *Cx. tarsalis* abundance, part of which is correlated across the entire region. Summer *Cx. tarsalis* fluctuations have correlated positively with moisture accumulation in the region, as indicated by precipitation, streamflow and snow water content in the Sacramento-San Joaquin watersheds. Prediction of summer *Cx. tarsalis* abundance from prior winter moisture variables may provide a useful look ahead at several months lead-time.

INTRODUCTION

As part of a collaborative NOAA Office of Global Programs grant, we are examining temporal changes in monthly mosquito abundance and their links to short term climate variations. Seasonal climate variations are substantial in California (Cayan and Riddle, 1993; Stahle *et al*, 2001; Knowles, 2002) and they have large regional footprints, especially in winter (Cayan and Peterson, 1989; Cayan 1996; Dettinger *et al*, 1998). Seasonal climate forecasts are only modestly successful but, certain conditions, such as large El Nino events, may provide useful skill for predicting temperature and precipitation over California (Namias, 1978; Cayan *et al*, 1999; Dettinger *et al*, 1999; Gershunov *et al*, 2000). The objectives of this study, as stated by W. Reisen in his introduction, are to quantify relationships of vector counts with climate and, to develop and assess long-range (seasonal) forecasts. Our rationale is based on the premise that if climate contributes significantly to variations in mosquito abundance, then measurements of mosquitos across the California region should vary in a similar fashion and be linked to changes in temperature and water availability.

DATA

Time series of monthly mosquito abundance (*Cx. tarsalis* females counts [/trap-day] were supplied by C. Barker of the Arbovirus Field Station in Bakersfield, California from New Jersey light trap records maintained by the Kern Mosquito and Vector Control District [MVCD} in the southern San Joaquin Valley, and by Sacramento/Yolo and Sutter/Yuba MVCDs in the Sacramento Valley of California. We have focused on female *Cx. tarsalis* because of its central involvement in the transmission of encephalitis viruses. The three data sets considered here cover the periods 1973-2000, 1986-2000, and 1954-2000, respectively. Because many of the mosquito traps were unevenly sampled thru time or provided fewer than 10 years of data, for several of the analyses to follow, a subset of 28 mosquito trap series were selected from the original 54, 62 and 49 mosquito trap histories from Kern, Sacramento/Yolo and Sutter/Yuba Mosquito Districts collections, respectively. For some of the analyses, the monthly mosquito abundance data were log transformed to provide more normally distributed time series.

In our search for climatic links, monthly precipitation from the NOAA San Joaquin and Sacramento climate divisions, monthly flows from the U. S. Geological Survey Kern River gage, and April 1 snow water content from selected California Department of Water Resources Cooperative Snow Surveys snow courses in the southern and central Sierra Nevada were employed. All of these climate data were available from 1954-2000.

ABUNDANCE AND SEASONALITY

The overall monthly averages of *Cx. tarsalis* females at representative mosquito traps from the three Districts varied considerably across the region, with nearly an order of magnitude difference between individual traps (Figure 1, *right*). Cx. tarsalis abundance peaked in the summer between June and September in almost all traps (Figure 1, *left* and figure 2, *upper*). Interestingly, the mosquito series from the southernmost traps, those in Kern County and the southern portion of Sacramento/Yolo Counties, had peak abundance in later summer (August and September), while the northernmost traps, those from the Sutter/Yuba District and the western and northern locations from the Sacramento/Yolo District, had peak abundance in June and July.

MONTHLY AND INTERANNUAL VARIABILITY

Inspection of the Cx. *tarsalis* abundances reveals relatively large monthly and interannual variability and, in many cases, remarkable trends over the duration of the sampling period. Many of these trends are towards decreasing abundances with time; perhaps because environmental conditions and light levels at the trap sites have changed. Therefore, the linear trend was removed to yield (detrended) residual anomalies for the subsequent correlation analyses.

Considering the variability of individual months, the relative variability (coefficients of variate of the log transformed monthly counts) tend to be greatest in the transition months before and

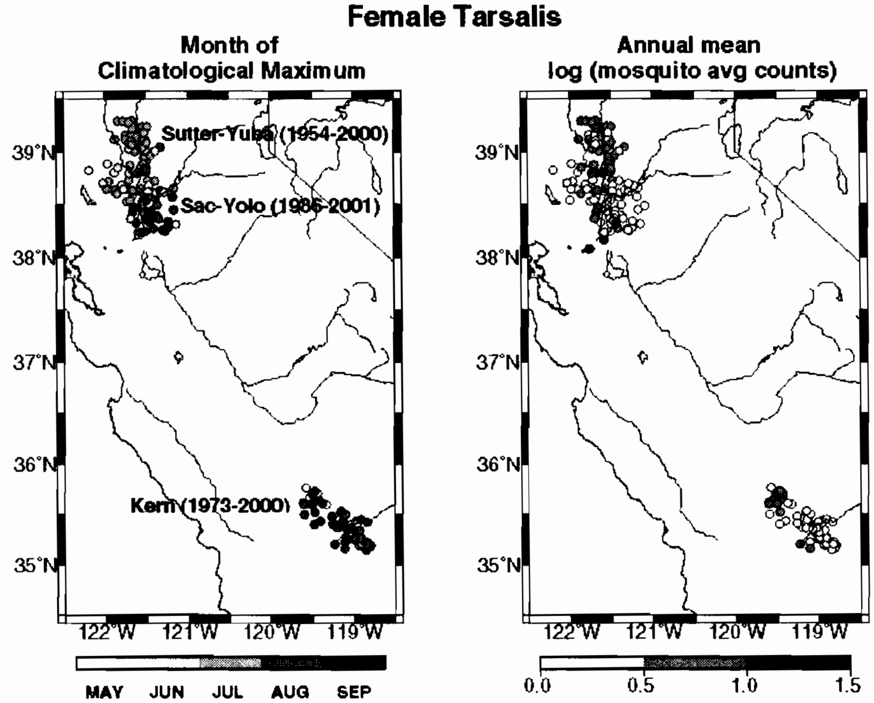


Figure 1. Figure 1: Month of climatological maximum (*left*) and annual mean of $\log_{10}$ (monthly counts) of *Cx. tarsalis* females captured in New Jersey light traps of the Sutter/Yuba, Sacramento/Yolo and Kern MVCDs. Years of record as shown on map.

after the summer peak period (Figure 2, *lower*). This coefficient of variation measure ranged from 0.2 to nearly 1.0.

SPATIAL COHERENCE

By correlating the anomalous, detrended monthly mosquito counts at one selected reference series from each of the three MVCDs, with those from each of the other mosquito trap series, an idea of the typical spatial span of higher and lower than normal mosquito abundances can be determined. Monthly anomalies were calculated by subtracting the monthly long-term average (detrended) value from the value (detrended) for each particular month. This exercise, performed for the early summer (May-July) and late summer (August-October) months, shows modest coherence among nearby and relatively distant mosquito traps, especially in late summer.

SHORT PERIOD CLIMATE LINKAGES

Because part of the mosquito life cycle is keyed to water availability, tests for linkages between anomalous monthly moisture-related climate variations and mosquito abundances were conducted. For the Kern MVCD series, we used winter precipitation, spring snow accumulation and water year streamflow from representative gages. The correlations among these series and monthly-detrended mosquito abundances were computed for a subset of the mosquito series containing 10 or more years of data. Using April 1 snow water content as an index, these correlations were computed with the April-October monthly mosquito abundance data. Correlations that were significant, different from zero, were nearly all positive. Strongest correlations from the Kern dataset occurred in late spring and early summer (April – June); they are relatively weak in July – October (Figure 3).

In contrast, correlations of winter (December-January) precipitation with Sacramento/Yolo and Sutter/Yuba detrended mosquito abundance are weak (or even negative) in April and May, but increase in summer, with maximum positive correlations in August and September (Figure 4). From temperature correlations, we see that warmer temperatures in winter are associated weakly with higher AMJ female Cx. tarsalis abundances at Kern MVCD, but this connection is not as strong as were the connections for winter and spring precipitation.

TOWARDS SEASONAL FORECASTING

Importantly, it appears that for several of the female *Cx. tarsalis* series, there is a positive correlation with winter precipitation (or spring snow accumulation) and summer abundances, meaning that prior season moisture indices may be useful predictors of summer mosquito abundance. As a preliminary test of this, linear regression was developed for predicting the average of Kern MVCD abundance series from April 1 snow water. This model, when fitted to the early (1973-1990) record, captured 40% of the year-to-year variance of April-June mosquito abundance; when applied to the remaining 10 years of independent data (1991-

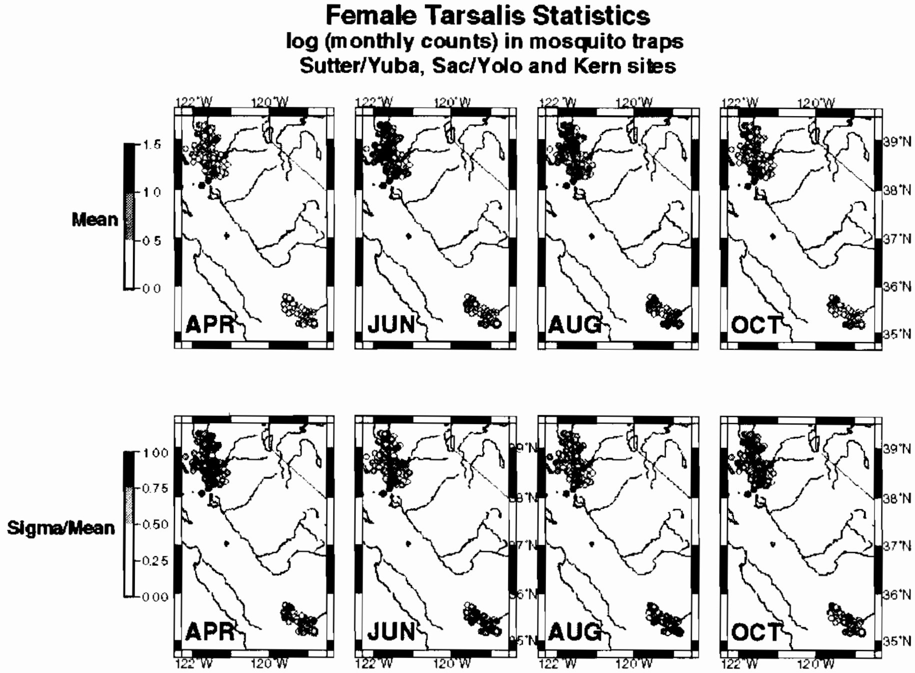


Figure 2. Figure 2: Monthly mean (*upper*) and coefficient of variation (standard deviation/mean) of log transformed counts of *Cx. tarsalis* females at selected light traps.

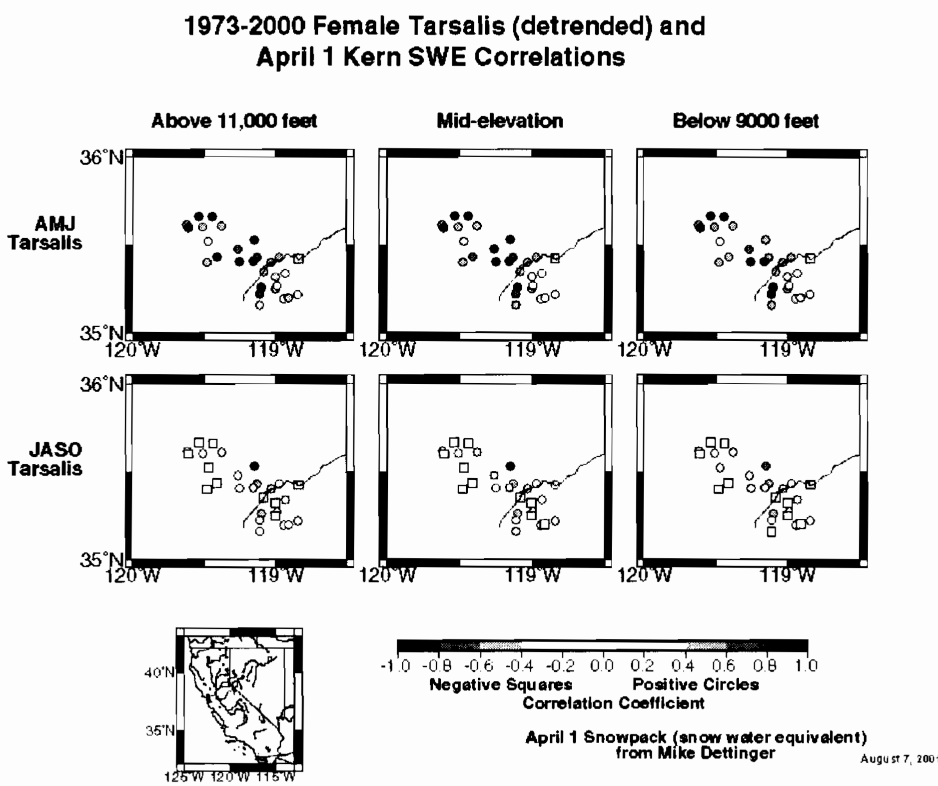


Figure 3. Figure 3. Correlations between April 1 snow water contents at various elevation zones in the Kern River watershed and Kern MVCD. April – June female *Cx. tarsalis* (*upper*) and July – September female *Cx. tarsalis* (lower).

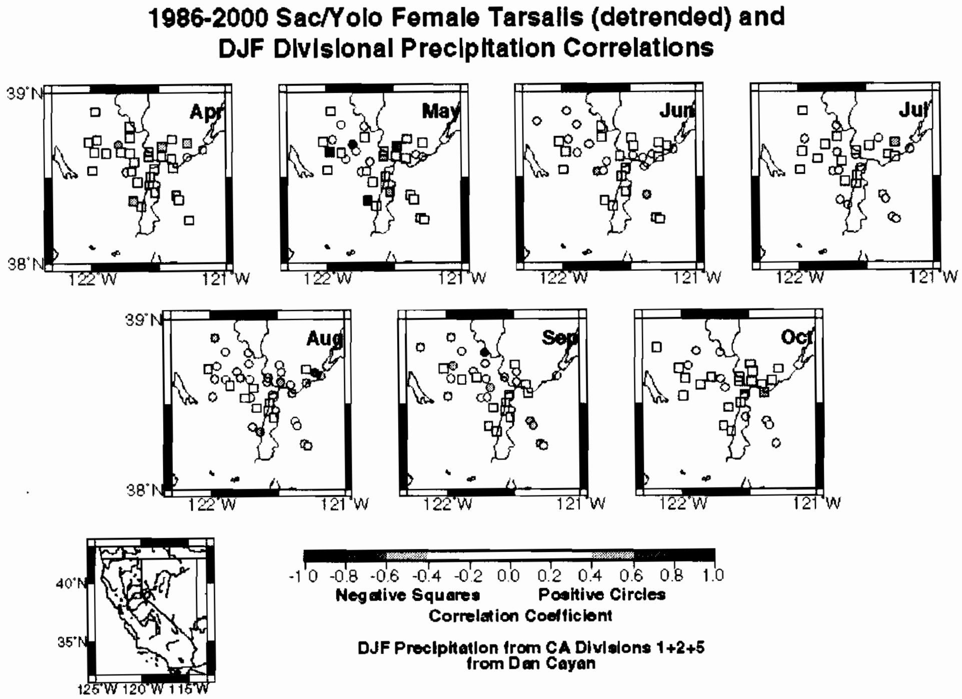


Figure 4. Figure 4: Correlations between December – February Sacramento climate division precipitation and selected Sacramento/Yolo MVCD monthly female *Cx. tarsalis* counts.

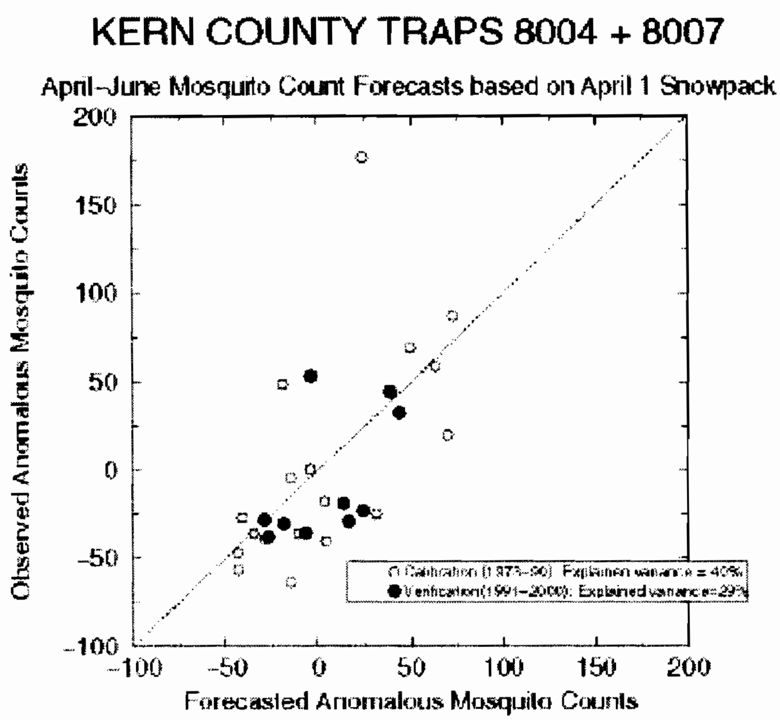


Figure 5. Comparison of observed and "forecasted" April-June female *Cx. tarsalis* counts, based on 1973-1990 linear regression with April 1 snow water content in Kern River watershed; *Cx. tarsalis* counts are averages from Kern MVCD traps 8004 and 8007.

2000), predicted 29% of the variance of April-June mosquito abundances (Figure 5).

Further work will relate other regional moisture variables (streamflow, soil moisture) and broader scale climate measures (atmospheric circulation, tropical Pacific El Nino/Southern Oscillation conditions, and Pacific sea surface temperature) to mosquito abundances. Mosquito trap series are being compiled from additional MVCDs and will be assembled in electronic format, so these will also be examined. The potential for useful, seasonal predictions also needs to be more fully explored and developed.

REFERENCES

Cayan, D.R., K.T. Redmond, and L.G. Riddle, 1999: ENSO and hydrologic extremes in the Western United States. *J. Clim.*, 12(9), 2881-2893.

Cayan, D.R., 1996: Interannual climate variability and snowpack in the western United States, *J. Clim.*, 9(5), 928-948.

Cayan, D.R. and L. Riddle, 1993: Atmospheric circulation and precipitation in the Sierra Nevada. *Proceedings of the AWRA 28th Annual Conference and Symposium*, Reno, Nevada, November 1-5, 1992, *Managing Water Resources During Global Change*, R. Herrmann, Editor, 711-720.

Cayan, D.R. and D.H. Peterson, 1989: The influence of North Pacific atmospheric circulation on streamflow in the West. *Geophys. Monograph 55: Aspects of Climate Variability in the Pacific and the Western Americas*, December 1989, 375-397.

Dettinger, M.D., D.R. Cayan, and K.T. Redmond, 1999: United States streamflow probabilities based on forecasted La Nina, winter –spring 2000. *Experimental Long-Lead Forecast Bulletin*, 8(4), 57-61.

Dettinger, M.D., D.R. Cayan, H.F. Diaz, and D.M. Meko, 1998: North-south precipitation patterns in western North America on interannual-to-decadal time scales. *J. Clim.*, 11(12), 3095-3111.

Gershunov, A., T.P. Barnett, D.R. Cayan, T. Tubbs, and L. Goddard, 2000. Predicting and Downscaling ENSO Impacts on Intraseasonal Precipitation Statistics in California: The 1997/98 Event. *J. Hydromet*, 1, 201-210.

Knowles, N. (2002): Natural and human effects in the San Francisco Bay-Delta Estuary and watershed. *Water Resources Research, (in press).*

Namias, J., 1978: Multiple causes of the North American abnormal winter 1976-77. *Mon. Wea. Rev.*, 106, 279-295.

Stahle, D.W., M.D. Therrell, M.K. Cleaveland, D.R. Cayan, M.D. Dettinger, and N. Knowles, 2001. Ancient Blue Oaks reveal human impact on San Francisco Bay salinity. *EOS, Transactions, American Geophysical Union,* 82(12), 141, 144-145.

Degree-day Models: Relationship to Mosquito Seasonality and Virus Occurrence

Farida Mahmood

Arbovirus Field Station, Center for Vector-borne disease Research, School of Veterinary Medicine, University of California, Davis, 4705 Allen Road, Bakersfield CA 93312

ABSTRACT: A degree-day model was constructed based on the developmental rates of overwintering *Culiseta melanura* immatures and validated using historical mosquito surveillance data from Dennisville, Cape May County, New Jersey. The model effectively predicted adult emergence peaks from the age structure of the overwintering immature population of *Culiseta melanura*. The timing and magnitude of adult *Cs. melanura* phenology was related to the vernal amplification and transmission of eastern equine encephalomyelitis virus to the avian fauna.

INTRODUCTION

Mosquitoes are poikilotherms and have eight distinct developmental stages in their life cycle (Figure 1). Each of these stages requires certain amount of heat to complete development and progress to the next stage. The total amount of heat required for the completion of a developmental stage is referred to as physiological time and is expressed in units called degree-days. Degree-day models are built by conducting experiments in which mosquitoes are reared in environmental chambers at a range of constant temperatures to determine their minimum and maximum threshold temperatures. Minimum threshold or thermal minimum is the temperature below which development is halted, whereas, at the thermal maximum or the maximum threshold temperature, rate of development slows down and/or stops. The minimum and maximum threshold temperatures as well as the number of total degree-days required for complete development are species specific and constant.

In the present study, a relationship between a degree-day model for the development of *Culiseta melanura* and its relation to the maintenance of eastern equine encephalomyelitis virus (EEE) in the avian fauna is discussed. Eastern equine encephalomyelitis is an *Alpha* virus related closely antigenically to western equine encephalomyelitis virus (WEE) and is maintained as an enzootic infection within avian populations (Figure 2).

In the EEE cycle, in New Jersey, *Cs. melanura* is the primary enzootic vector of EEE to birds and preferentially feeds on them. *Coquillettidia perturbans* and *Aedes sollicitans* are the presumptive vector that transmits EEE to horses in the inland areas and to humans in the coastal areas respectively (Crans 1977, Crans and Schulze 1986, Crans et al. 1986). Humans and horses never develop high enough viremias and are the dead-end hosts.

The chronological time sequence of various components of EEE cycle is presented in Figure 2. In early spring, after hatching year (AHY) summer resident birds return to the study site (Cape May County, NJ) and start nesting from April to June. Fledglings are present from mid-June and Juveniles are on the wing from first week of July. The juveniles increase in number from the first week of July and reach a maximum in mist net collections during August. The summer residents (SR) start migrating south in September and at the same time winter residents (WR) start returning to their overwintering grounds. Most of the winter residents are hatching year birds (HY) that are considered AHY from January onwards in the second year and they start migrating out in March. Permanent residents (PR) birds are present year around.

A large percentage of SR and PR birds have EEE antibodies in spring compared to WR birds (Crans et al. 1994). In April, a large number of PR and SR, AHY birds have EEE antibodies that start to decline from mid-June to mid-July. Percentage of birds with EEE antibodies begins to rise in mid-July, reaches a maximum in August and decreases in fall due to the arrival of WR birds (Crans et al. 1994). The overwintering mechanism[s] of EEE remains a mystery. Crans et al. (1994) hypothesized that birds are the overwintering reservoir of EEE; whereas Morris (1988) hypothesized that an avirulent form of EEE is transmitted to the birds by *Cs. melanura* in early spring, that later changes to a virulent form in birds due to hormonal changes in the nesting season. Such a mechanism[s] would induce antibodies in HY birds early in the season.

Culiseta melanura overwinters as 1st - 4th instar larvae during winter (Joseph and Bickley 1969) and its populations in the eastern USA are subjected to winter minimal temperatures ranging from -5°C to above 10°C in January, indicating that this species would show different rates of larval development and times of adult emergence peaks depending on latitude (Figure 3). Mahmood and Crans (1998) showed that *Cs. melanura* has a bivoltine life cycle in the northeast but has more than 2 generations a year in the south where it experiences much higher temperatures during the year.

Culiseta melanura breeds in fresh water, subterranean swamp habitats called "crypts", having an acidic (pH 5.0-6.3) coffee-colored water (Burbutis and Lake 1956, Siverly and Schoof 1962, Joseph and Bickley 1969, Morris et al. 1976, Morris 1988). Larvae overwinter as 1st to 4th instars and adult emergence occurs early in May (Burbutis and Lake 1956, Gusciora 1961, Joseph and Bickley 1969, Orrell 1997). After emergence, adults stay within the swamp habitat for the first 4 days of life where swarming and mating occurs: thereafter adults move to the swamp perimeter

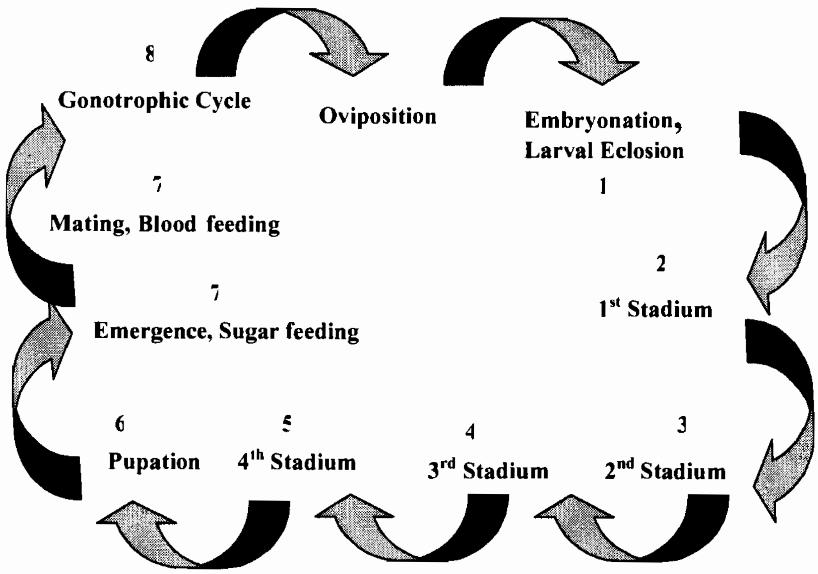


Figure 1. Different developmental stages in the life cycle of *Cs. melanura*.

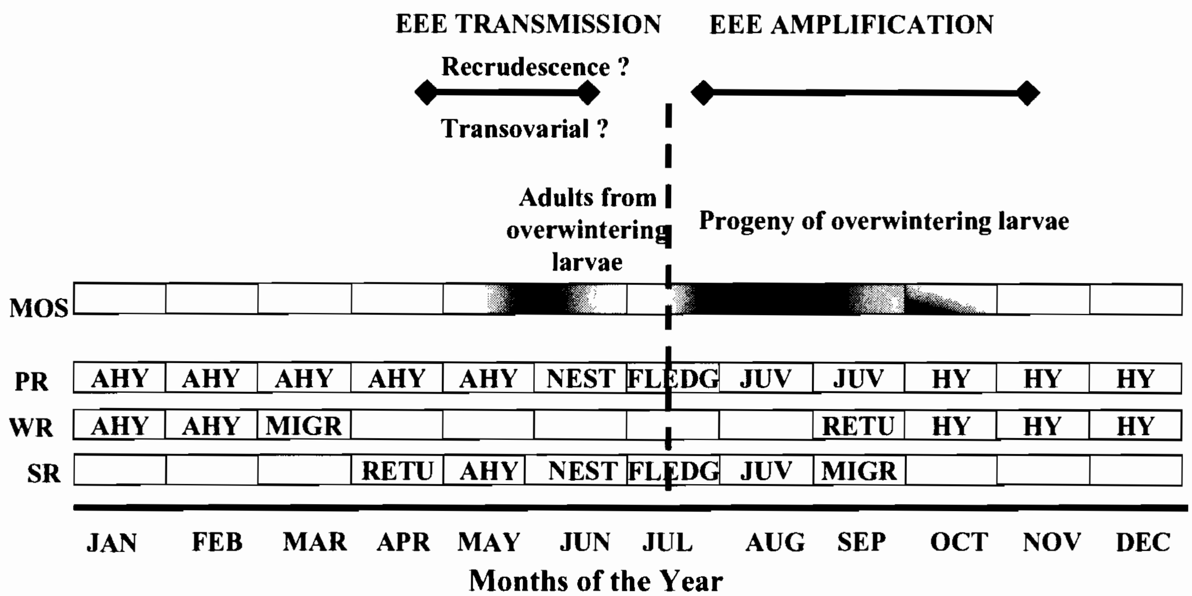


Figure 2. A chronological time sequence of the various components of EEE virus cycle in relation to the avian fauna of southern New Jersey. AHY = After hatching year birds, FLEDG = Fledgling, HY = Hatching year birds, JUV = Juvenile birds, MIRG = Migration, MOS = Mosquitoes, NEST = Nesting, PR = Permanent residents, RETU = Return of birds, SR = Summer residents, WR = Winter residents.

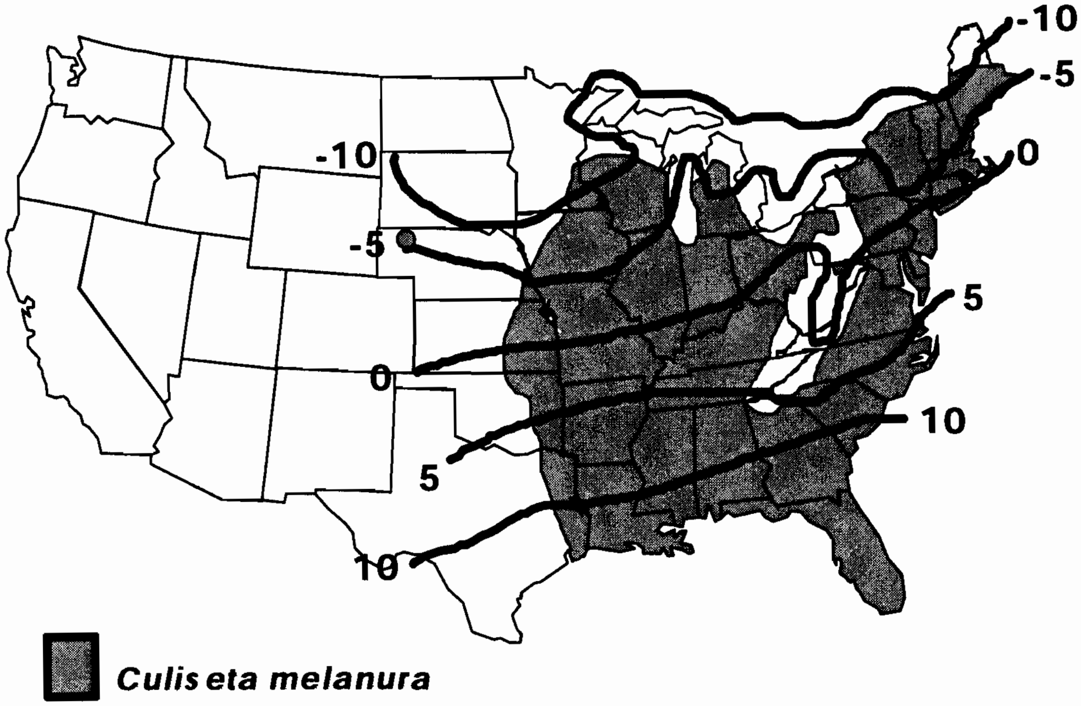


Figure 3. Distribution of *Culiseta melanura* in the USA and the mean minimum daily isotherm during January.

(Hayes 1958, Morris 1984). Females take a blood meal 6 or more days after emergence and blood-fed females also sugar feed (Morris 1984). Host-seeking females have a longer flight range compared to resting females (4 km) (Howard et al. 1989). Females blood feed equally on birds resting at various heights of the trees (Nasci and Edman 1981). Host-seeking females of *Cs. melanura* transfer EEE from the swamp to upland habitats (Howard et al. 1989). There is no difference in the parity of females collected at the swamp edge or away from it, but more fed females are found near the swamp edge compared to a larger proportion of unfed nulliparous females that rest away from the swamp perimeter (Nasci and Edman 1981). In New Jersey, blood-fed females can be collected from the resting boxes as late as the 3rd week of November (Gusciora et al. 1972).

The main objective of the current study was to develop a model using maximum and minimum daily ambient temperatures to predict the emergence peaks of *Cs. melanura* in nature and to use the historical vector surveillance data to validate these patterns. Also explored are the applications of the model in establishing phenological relationships among emergence peaks of *Cs. melanura*, migration and nesting of resident and non-resident avian species, and amplification of EEE.

MATERIALS AND METHODS

In the present study, previously published degree-day models for 8 stages of the life cycle of *Cs. melanura* were used to predict the rate of development and the calendar date of *Cs. melanura* emergence in nature (Figure 1, Table 1).

Historical records of the abundance of *Cs. melanura* collected from the resting boxes at Dennisville, Cape May County from 1981-1984 (Crans 1995, Crans and McCustion 1993) were used to validate the predictive model. Air and water temperatures were noted twice a month from more than 20 subterranean crypts from January 1996 – June 1997 (Orrell 1998). For 1982 – 1983, EEE sero-prevalence and infection rates in birds were related to mosquito emergence peaks and abundance. These birds were captured at Cape May County, New Jersey from 11 May –20 October 1982 and 11 May to 23 August 1983 (Crans et al. 1994). Daily maximum and minimum temperatures for 1982-1984 were obtained from NOAA weather data records for Bellplain State Forest. Missing data were substituted with records from the nearest station or estimated via interpolation.

STATISTICAL ANALYSIS

Culiseta melanura spend most of their life as immature stages, and water temperatures inside the crypts directly affect the rate of immature development. Because data for the water temperature inside the subterranean crypts were not available for all the years, a regression model was used to predict crypt water temperatures from the daily mean air temperatures recorded concurrently during 1996-1997. Time required from embryonation of eggs to adult emergence was estimated using these predicted water temperatures. Time required for adult maturation and duration of the gonotrophic cycle was calculated using the maximum and minimum daily

Table 1. The total number of Degree-days required for the development of different stages and the minimum threshold temperature in the life cycle of *Cs. melanura*

Life Cycle Stage	Stage of Development	Total Degree-days above *t0*°C	Minimum threshold temperature *t0*°C	*R2*	Reference
1	**Embryonation**	38.46	9.38	0.96	Mahmood & Crans (1998)
2	**1st Stadium**	71.27	10.03	0.94	"
3	**2nd Stadium**	71.47	8.83	0.79	"
4	**3rd Stadium**	85.78	8.25	0.62	"
5	**4th Stadium**	210.57	5.51	0.51	"
6	**Pupation**	44.63	8.32	0.99	"
7	**Emergence, sugar feeding, mating, host seeking**	7 Days*			(Morris (1984), Mahmood & Crans (1997)
8	**Gonotrophic Cycle**	95.87	6.40	0.98	Mahmood & Crans (1997)

*Degree days were not estimated for these stages and number of days were assigned from literature.

ambient temperatures. The resulting phenological model predicted the timing of events in life history of *Cs. melanura* (Figure 2, Table 1).

Duration of larval development was calculated from the degree-days required for the completion of each stadium; i.e., time required for molting from one instar to the next. Seven days were added from emergence to blood feeding to allow for sugar feeding and mating (Morris 1984). Mean age at molting was related inversely to temperature; i.e., larvae spent more days in each stadium as the temperature decreased (Mahmood and Crans 1998). Adults emerged after 32 days at 26°C as compared to 244 days at 10°C; all larvae died at 34°C. Therefore, 32°C was considered as the upper threshold temperature (Mahmood and Crans 1998).

The number of degree-days accumulated daily were predicted using the Single Sine and horizontal cut off methods. This method is used in circumstances where the daily air temperature lies entirely within the range of the maximum and minimum threshold temperatures. Degree-days (°D) accumulated daily were calculated as:

°D=(Tmax+Tmin/2) – TL
TU=Upper threshold = 32°C
TL= Lower threshold
Tmax = Maximum temperature
Tmin= Minimum temperature
A = (Tmax –Tmin)/2

In the Single Sine method a day's minimum and maximum temperatures were used to produce a sine curve over a 24 h period to estimate degree-days for that day by calculating the area above the minimum threshold and below the curve. This method assumes the temperature curve is symmetrical around the maximum temperature. Horizontal cutoff method assumes that development continues at a constant rate at temperatures in excess of the upper threshold. Mathematically, the area above the upper threshold is subtracted from the area above the lower thresh hold.

January 1 was taken as the biofix point or the date to start accumulating degree-days to determine the date of emergence of each overwintering larval instar. January 1 was chosen because mean ambient temperatures were below the minimum threshold temperature for the development of the immature stages of *Cs. melanura.*

Degree-days were calculated for the 4 overwintering instars and late 4th instars and/or early pupal stages of *Cs. melanura* using the predicted water temperatures that were calculated using the average daily temperatures obtained from NOAA. A hypothetical model is presented to show how the development of different overwintering larvae creates a cascade of adult emergence peaks during May – June. Therefore, the late 4th instars and/or early pupae would emerge first during the last week of April or in May and their progeny would exhibit a second peak of emergence in July (Figure 4). Early 4th, 3rd, 2nd and 1st instars would emerge in succession to the late 4th instar or early pupae. After adult emergence, the number of days required for sugar feeding, mating, blood feeding, oviposition and egg hatching were added to the development of a particular overwintering instar. Because temperatures were higher in spring and summer months, a comparatively shorter time was taken for the completion of the summer generation, i.e., the progeny of the overwintering generation resulting in the next peak of emergence.

Three years were selected to show concordance between predicted and observed emergence peaks of *Cs. melanura* and

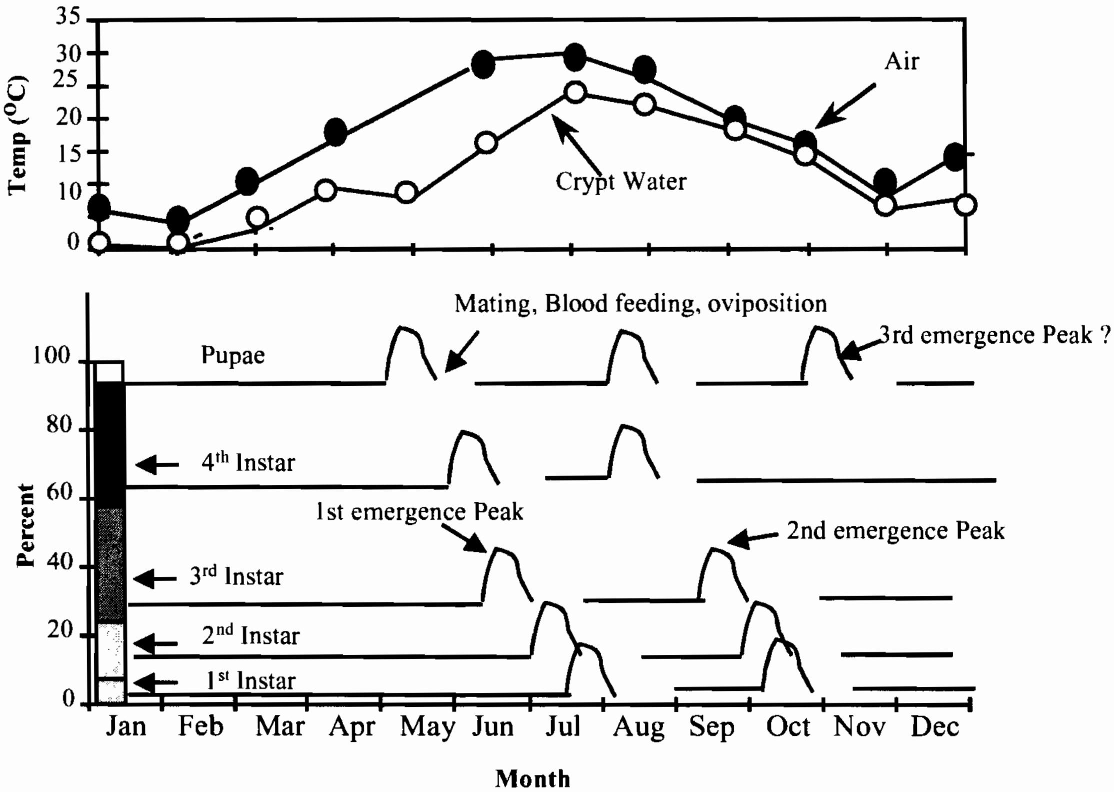


Figure 4. A hypothetical model showing adult emergence peaks of various over wintering instars of *Cs. melanura* and changes in water temperature of the subterranean crypts in relation to the changes in ambient temperatures.

their relationship to the detection of EEE infection in mosquitoes. The minimum field infection rate per 1,000 females (MFIR) varied among these 3 consecutive years (Figure 5)(Crans et al. 1994). The adult emergence peaks for these years were calculated by using the resting box data from Dennisville, Cape May County (Figures 6A-C). The weekly Williams means were calculated for 20 - 50 boxes to control variability among boxes (Williams 1947). Arrows mark the predicted emergence peaks of various overwintering immature stages calculated from the total number of degree-days that were accumulated for each stadium (Figures 6A-C).

RESULTS

The MFIR varied during 1982 to 1984 (Figure 5). In 1982 the MFIR was distributed like a "bell shaped" curve over July, August, September and October (Figure 5). In contrast, only 1 EEE infected mosquito pool was detected in August 1983. No EEE infected pools were detected in July 1983. In 1984 the MFIR formed a curve that was shifted towards the right. Comparatively lower MFIR (2.97 / 1000 females) was observed in August 1984 compared to August 1982 (5.14 / 1000 females).

During 1982, the first predicted emergence of spring generation of adults occurred on May 1 from overwintering late 4th instar larvae followed by emergence of early 4th instars during week 24. Third, 2nd, and 1st instar larvae formed the last emergence peak of the spring generation during weeks 26, 27, and 29 respectively. Maximum mean numbers of adults were collected during weeks 24, 32 and 33. The summer generation showed 3 well-defined emergence peaks. The tight emergence peak during weeks 32 and 33 consisted of the progeny of overwintering late 4th instars or early pupae and the early 4th instars. The progeny of overwintering 3rd instars emerged in week 36 followed by progeny of 2nd instars in week 40. Mosquito abundance during weeks 24 and 34 was not significantly different but they played different roles in amplification and transmission of EEE respectively. Weeks 30, 35, and 42 had the lowest population of the season. The last predicted emergence peak of 1982, consisting of the progeny of overwintering 1st instars was in fall during week 46 (11/6/1982) (Figure 6A).

Favorable ambient temperatures during 1982, ranged from 21-30°C in March - April, to 24-30°C during July and August and 12 – 25°C in September might be responsible for high immature survival that resulted in large adult emergences of the summer generation in 1982 (Mahmood and Crans 1998).

The dates of occurrence of Highland J virus (HJ) and EEE infected mosquito pools and trophic status of the females revealed that adults emerging from overwintering 3rd, 2nd and 1st instars blood fed from mid-July to the first 2 weeks of August and progeny of the overwintering late and early 4th instars was involved in the transmission of EEE from mid-August to mid-September (Table 2). The progeny of overwintering 3rd instars most likely transmitted EEE from mid-September to mid-October. Only the progeny of the 2nd instars might be involved in virus transmission

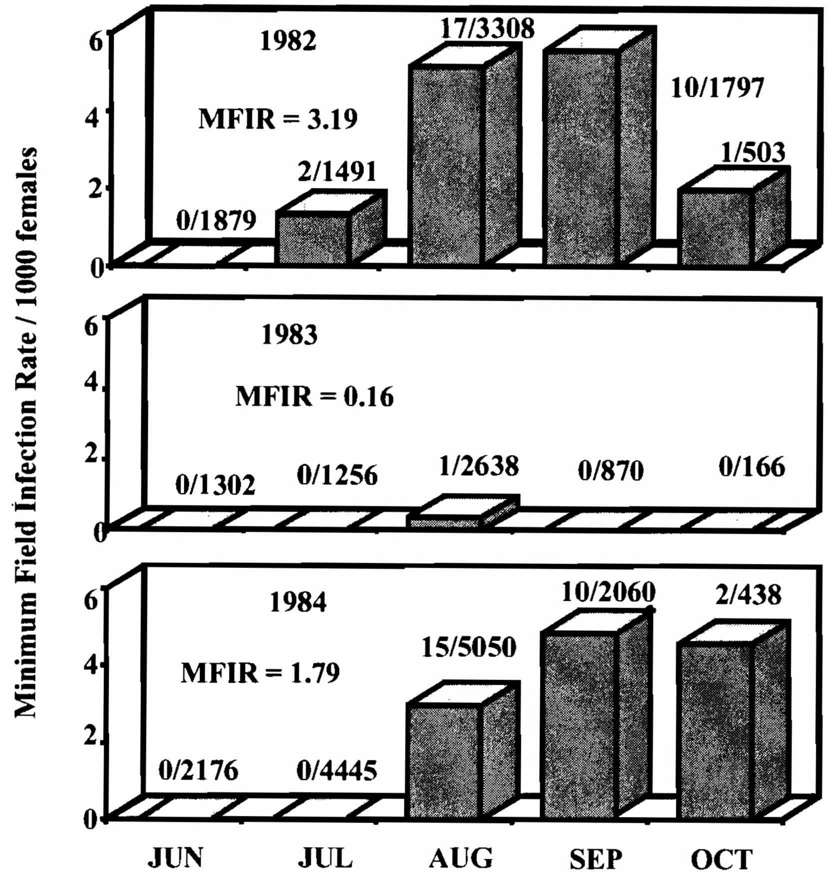


Figure 5. Variations in the minimum field infection rates of *Cs. melanura* populations from 3 different years (Crans et al. 1994).

Table 2. The virus infected mosquito pools and the feeding status of *Cs. melanura* during 1982. Numbers in parentheses represent number of pools collected on a date.

Dates of collection	**Feeding status of females**
Highland J virus	
29, July	Late fed
19, August	Late fed
12, August	Gravid
5, August	Unfed and / or sugar fed
27, September	Fresh fed
Eastern equine encephalomyelitis virus	
15, July	Late fed
29, July	Unfed and / or sugar fed
2, 9 (2), 12, August	Late fed
8, 9, 16, August	Fresh fed
9, 12, 26 August	Gravid
9, 12 (4), 23 (2), August	Unfed and / or sugar fed
30, September	Late fed
9, September	Fresh fed
13, 16, September	Gravid
13(2), 16, 23, 27, 30, September	Unfed and / or sugar fed
14, October	Unfed and / or sugar fed

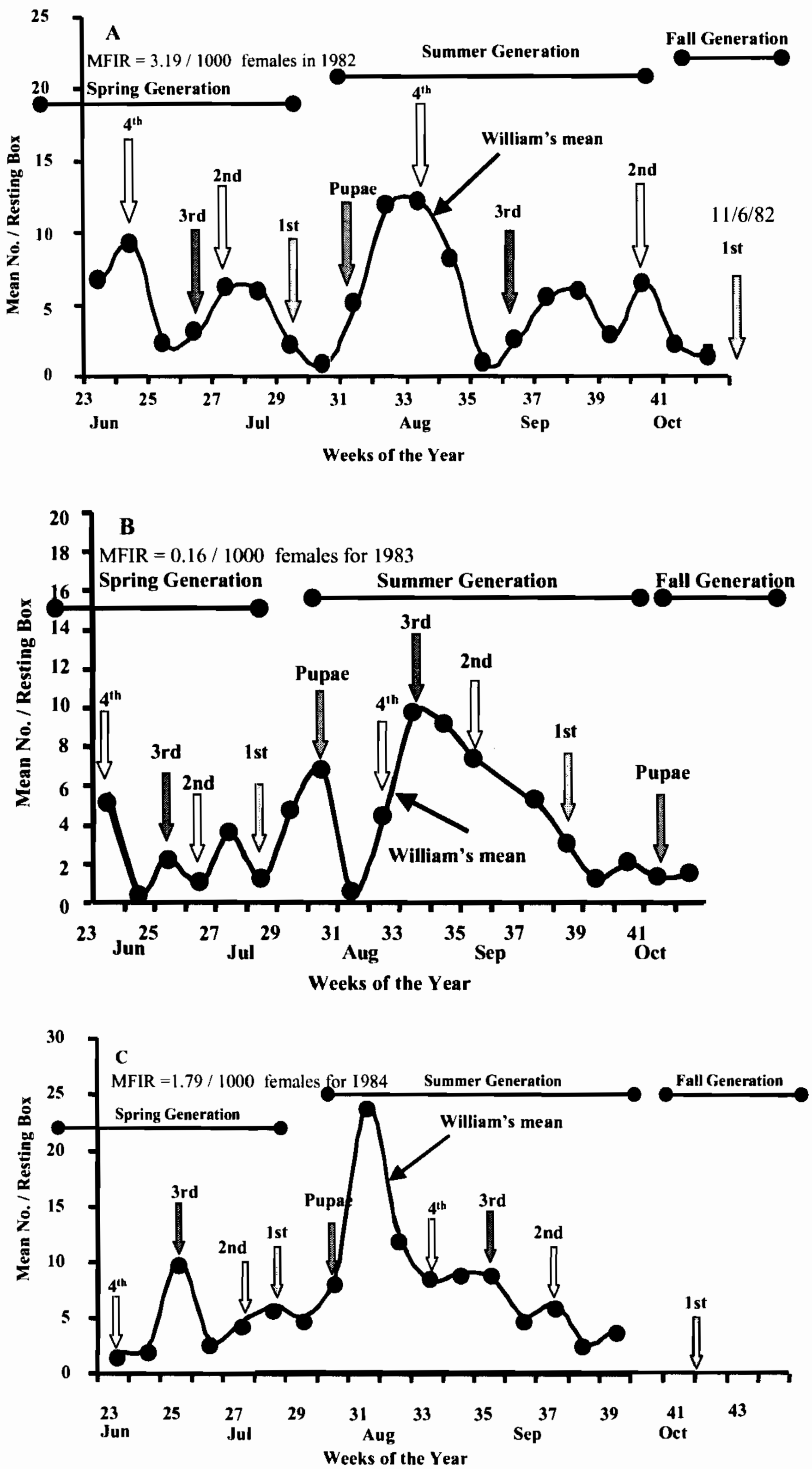


Figure 6. Population dynamics of *Cs. melanura* at Dennisville, Cape May County during 3 consecutive years, 1982 (A), 1983 (B), and 1984 (C), showing back transformed Williams mean number of females collected in the resting boxes per week (closed circles) and predicted population emergence peaks calculated using a degree-day model. Arrows indicate times of predicted emergence peaks of various overwintering immatures and their progeny.

in October and the last predicted peak from the progeny of the 1st instars possibly could not play any role in virus transmission (Figure 6A).

In contrast to 1982, 1983 had higher ambient temperatures with maximum temperatures reaching 36°C resulting in low mosquito survival and/or modulation of EEE infection, because only 1 EEE infected pool was collected on 29 August (Figures 5 and 6B, Table 3). In contrast, 8 HJ pools were detected in August and 5 in September. All stages of blood digestion were represented in HJ infected mosquito pools.

Five actual adult emergence peaks were observed in 1983, and 3 of these peaks constituted the spring generation and 2 peaks represented the summer generation. Adult female abundance differed significantly among weeks. Week 24 had the lowest adult abundance because the predicted peak of overwintering 4th instars occurred a week earlier due to warm temperatures (Figure 6B). The actual emergence peaks coincided well with the predicted peaks of the spring generation and predicted adults emerged from the 3rd, 2nd, and 1st instars on weeks 25, 26, and 28. The surveillance data showed small individual peaks instead of a combined large peak as was observed in 1982 for weeks 26, 28 and 29, thereby possibly reducing vector and host contact in 1983. The first emergence peak of the summer generation or progeny of late 4th instars and/or early pupae was during week 30, 2 weeks earlier than in 1982 and was separated from the predicted emergence peak of the early 4th instars compared to 1982. This separation was a direct result of high temperatures that ranged from 22-35°C during June. The second peak of summer generation was observed in weeks 33-35 and had the highest abundance, and occurred 1 week later than that observed in 1982

Table 3. The virus infected mosquito pools and feeding status of *Cs. melanura* during 1983. Numbers in parentheses represent number of pools collected on a date.

Dates of collection	Feeding status of females
Highland J virus	
19, 25, August	Late fed
1, 4, 19, August	Fresh fed
15, 25(2), August	Unfed and / or sugar fed
1, September	Late fed
1, September	Gravid
12, 30, September	Fresh fed
1, September	Unfed
Eastern equine encephalomyelitis virus	
29, August	Fresh fed

Table 4. The virus infected mosquito pools and feeding status of *Cs. melanura* during 1984. Numbers in parentheses represent numbers of infected pools collected on that date.

Dates of collection	Feeding status of females
Highland J virus	
9 (3), 19, 26, 30 (2), July	Unfed and / or sugar fed
2, 5, 12, 16, 23, July	Fresh fed
2, 9, 12, 16, 19, 26, 30, July	Late fed
5, 30, July	Gravid
2,9 (2), 20, August	Unfed and / or sugar fed
13, August	Fresh fed
2(2), 6, 13, August	Late fed
2, 9, 13, August	Gravid
20, 24, September	Unfed
6, September	Fresh fed
24 September	Gravid
Eastern equine encephalomyelitis virus	
6, 13, 16, 20 (2), 23, 27, August	Unfed
2 (2), 9 (2), 13, 16, 20, 23, August	Fresh fed
4, 10, 13, September	Unfed
4, 13, 24 (2), September	Fresh fed
4, 6, 27, September	Gravid
October	Not available

(weeks 32-33) (Figures 6A and B). Although the 4th instar larvae emerged on week 33 as in 1982, adults from the progeny of 3rd instars emerged 2 weeks earlier in week 34 resulting in the concurrent emergence of the progeny of 3rd and 4th instars. However, the total number of adults per resting box was still fewer than in 1982, perhaps as a result of high ambient maximum temperatures (27 - 36°C) in July thus resulting in lower mosquito to bird contact (Figure 6B). Lowest mosquito abundance with comparable number of females was observed in weeks 27, 29, and 32 (Figure 6B).

In 1984, 2 major and 3 minor adult emergence peaks were observed from the surveillance data (Figure 6C). The first predicted emergence peak of spring generation was of late 4th instars or early pupae in the first week of May. The predicted early 4th instars emergence peak in week 23 was absent. The predicted emergence peak of 3rd instars during week 25 coincided with the observed peak in week 25. The predicted peak of overwintering 2nd instars was during week 26 followed by the predicted peak of first instars during week 28 (Figure 6C). The presence of only a few adults during week 23 in 1984 compared to 1983 indicated that a small number of early 4th instars overwintered in 1983. Small mosquito abundance during July (weeks 27 - 30) resulted in the absence of EEE infected pools in July (Figure 5). The predicted second wave of emergences started from the last week of July from late 4th instars or early pupae and resulted in significantly larger number of adults in week 31. This was the largest peak emergence of the season and was one week earlier than in 1982 and 2 weeks earlier than in 1983 (Figure 6C). Predicted progeny of 4th instars emerged in the 33rd week followed by 3rd instars in week 35, 2nd instars in week 38 and last emergence of the summer generation was the progeny of overwintering 1st instar larvae around October 15 (week 42). Ambient temperature was within the favorable range of immature development of *Cs. melanura* in June (21°-31°C) and in July-August (21°-33°C). There was no difference in population abundance among weeks 25, 32, and 34. The smallest number of mosquitoes was found during weeks 23, 24, 26, and 38.

Most of the HJ infected mosquito pools were collected in July (21), followed by August (12), and the smallest number was collected in September (4). In comparison, no EEE infected pools were collected in July, and 15 EEE infected pools were collected in August, followed by 10 pools in September and 2 in October (Figure 5, Table 4). Only 1 infected pool of gravid females was detected in September (Table 4). This was supported by a sharp decline in population from more than 20 females per resting box in week 31 to 10 females per box in week 32 to less than 6 mosquitoes per box in week 33 (Figure 6C).

DISCUSSION

The chronology of events among the three components of the EEE cycle is presented in Figure 1. Comparison of the above 3 years shows that the first adult emergence from late 4th instars occurred in the last week of April and or first week of May depending on ambient temperature and transmits EEE from recrudescing AHY, SR and PR birds to non-immune AHY birds. Mosquitoes emerging from the overwintering early 4th to 1st instars during June transmit EEE from the AHY birds to the HY fledglings. In summer generation, the progeny of late 4th instars and early 4th instars plays a major role in August amplification of the virus between AHY and Juvenile populations. The concurrence of a large population peak in week 33 with a large avian population triggers amplification of the virus in non-immune juveniles. The proportion of various overwintering instars in the preceding fall determines the fate of EEE transmission and amplification in the following spring. The temperatures in the following year control not only the timing of adult emergence but also female survival and the extrinsic incubation and modulation of EEE in spring and summer.

Crans et al. (1994) hypothesized that EEE overwinters in southern New Jersey as a latent infection in previously infected birds and recrudesces in spring and early summer due to the stress related to nesting. They suggested that an influx of newly emerged nulliparous mosquitoes was needed at the same time as the presence of non-immune juvenile hosts to initiate amplification. In 1982, the predicted emergence peak from the 4th instar larvae indicated that these mosquitoes fed during May on AHY permanent and SR birds. Emergence of the 3rd, 2nd, and 1st instars formed a tight peak during weeks 27 – 28 and resulted in mosquitoes blood feeding on potentially infective AHY parents and transmitting the virus to non-immune HY fledging juveniles resulting in the amplification of EEE. Six viremic birds were collected 7-51 days before isolations were made from mosquitoes during 1981-82. Six different species of birds had early viremias and of these, only one was an AHY bird. A Gray catbird recaptured on 13 May, 1981 had a PRNT titer of 1:20, and then was viremic on 8 June 1982 showing the spring recrudescence of EEE. Crans et al., (1994) showed that antibody prevalence was relatively high in early spring, but progressively decreased in May or June when new HY birds were added to the population and mosquito abundance was very low, so little EEE transmission might be occurring at this time. The antibody prevalence increased again in late summer due to transmission by large adult emergence peaks. Observations made by Crans et al. (1994) were supported by later studies using a more sensitive virus detection technique. Reverse transcriptase- polymerase chain reaction (RT-PCR) that detected EEE RNA in freshly engorged and late fed mosquitoes in spring (Monroy et. al 1996, Mahmood 1996, Mahmood & Crans 1995). The earliest detection of infectious virus using a wet chick assay was in the first week of July during 1995 (Mahmood 1996). The inability to detect early season infectious virus might be due to long gonotrophic cycles (11-12 days in May and up to 8 days in June) and extrinsic incubation periods at cool temperatures during June (Mahmood & Crans 1997, Takahashi 1976). In 1982, the maximum percentage of juvenile birds was collected during the last 2 weeks of August and this overlapped with the peak in *Cs. melanura* population during weeks 32 and 33.

In 1983, high ambient temperatures resulted in faster immature development resulting in several small early peaks for each overwintering larval instar and abundance levels that averaged less than 2 mosquitoes per resting box during June. The small number of adults might also have been the result of low female survivorship and few females might have completed the first gonotrophic cycle in June, thus resulting in low transmission of EEE from the AHY birds to non-immune HY nestlings. In July 1983, maximum ambient temperatures ranged from 27 – 36 °C and were intolerant for immatures as well as adult mosquitoes and might have caused increased larval mortality at molting and low adult survival after first oviposition (Mahmood and Crans 1998). Detection of only 1 EEE infected pool might have been due to low adult survivorship during the transmission season. In 1983, none of 40 recaptured birds, showed evidence of seroconversion (Crans et al., 1994).

Another discrete possibility is that high ambient temperatures interfere with multiplication of EEE in the mosquitoes resulting in modulation of EEE. Because HJ infected mosquito pools were collected from the first week of August to the end of September, suggesting that mosquitoes were living long enough to allow for extrinsic incubation of the virus. Although EEE activity was very low in coastal areas including the Dennisville study site, there were equine deaths in inland areas of New Jersey (Crans 1984), indicating differences in temperature between the 2 areas resulted in amplification of EEE in avian fauna of the inland sites and its transmission to mammals by a mosquito other than *Cs. melanura*. Differences of a few weeks in *Cs. melanura* emergence times might have resulted in few mosquitoes feeding on recrudescing AHY birds and thus very low transmission of EEE to the HY birds. Progeny of the late 4th instars and early pupae emerged and formed a sharp peak during week 30 instead of week 32 as observed in 1982, where they formed a peak with early 4th instars that emerged during week 33. In 1983 the population peak during weeks 33-36 was formed by early 4th, 3rd and 2nd instars but the adult abundance was lower than 1982. So the low amplification of virus was accompanied with low transmission resulting in low EEE detection during 1983.

Eastern equine encephalitis was very active in 9 of the 21 counties in New Jersey during 1984, and pheasant and equine deaths and human cases were reported (Crans 1985). The first equine case was reported on 28 July from Middlesex County and the last from Atlantic County on November 2 (Crans 1985). The amplification of EEE virus in AHY birds was due to late 4th instar and early pupae that emerged during the first week of May. The predicted peak of 4th instars was during week 23 and a small number of adults were collected at this time due to low survival of adults in the previous year. The transmission of EEE from AHY birds to HY birds in June and first 2 weeks of July was by females emerging from overwintering 3rd, 2nd, and 1st instars. Highland J was active from July to September and EEE infected pools were collected from the 1st week of August to the end of September and 1st week of October. Second generation females were involved in amplification and transmission of EEE to Juveniles and AHY birds. Mean number of mosquitoes was >24 per box during week 31, a time when the bird population comprised of >60% HY birds (Crans et al. 1994).

Analysis of the timing of mosquito emergence peaks during 1982-1984 indicated that an overlap between mosquitoes and avian fauna within certain weeks of the year was a perquisite for virus amplification (Scott 1988). Effect of high temperature on the upper threshold of EEE development and/or multiplication of EEE is not well understood. The effect of high temperatures on host seeking in nature also is not well documented for *Cs. melanura* and further studies are required.

In summary, differences in seasonal temperatures resulted in differences in the MFIR rate in the *Cs. melanura* populations and changes in the emergence patterns of adults overwintering as different instars. A shift in emergence peaks resulted in mosquitoes blood feeding on a younger or older population of the birds, thus a synchrony between mosquito emergence and non-immune avian population was a key to successful amplification of EEE. The degree-day model was useful in predicting the population dynamics of *Cs. melanura* and various events in the EEE transmission cycle. The extant of EEE amplification is related directly to the size and survival of overwintering larval cohorts. Because a certain minimal level of mosquito abundance was required at the time of EEE recrudescence in birds, the role of mosquito feeding as a stress and/or the role of mosquito saliva in initiating recrudescence of the virus in birds is not known.

An alternative hypothesis to explain focal virus persistence is that EEE overwinters in vertically infected *Cs. melanura* larvae. Blood feeding stimulated these infections to replicate and become infectious during the first vernal gonotrophic cycle and a certain number of infected mosquitoes were required for amplification of EEE to occur in nature.

Acknowledgments

This research was conducted at the Mosquito Research and Control, Headlee Research Laboratories, Department of Entomology, Rutgers the State University, New Brunswick, New Jersey. I greatly appreciate the contributions of Dr. W. J. Crans for providing the historical data for mosquito population analysis. The contribution of Dr. Donald F. Caccamise, Ellen P. Orrell, Linda McCustion for collection of some of the data used in this publication. Judy Hansen and staff of Cape May County provided facilities during collection of this data. The research was made possible by funding provided by New Jersey State Mosquito Control Commission, U.S. Hatch funds, and a grant by New Jersey Water Research Institute. I thank Dr. W. K. Reisen for critically reviewing the manuscript.

REFERENCES

Burbutis, P. P. and R. W. Lake. 1956. The biology of *Culiseta melanura* in New Jersey. Proc. N. J. Exterm. Assoc. 43: 155-161.

Crans, W. J. 1977. The status of *Aedes sollicitans* as an epidemic vector of eastern equine encephalitis in New Jersey. Mosq. News 37: 85-89.

Crans, W. J. 1984. Eastern equine encephalitis in New Jersey during 1983. Proc. N. J. Mosq. Control Assoc. 71: 34–36.

Crans, W. J. 1985. Eastern equine encephalitis in New Jersey during 1984. Proc. N. J. Mosq. Control Assoc. 72: 34–37.

Crans, W. J. 1995. Resting boxes as mosquito surveillance tools. Proc. N. J. Mosq. Control Assoc. 82: 53-57.

Crans, W. J. and L. J. McCuiston. 1993. New Jersey's approach to encephalitis prevention. Bull. Soc. Vector Ecol. 11: 178-184.

Crans, W. J. and T. L. Schulze. 1986. Evidence incriminating *Coquillettidia perturbans* as an epizootic vector of eastern equine encephalitis I. Isolation of EEE virus from *Cq. perturbans* during an epizootic among horses in New Jersey. Bull. Soc. Vector Ecol. 11: 178-184.

Crans, W. J., D. F. Caccamise and J. R. McNelly. 1994. Eastern equine encephalomyelitis virus in relation to the avian community of a coastal cedar swamp. J. Med. Entomol. 31: 711-728.

Crans, W. J., J. McNelly, T. L. Schulze and A. J. Main. 1986. Isolation of eastern equine encephalitis virus from *Aedes sollicitans* during an epizootic in southern New Jersey. J. Am. Mosq. Control. Assoc. 2: 68-72.

Gusciora, W. R. 1961. The resting box technique for the sampling of *Culiseta melanura* (Coquillett). Proc. N. J. Mosquito Exterm. Assoc. 48: 122-125.

Gusciora, W., D. Adam, G. Bordash, O. Sussman and M. Goldfield. 1972. A search for a possible overwintering arthropod reservoir for EEE virus. N. J. Mosq. Exterm. Assoc. 59: 147-164.

Hayes, R. O. 1958. Observations on the swarming of *Culiseta melanura* (Coquillett). Mosq. News 18: 70-73.

Howard, J. J., D. J. White and S. L. Muller. 1989. Mark-recapture studies on the *Culiseta* (Diptera: Culicidae) vectors of eastern equine encephalitis virus. J. Med. Entomol. 26: 190-199.

Joseph, S. R. and W. E. Bickley. 1969. *Culiseta melanura* (Coquillett) on the eastern shore of Maryland (Diptera: Culicidae). Univ. Maryland Agric. Exp. Sta. Bull. A-161. 84 p.

Mahmood, F. 1996. Detection of eastern equine encephalitis virus in wild-caught *Cs. melanura* females by the reverse transcriptase polymerase chain reaction: The 1995 trials. Proc. N. J. Mosq. Control Assoc. 83: 84-94.

Mahmood, F. and W. J. Crans. 1995. Detection of eastern equine encephalitis virus in wild-caught *Culiseta melanura* by the reverse transcriptase polymerase chain reaction (RT- PCR) during 1994 vector surveillance season in southern New Jersey. Proc. N. J. Mosq. Control Assoc. 82: 17-31.

Mahmood, F. and W. J. Crans. 1997. A thermal heat summation model to predict the duration of the gonotrophic cycle of *Culiseta melanura* in nature. J. Am. Mosq. Control Assoc. 13: 92-94.

Mahmood, F. and W. J. Crans. 1998. Effect of temperature on the development of *Culiseta melanura* (Diptera: Culicidae) and its impact on the amplification of eastern equine encephalomyelitis virus in birds. J. Med. Entomol. 35: 1007-1012.

Monroy, A. M., T. W. Scott and B. A. Webb. 1996. Evaluation of reverse transcriptase polymerase chain reaction for the detection of eastern equine encephalomyelitis virus during vector surveillance. J. Med. Entomol. 33: 449-457.

Morris, C. D. 1984. Phenology of trophic and gonobiologic states in *Culiseta morsitans* and *Culiseta melanura* (Diptera: Culicidae). J. Med. Entomol. 21: 38-51.

Morris, C. D. 1988. Eastern equine encephalomyelitis. In: T. P. Monath. (Ed.). The arboviruses: epidemiology and ecology. Vol. 3. CRC Press, Inc. Boca Raton, FL. 1-20

Morris, C. D., R. H. Zimmerman and L. A. Magnarelli. 1976. The bionomics of *Culiseta melanura* and *Culiseta morsitans* dyari in Central New York state (Diptera: Culicidae). J. Med. Entomol. 69: 101-105.

Nasci, R. S. and J. D. Edman. 1981. Vertical and temporal flight activity of the mosquito *Culiseta melanura* (Diptera: Culicidae) in southeastern Massachusetts. J. Med. Entomol. 18: 501-504.

Orrell, E. P. 1997. The population dynamics of *Culiseta melanura* larvae at an endemic focus for eastern equine encephalitis virus in a New Jersey cedar swamp. Proc. Northeastern Mosq. Control Assoc. 43: 38-39.

Orrell, E. P. 1998. Population dynamics and overwintering mechanisms of eastern equine encephalitis virus in *Culiseta melanura* (Coquillett). Ms. Thesis, Rutgers the State University of New Jersey. 61 p.

Siverly, R. E. and H. F. Schoof. 1962. Biology of *Culiseta melanura* (Coquillett) in southeast Georgia. Mosq. News 22: 274-282.

Scott, T. W. 1988. Vertebrate host ecology. In: T. P. Monath. (Ed.), The arboviruses: epidemiology and ecology. Vol. 1. CRC Press Inc. Boca Raton, FL. 257–278.

Takahashi, M. 1976. The effects of environmental and physiological conditions of *Culex tritaeniorhynchus* on the patterns of transmission of Japanese encephalitis virus. J. Med. Entomol. 13: 275-284.

Williams, C. B. 1947. The logarithmic series and its application to biological problems. J. Ecol. 34: 255-272.

Evaluation of the California Encephalitis Epidemic Risk Assessment Scheme Using Conditional Simulations with Historical Data*

Christopher M. Barker[1], William K. Reisen[1], and Vicki L. Kramer[2]

[1]*Arbovirus Research Unit, Center for Vector-Borne Disease Research, School of Veterinary Medicine, University of California, Davis, CA 95616*
[2]*Vector-Borne Disease Section, California Department of Health Services, 601 North 7th Street, Sacramento, CA 94234*

ABSTRACT: The California Mosquito-borne Virus Surveillance and Response Plan was evaluated using retrospective conditional simulations based on historical data to determine whether the risk levels would have been appropriate during periods with differing levels of virus activity. For the majority of the periods and regions examined, the model would accurately have represented the actual situation, and during epidemic years, it would have provided some early warning of epidemic activity that would follow. During 1989 in Kern County, however, an epidemic of St. Louis encephalitis was undetected by the model, and risk for virus activity was at normal levels during the period prior to the first human case. The need for separate models for western equine encephalomyelitis and St. Louis encephalitis viruses and possible future modifications to the model are discussed.

INTRODUCTION

The California Mosquito-borne Virus Surveillance and Response Plan was developed by the California Department of Health Services, the Mosquito and Vector Control Association of California, and the University of California to quantify the risk for western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) virus activity within the state and to provide appropriate graded intervention response recommendations when risk increases (California Department of Health Services 2001). The model quantifies factors related to environmental conditions, vector abundance, and virus amplification, and overall risk is divided into three categories: normal season, emergency planning, and epidemic conditions. During normal conditions, routine vector control and virus surveillance activities are conducted, but when the lower threshold for emergency planning conditions is reached, public awareness and vector control measures are intensified to avoid reaching epidemic conditions. By definition, human cases occur during epidemic conditions, and it is these cases that the surveillance and response system is designed to prevent.

MATERIALS AND METHODS

The overall risk for virus activity in several regions of the state was estimated for years with differing levels of virus activity based on the response plan which considers environmental conditions, adult mosquito abundance, virus isolation rates in mosquitoes, sentinel chicken seroconversions, equine cases (for WEE), human cases, and human population density in areas where virus activity is detected. For calculating retrospective risk levels, the year was divided into 24 half-month periods, and the risk level for each half-month was based on data from the previous half-month. Each of the seven factors was assigned a value between 1 and 5, and all were averaged to determine an overall risk value, also scaled from 1 to 5 (Table 1). Overall risk fell into one of three categories: normal season (1.0-2.5), emergency planning (2.6-4.0), and epidemic conditions (4.1-5.0).

Environmental conditions were comprised of water supply and temperature. The water component was the average of runoff[1] and rainfall[2] from November through May, but because rainfall during the remainder of the year is negligible in most of the state, runoff alone was used to calculate the water component during summer and early fall. In highly urbanized areas where runoff from snowmelt does not appreciably affect availability of mosquito larval sites, rainfall was the sole water factor used for calculating risk. Other water sources such as lawn irrigation and their resulting runoff also affect larval habitat availability, but quantification of these factors is not possible for most areas. As a way of relating environmental variables for the study period to historical data, rainfall, runoff, and temperature values[2] for the past 50 to 60 years were divided into quintiles, and risk levels were assigned for a given year by the quintile into which the measurements for each half-month fell.

Risk level assignments for other factors were subjective, relying heavily on expert opinion rather than calculated thresholds. Adult female *Culex tarsalis* abundance[3] for each half-month of

---

**An expanded description of this research has been submitted for publication in the American Journal of Tropical Medicine and Hygiene.*

[1] Runoff data were obtained from the online California Data Exchange Center, maintained by the California Department of Water Resources at http://cdec.water.ca.gov.
[2] Precipitation and temperature data were obtained from the website of the University of California Statewide Integrated Pest Management Project at http://www.ipm.ucdavis.edu/ WEATHER/ wxretrieve.html.
[3] Adult *Cx. tarsalis* data were obtained from collection records provided by the Greater Los Angeles County VCD, Kern MVCD, Sacramento-Yolo MVCD, and Sutter-Yuba MVCD.

Table 1. Thresholds for assignment of risk levels for each factor in the response plan. PF = positive flocks; CPPF = conversions per positive flock.

Risk Level	Environmental Conditions			Adult Mosquito Abundance	Mosquito MIR/1,000	Chicken Seroconversions	Equine Cases	Human Cases	Proximity of Virus Activity to Populated Areas
	Rain	Runoff	Temperature						
1	Well Below Average			< 50% 10-yr. Avg.	0	0 conversions	0 Statewide	0 Statewide	Remote Area
2	Below Average			50-90% 10-yr. Avg.	0.1-1.0	1 conversion			Rural Area
3	Average			91-150% 10-yr. Avg.	1.1-2.0	PF > 1 CPPF ? 1	? 1 Statewide 0 Local	? 1 Statewide 0 Local	Small Town
4	Above Average			151-300% 10-yr. Avg.	2.1-5.0	PF > 1 1 < CPPF ? 3	1-2 Local		Suburban Area
5	Well Above Average			> 300% 10-yr. Avg.	> 5.0	PF > 1 CPPF > 3	> 2 Local	? 1 Local	Urban Area

Overall Response Level	
	Normal Season (1.0 to 2.5) **Emergency Planning** (2.6 to 4.0) **Epidemic** (4.1 to 5.0)

the simulated year was compared to the average for the same half-month over the previous 10 years, or if the historical dataset was <10 years, the average was calculated from all available years. The risk level was determined by the current abundance expressed as a percentage of the 10-year average (Table 1).

Virus isolation rates in *Cx. tarsalis*[4] were a direct measure of virus activity, and risk levels increased as minimum infection rates increased. Sentinel chicken seroconversions[4] also measured enzootic virus transmission, and numbers of positive flocks and seroconversions per positive flock provided a measure of the extent and intensity of virus activity; therefore , the risk level increased as the number of positive chickens or the number of positive flocks increased (Table 1).

Equine and human cases[5] also were indicative of WEE transmission (human cases only for SLE), and risk levels increased when cases occurred within the state and were highest when cases occurred within the local region. An additional risk factor represented the size of the human population potentially exposed to viral infection[6], and this factor was included only after virus activity was detected. The lowest possible risk value is 1 for virus activity found in a remote, unpopulated area, such as a wildlife refuge, and the highest risk value is 5 for virus activity in an urban area (Table 1).

To evaluate the response plan, historical data were assembled to simulate each half-month of years with varying levels of virus activity, including: Kern County – 1952 (WEE, SLE epidemic), 1983 (WEE enzootic activity), 1989 (SLE epidemic), 1995 (no virus activity), and 1996 (WEE enzootic activity); Greater Los Angeles – 1984 (SLE epidemic), Sacramento and Yolo Counties – 1993 (WEE enzootic activity), and Sutter and Yuba Counties – 1993 (WEE enzootic activity).

RESULTS

The 1952 epidemic of WEE produced the greatest number of encephalitis cases ever reported in California. SLE also reached epidemic levels in that year, and in Kern County there were 100 human cases of WEE and 16 cases of SLE. Based on weather and surveillance data, the risk model would have accurately represented the situation during most of the year for both viruses, and several weeks of emergency planning conditions during winter and spring would have provided some warning of the pending epidemic that began in early summer. For both viruses, a period of emergency planning conditions preceded the first human case, and the overall risk level immediately exceeded the epidemic threshold following the first human cases.

Human SLE case numbers during 1989 in Kern County were similar to those in 1952, and in both years, periods in the emergency planning range preceded the epidemic. During 1989, emergency planning conditions were reached briefly in late April, but risk levels quickly declined to normal and remained there until the first human SLE case occurred. Mosquito populations were high during the spring virus amplification period, but were well below average thereafter. Despite low vector abundance, SLE activity increased dramatically beginning in August, but even during the late summer period when human cases were occurring, the overall risk level failed to reach epidemic conditions. WEE activity was not detected in 1989 in Kern County, and as a result, overall risk remained within the normal range for the entire year.

During 1984 in Los Angeles, risk based on environmental conditions was close to normal levels during winter and spring. *Cx. tarsalis* adults were more abundant than usual in winter but declined to normal levels during spring. As usual, SLE was not detected during these early months, but overall risk levels reached

[4] Virus isolation rates in *Cx. tarsalis* and sentinel chicken seroconversion rates were assimilated from the Proceedings and Papers of the Mosquito and Vector Control Association of California, weekly Arbovirus Surveillance Bulletins published by the California Department of Health Services, data provided by individual MVCDs, and from Reeves and Hammon 1962.
[5] Equine and human case data were obtained from the Proceedings and Papers of the Mosquito and Vector Control Association of California.
[6] Information on the human population densities of areas with virus activity was obtained from individual MVCDs.

emergency planning conditions several times before human cases and other SLE activity were detected in early August. During the same year, precipitation levels in Los Angeles were below normal during the early parts of the year and at or near normal during the summer months preceding the epidemic (normal precipitation in L.A. is essentially zero during summer). *Culex quinquefasciatus* populations were below average through spring and early summer, but were above average during late August and September when many of the human SLE cases occurred.

Extensive enzootic WEE activity occurred over much of the Sacramento Valley during 1993, with many virus isolations from mosquito pools and seroconversions in sentinel chicken flocks. The overall risk levels in Sacramento-Yolo and Sutter-Yuba MVCDs were virtually identical for the entire year, with risk levels remaining in emergency planning conditions from July through September. Rainfall in Sacramento-Yolo and Sutter-Yuba during 1993 began the year at higher-than-normal levels and then followed an up-and-down pattern into the summer when precipitation lapsed into the usual pattern of zeros. Temperatures for both districts were generally near normal, except for warm periods in early spring and early summer that might have accelerated larval development and virus incubation. *Cx. tarsalis* populations were well above normal through the spring and early summer, but were slightly below normal during most of the period of virus activity for both districts.

During 1983 and 1996, enzootic WEE activity in Kern County pushed risk levels into the emergency planning range by late July or early August. The overall risk levels during 1983 and 1996 were similar, with normal conditions for most of the year, then emergency planning conditions during late summer and early fall. No virus activity was detected in Kern County during 1995, and risk levels remained within the normal range for the entire year despite high risk levels for environmental conditions and *Cx. tarsalis* abundance during several months.

DISCUSSION

The current encephalitis virus response plan would have reflected appropriate risk levels during most of the periods simulated in our study using retrospective data (Table 2). Generally, periods without virus activity and with benign environmental conditions fell within the normal range for overall risk, periods with enzootic virus activity required emergency planning, and periods with human cases were classified appropriately as epidemic conditions. A notable exception was the 1989 SLE epidemic in Kern County, when epidemic conditions would have been undetected by the risk model. Also, just before the 1989 epidemic activity began, normal conditions prevailed, even though emergency planning conditions were reached briefly earlier in the season. This potential failure of the model to forecast or detect current SLE activity indicated the need for modifications tailored to SLE. In the future, separate models will be developed to account for differences between these viruses. Another consideration for future development of the risk model is the potential introduction of West Nile virus (WN) into California. WN is related closely to SLE, but because virus testing of dead birds is an important component of surveillance for WN, a third risk model may be necessary.

Human and horse encephalitis cases are less sensitive indicators of virus activity than other surveillance tools, because they occur tangentially to the primary amplification cycle. Clinical diagnosis of human cases of encephalitis is frequently non-specific, and as a result, cases of human infection with mosquito-borne viruses often are undetected, even during epidemic periods (Tueller 1990). Also, many infections are asymptomatic or may cause subclinical symptoms. Further, most horses are vaccinated for WEE, so the number of horses susceptible to illness is relatively small. For these reasons, it may improve the risk assessment to combine human and horse cases as a single WEE risk factor to reduce their weight in the model.

Table 2. Summary indicating earliest period of emergency planning and epidemic conditions for years with various levels of virus activity. Dates are listed by month and half-month. KERN = Kern MVCD; SAYO = Sacramento-Yolo MVCD; SUYA = Sutter-Yuba MVCD; GRLA = Greater Los Angeles County VCD.

Virus	Year and District	Earliest Emergency Planning Conditions	Earliest Epidemic Conditions
WEE	KERN 1952*	February 1, April 2	June 2 following 1st case
	KERN 1983	July 2	Did not occur
	SAYO 1993	July 1	Did not occur
	SUYA 1993	July 1	Did not occur
	KERN 1996	August 1	Did not occur
SLE	KERN 1952*	February 1, April 2	August 2 following 1st case
	GRLA 1984*	January 2, February 2	August 2 following 1st case
	KERN 1989*	April 2	Did not occur
No Activity	KERN 1989 (WEE)	Did not occur	Did not occur
	KERN 1995	Did not occur	Did not occur

A comparison of risk levels during epidemic and enzootic years revealed that emergency planning conditions generally would have been reached during late winter or early spring in epidemic years, but in years with only enzootic activity, emergency planning conditions would not usually occur until middle to late summer (Table 2). This temporal difference between epidemic and non-epidemic years may be useful for forecasting whether human cases are likely to occur in a given year based on the date when emergency planning conditions are first reached.

Acknowledgments

We sincerely thank the Kern MVCD, Greater Los Angeles County VCD, Sacramento-Yolo MVCD, and Sutter-Yuba MVCD for providing historical data. We are particularly grateful to Debbie Lemenager, Richard Takahashi, Rhonda Laffey, Ken Boyce, Jacqui Spoehel, Minoo Madon, and Deborah Dritz for assistance in obtaining the data. Funding was provided by a grant from the Office of Global Programs, National Oceanic and Atmospheric Administration.

REFERENCES

California Department of Health Services, Mosquito and Vector Control Association of California, and University of California. 2001. California state mosquito-borne virus surveillance and response plan (V.L. Kramer, ed.). <http://www.dhs.ca.gov/ps/dcdc/disb/pdf/CA%20Mosquito%20Response%20Plan%206-01.pdf>.

Reeves, W.C. and W. McD. Hammon. 1962. *Epidemiology of the arthropod-borne viral encephalitides in Kern County, California, 1943-1952.* Berkeley, CA: University of California Press.

Tueller, J.E. 1990. An outbreak of illness due to St. Louis encephalitis virus in the southern San Joaquin Valley, California, 1989. Proc. Calif. Mosq. Vector Control Assoc. 58:12.

Effect of Vegetation Management on the Abundance of Mosquitoes at a Constructed Treatment Wetland in Southern California

J. B. Keiper[1], J.A. Jiannino, M.R. Sanford, and W.E. Walton

Department of Entomology, University of California, Riverside, CA 92521

ABSTRACT: We studied the spatial distribution and abundance of mosquitoes in a 186-ha constructed treatment wetland designed to remove nitrate from river water in southern California. Relative to constructed treatment wetlands receiving comparatively poor quality water, marshes of the Prado Wetland that were flooded continuously for 2-3 years consistently produced few mosquitoes. Mosquito abundance increased directly with emergent vegetation coverage as the wetland aged. *Culex erythrothorax* was prevalent in carbon dioxide-baited CDC trap collections. Dense, central areas (2-5 m from periphery) of plant stands produced ~ 75% of all emergent mosquitoes. Newly flooded areas of the wetlands that had undergone a drawdown-vegetation knockdown-reflooding cycle for vegetation management produced significantly more mosquitoes (maximum of > 50 larvae·dip^{-1}) than did control marshes which had not undergone vegetation management and marshes in which knocked down vegetation was reduced by grading the pond bottom. *Culex tarsalis* was the species most commonly taken following vegetation management, with a mean of > 25 larvae·dip^{-1} at 7 wk postflooding. *Culex stigmatosoma, Cx. quinquefasciatus, Cx. restuans,* and *Anopheles hermsi* were present but not abundant. Although this constructed wetland produced few mosquitoes under normal operating conditions, mosquito production increased markedly following vegetation management. Mosquito abatement efforts need to be concentrated in areas undergoing vegetation management and may need to be carried out for 2 months following reflooding.

Constructed wetlands have been designed for numerous purposes, including waste water treatment, flood control, and the replacement of damaged natural marshes (Kadlec and Knight 1996, Sartoris et al. 2000). Constructed wetlands also provide secondary benefits such as wildlife habitat and recreational activities. Even though man-made wetlands provide many benefits, they may create a potentially serious public health threat when large populations of pathogen- transmitting mosquitoes are produced (Walton and Workman 1998, Walton 2002). Control efforts frequently are hampered by problems such as dense emergent vegetation (de Szalay et al. 1995, 1999; Walton et al. 1999a). Furthermore, applying chemical pesticides to constructed wetlands that provide water destined for public consumption is not desirable.

Immature mosquitoes show considerable selection for particular microhabitats in nature, and microhabitat/mosquito associations often differ among closely related species (Meyer and Durso 1998). Some species, such as the tule mosquito, *Culex erythrothorax* Dyar, exhibit an affinity for vertical structure such as dense stands of emergent vegetation (Workman and Walton 2000). Conversely, *Cx. tarsalis* Coquillett may become abundant in newly flooded areas before vegetation develops (Walton et al. 1990). *Culex quinquefasciatus* Say is numerous in highly eutrophic conditions such as water contaminated by sewage or cattle runoff (Mian et al. 1990). Wetlands support a mosaic of microhabitats allowing potentially species-rich mosquito communities to develop.

The increasing use of constructed wetlands in close proximity to dense human habitation creates a need for control efforts that focus on specific areas of a site that are most likely to produce problematic mosquito populations. Optimally, this would entail a significant reduction in mosquito breeding with concurrent savings in time, money, and equipment. In this study, we report the results of intensive larval and adult sampling of mosquitoes from a constructed treatment wetland in southern California. The goals of this study were to identify areas within this wetland that support high mosquito production and to examine the impact of wetland vegetation management practices on mosquito production.

MATERIALS AND METHODS

The Prado Constructed Wetland is located ~ 6.5 km NW of Corona (Riverside County) CA. The 186-ha wetland is operated by the Orange County Water District (OCWD) and consists of 46 marshes/ponds interconnected by water control structures (Fig. 1; Keiper et al. 1999, 2000). The primary function of the wetland is to remove nitrogen from the Santa Ana River. The wetland receives ~ one-half the flow (1.7-2.3 $m^3 \cdot s^{-1}$) of the Santa Ana River and moderate levels of nitrogen (NO_3-N: ~ 10.0 mg·L^{-1}; NH_4-N: ~ 0.2 mg·L^{-1}) loading. The nitrate concentration of water exiting the wetland is reduced to < 1 mg·L^{-1} by biological processes (OCWD 2001).

The components of the wetland can be categorized into 2 types based on vegetation cover and water depth: shallow marshes containing emergent vegetation and deep ponds that are devoid of emergent vegetation. Water depth ranges from ~ 0.5 m in the marshes at the inflow at the eastern end of the wetland to ~ 2 m in the deeper polishing ponds at the outflow on the western end of

[1] *Current address: Department of Invertebrate Zoology, Cleveland Museum of Natural History, Cleveland, OH 44106*

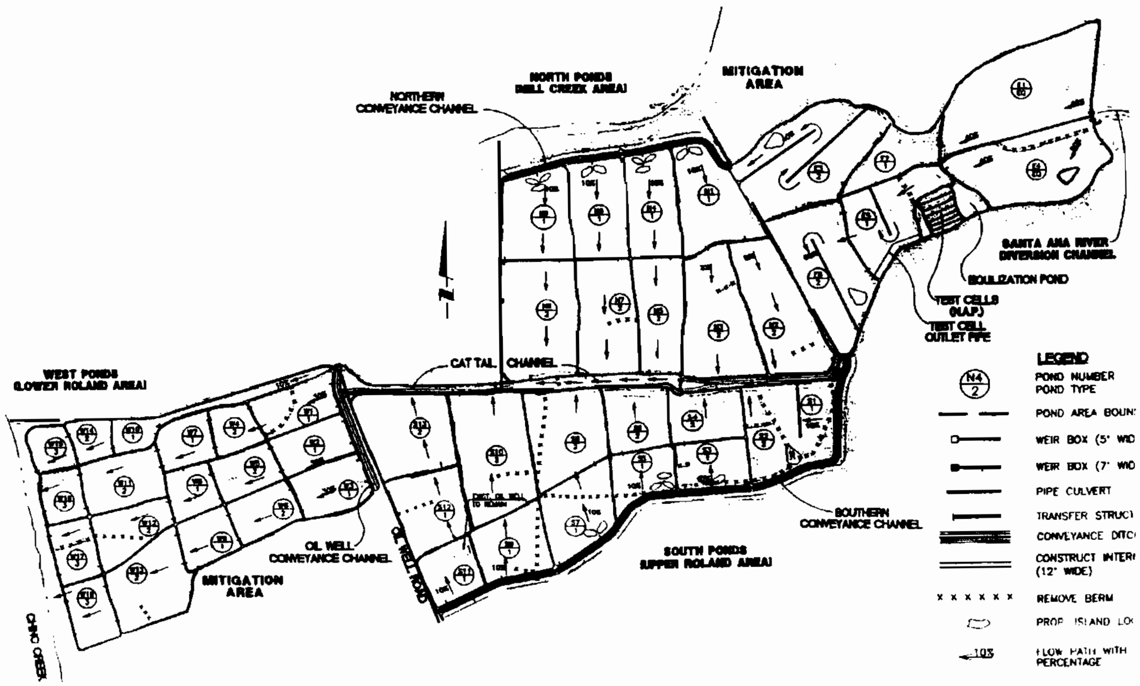


Figure 1. The 186 ha Prado Wetland, Corona, CA. Ponds are numbered within 4 areas designated by compass direction (N = north; E = east, W = west, S = south). Arrows indicate water flow.

the wetland. Marshes ≤ 1.0 m deep support emergent vegetation, most notably California bulrush (*Schoenoplectus* [=*Scirpus*] *californicus* [Meyer] Sojak), cattails (*Typha* sp.), buttercups (*Ranunculus flammula* var. *ovalis* [Biegel.]), water pennywort (*Hydrocotyl ranunculoides* L.), and smart weeds (*Polygonum* spp.); bulrush and cattails are the dominant plants. A ratio of ~ 50% vegetation to 50% open water is desired in the marshes, although rapid proliferation of macrophytes often generates proportionately greater vegetation coverage. Management of vegetation consists of draining ponds, felling the vegetation with a bulldozer or excavator, and reflooding. The wetlands are usually stocked with mosquitofish (*Gambusia affinis* Baird and Girard) several times per year by the Northwest Mosquito and Vector Control District (NWMVCD).

Host-seeking adults. An east-west transect of 5 CO_2-baited suction traps, without lights, (Model 912, J. W. Hock Co., Gainesville, FL) was positioned within the wetland from April 1999 - October 2000. Traps were placed near the inflow channel adjacent to pond E4 (Fig. 1), at the wetland research cells ~ 1/5 the distance across the wetland, adjacent to heavily vegetated ponds ~ 2/5 the distance across the wetland (near pond S6), at the transition between heavily vegetated and sparsely vegetated ponds at ~ 3/5 the distance across the wetland (near Pond S10), and between ponds W4 and W5 in the deep, non-vegetated polishing ponds ~ 4/5 the distance across the wetland. Each trap was hung ~ 0.75 m above the top of the berms using a tripod. Traps were run for 24 h approximately weekly from May - September, and biweekly or monthly during periods of lower host-seeking activity between October and April. The mean catch for the 5 traps was calculated for each sampling date.

Larval sampling: routine and basin-wide surveys. During 1998 and 1999, 52 and 36 permanent larval sampling stations were established, respectively, within emergent vegetation. At each station, 3 samples were taken with a standard 350-ml dipper, combined in a concentrator cup, and placed in a vial with 95% ethanol; the water included with the sample diluted the ethanol to ~ 50%. Twenty-four (1998) and 16 (1999) stations were established in stands of cattails, one each at the periphery and 3-5 m within the stands. Twenty-two (1998) and 16 (1999) were established within bulrush, half at the periphery and half at 2-3 m within the stands. Six (1998) and four (1999) additional stations were positioned within dense growths of buttercups. Samples were taken every 1-2 wk from July - October 1998 and every 2-3 wk from May - September 1999.

Fish and macroinvertebrates were sampled with a D-frame aquatic net along a 2-m transect at each dip station. Macroinvertebrates were identified using Merritt and Cummins (1996).

Four dip surveys across the entire wetlands were conducted between May and August 1999. Sample locations were representative of as many larval mosquito microhabitats as possible, including shoreline, emergent plant, and submerged macrophyte areas. Three samples were taken at each station with a standard 350-ml dipper, combined in a concentrator cup, and the number of *Culex* and *Anopheles* larvae obtained, were enumerated and recorded. If a sample contained too many immature mosquitoes to count in the field, the sample was preserved with 95% ethanol and returned to the laboratory for enumeration.

Larval sampling: effects of vegetation management. Two larval mosquito surveys were carried out in conjunction with vegetation management performed by the OCWD. During August 1999, two vegetation management practices were carried out in three 3- to 5-ha marshes. The marshes were drained, dried for several weeks and the macrophytes (primarily *Typha* sp.) were knocked down using a bulldozer. The knocked down vegetation was then either graded by removing the vegetation and the top 31-61 cm of the pond bottom (pond N2) or left intact and inundated. The vegetation coverage in the latter treatment was either 50% (pond N3) or 90% (pond N1) coverage prior to vegetation management. Mosquito abundance was assessed by taking 36 350-ml dips in each pond on 4 dates between August 23 and November 1.

During April 2000, OCWD personnel drained 5 marshes and knocked down the vegetation prior to reflooding. To provide a quantitative comparison of the larval production in newly manipulated / managed vs. older established marshes, dips were collected at the margins of the 5 newly flooded marshes (ponds E1, N6, N7, S3, S4: Fig. 1) and 5 adjacent older marshes (ponds E4, N8, N9, S7, S8) that served as controls. In each marsh, composite dip samples (three 350-ml dips) were taken at each of 12 stations along the margins of each marsh. Fluctuating water levels and/or the development of dense vegetation occasionally caused us to reduce the number of samples taken. Sampling was conducted at 2- to 3-wk intervals from April - June.

During both surveys, mosquito abatement in the study ponds was carried out by the NWMVCD if mosquito abundance was high enough to warrant concern. VectoLex (*Bacillus sphaericus* Neide) was applied at a rate of 5.60-11.25 kg·ha^{-1} on 3 dates during autumn 1999 to the ponds containing 50% and 90% vegetation coverage and on 25 April, 16 May, and 13 June 2000 to the newly flooded marshes.

Adult emergence. From May - September 1999, one 0.25-m^2 pyramidal emergence trap (Walton et al. 1999b) was deployed biweekly at each of the 36 permanent larval dipping stations. Each trap was placed over emergent vegetation that was cut with shears to 4-6 cm above the water surface so that the physical structure of the plants remained intact below water. Capture jars were placed on top of each trap and collected after 5 d.

Voucher specimens for invertebrates collected during this study were deposited at the Entomology Research Museum of the University of California, Riverside, and at the Cleveland Museum of Natural History.

Data analysis. Routine larval sampling produced too few individuals for statistical analysis of the effects of vegetation type on larval abundance. These data were combined and the abundance per dip was calculated and graphed to illustrate year to year differences in abundance. Similarly, few emergent mosquitoes were produced during routine sampling, therefore data for emergent adults were combined and a *Z*-test performed to determine if the proportion of adults produced in cattail vs. bulrush central and peripheral areas was statistically different.

The effects of vegetation management on larval mosquito abundance were analyzed by ANOVA. If data were not normally distributed, they were $\log_{10}(n+1)$ transformed prior to analysis. Transformation did not improve the normality of the distribution for *Cx. tarsalis* data, therefore individual Mann-Whitney Rank Sum Tests were used; a p value of ≤ 0.008 (i.e., 0.05/6) was used to correct for comparison-wise error.

RESULTS

Host-seeking adults. Host-seeking mosquito abundance increased annually as emergent vegetation coverage increased throughout the wetland. Mean host-seeking mosquito abundance was consistently low (<200 females·trap^{-1}·night^{-1}) during 1999 and increased about 2.5-fold during 2000. *Culex erythrothorax* was the dominant mosquito collected in CO_2-baited suction trap catches during 1999 – 2000 (Fig. 2), comprising 70% and 86% of the annual collections, respectively. The summer 2000 *Cx. erythrothorax* host-seeking population was 3 times larger than during summer 1999.

Culex tarsalis host-seeking female abundance was low during the summer and increased during late summer in both years (Fig. 2). This species also exhibited a vernal peak of host-seeking females in 2000. Unlike *Cx. erythrothorax* populations which increased over time, *Cx. tarsalis* catches did not increase appreciably and were ~ 3- to 13-fold lower than *Cx. erythrothorax*. *Culex tarsalis* was 13% and 9% of the host-seeking females collected annually in 1999 and 2000, respectively.

Culex stigmatosoma Dyar, *Cx. quinquefasciatus* and *Culiseta* spp. were collected less frequently than *Cx. tarsalis*. *Culex stigmatosoma* was < 1% of the host-seeking populations. *Culex quinquefasciatus* was 8% and 2% of the yearly host-seeking populations. The numbers of *Cx. quinquefascistus* collected in CO_2-baited CDC traps increased markedly during September and October in both years. *Culiseta particeps* (Adams) and *Cs. inornata* (Williston) were rare (<0.5%) in CDC trap collections. *Anopheles hermsi* Barr and Guptavanji abundance was typically < 5 host-seeking females·trap^{-1}·night^{-1} during spring and summer (Fig. 2). This species was 7% and 3% of the host-seeking populations in 1999 and 2000, respectively. The annual trend for host-seeking female catches was similar to that observed for *Cx. tarsalis*; a peak in *An. hermsi* numbers occurred in June 2000 and in both years during late September.

Established marshes. Larval mosquito sampling in established marshes (ca. 1-2 years old) produced < 1 larva·dip^{-1} (Fig. 3) during both 1998 and 1999. No more than 4 immature mosquitoes were taken per sampling date in 1998. Numbers increased as much as 10-fold in 1999, with peaks in June and late August. *Anopheles hermsi* was the most abundant species while *Cx. tarsalis* was the next most abundant species.

Only 42 adults were captured in emergence traps placed in established marshes during 1999 with a maximum of 1.22 emergent adults·m^{-2} recorded in mid June. The percentage of emerging adults taken at the periphery (23%) and center (22%) of bulrush stands was similar, while sampling in cattails captured emergent mosquitoes in central areas only (55% of emerging mosquitoes). Overall, proportionately more adults were taken in central areas of those plant stands (Z=6.93, p<0.001). A similar trend was observed for larval mosquitoes collected in the center vs. edge of emergent macrophyte stands (Fig. 4A).

Coexisting macroinvertebrates comprised mostly of collector/ gatherers (Fig. 4B), which were predominantly water boatmen

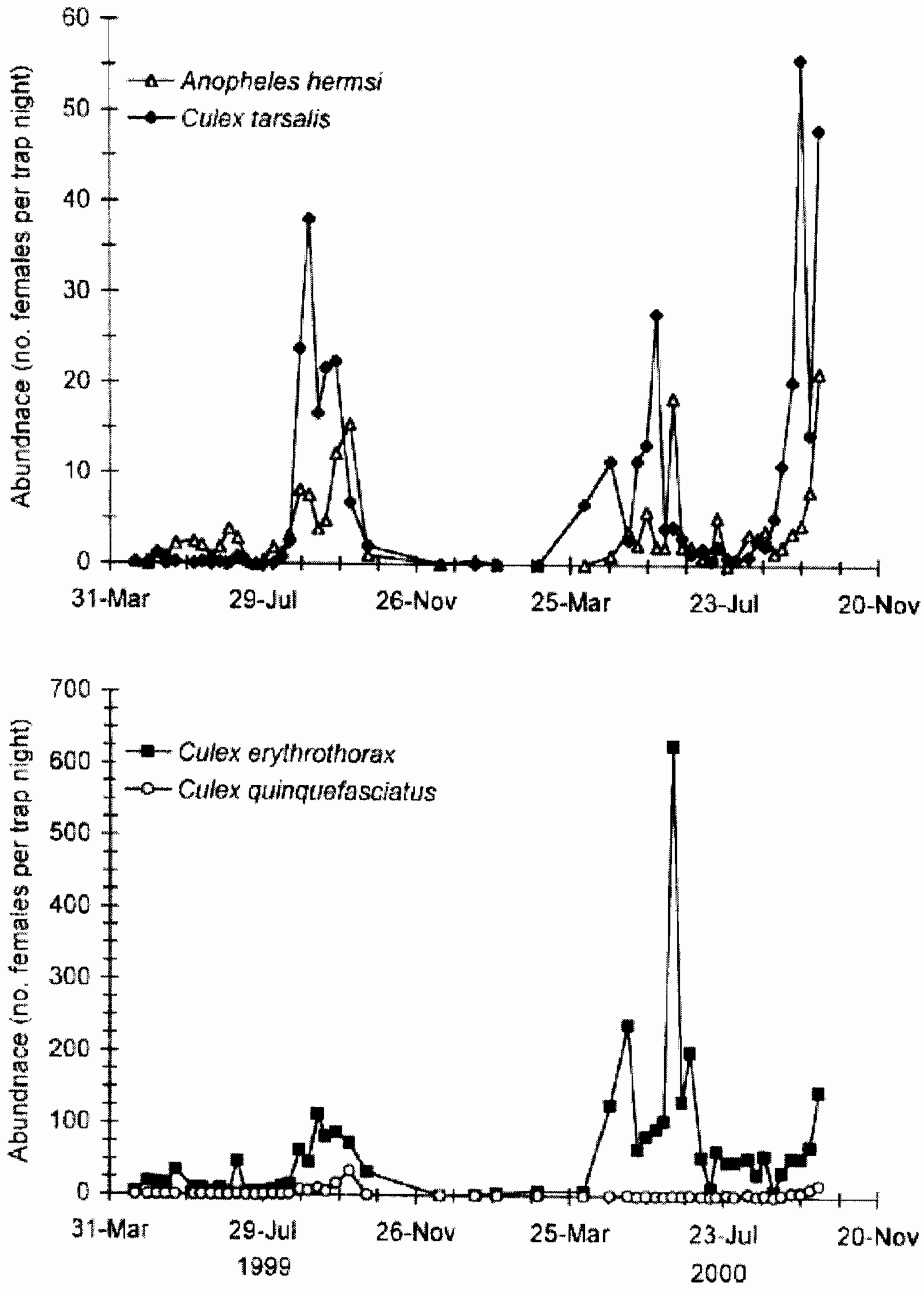


Figure 2. Mean abundance of host-seeking females of 4 mosquito species in CO_2–baited suction traps at the Prado Constructed Wetlands during 1999-2000.

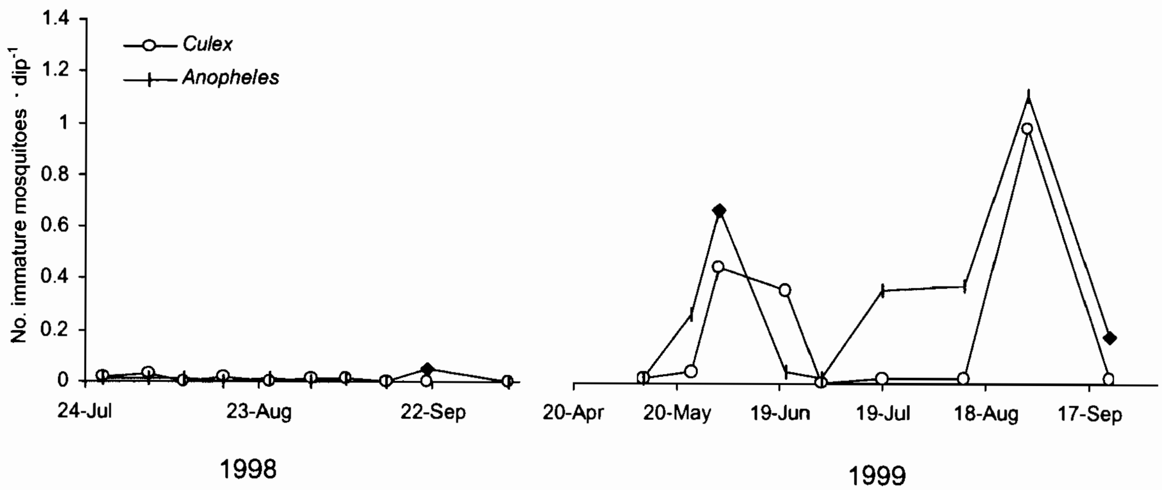
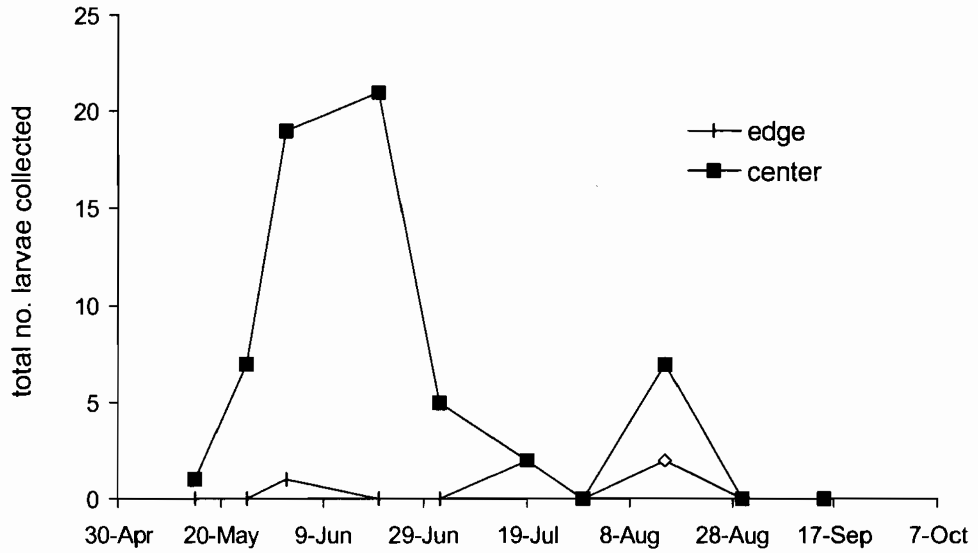


Figure 3. Number of immature *Culex* and *Anopheles* larvae per dip taken in established marshes of the Prado Constructed Wetlands from 1998-1999.

A.



B.

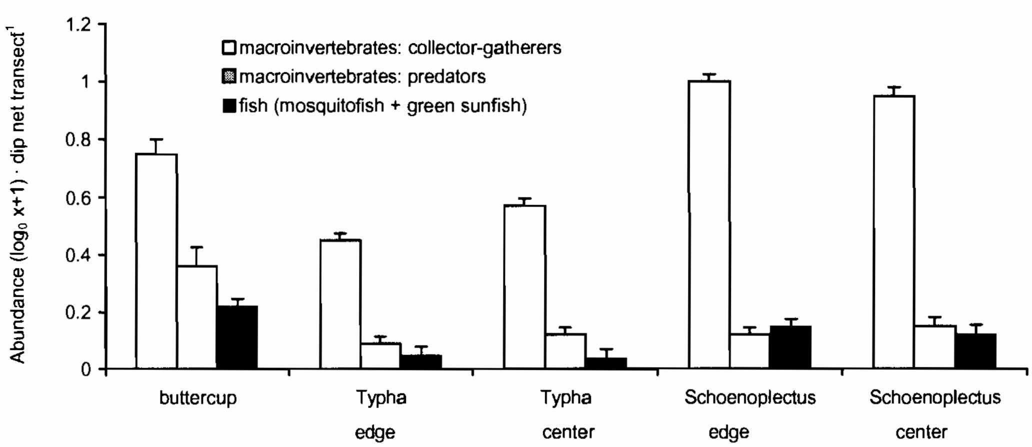


Figure 4. Total number of larvae collected at edge and center positions of emergent macrophytes (*Schoenoplectus* and *Typha*) during 1999 (A) and mean abundance of macroinvertebrates (collector-gatherers and predators) and mosquitofish collected per aquatic dip net sweep in buttercups, bulrush and cattails during 1998-1999 (B). Mean ± SE are shown.

(Hemiptera: Corixidae: *Corisella* sp.) and amphipod crustaceans (Hyallelidae: *Hyallela azteca* (Saussure)). More collector/gatherers were encountered in bulrush than peripheral areas of cattails ($H_4 = 17.83, p = 0.001$), while numbers in central areas of cattails and buttercups were similar to all other locations. Predators, mostly Odonata nymphs, were most abundant in the dense mats of buttercups, but this difference was significant only when compared to peripheral areas of bulrush and cattail stands ($H_4 = 12.71, p = 0.013$). Fish were equally abundant in buttercups and all areas of bulrush, and were in lowest abundance at central areas of cattail stands ($H_4 = 17.92, p = 0.001$).

Mosquito larvae were similarly rare in established marshes across the wetlands. Abundance in the 1999 basin-wide surveys was always < 1 larva·dip^{-1} (Table 1). The combined total for the 4 survey dates was 92 larvae taken in 587 samples (3 dips per sample; 0.16 larvae·sample^{-1}). The majority of *Culex* larvae were taken in late June while the peak in *Anopheles* occurred in late July. Pupae were taken only rarely.

Effects of vegetation management. During autumn 1999, the largest number of mosquito larvae was collected from the pond (N1) that previously supported 90% vegetation coverage and the dead, knocked down vegetation was inundated (Fig. 5). Larval mosquito abundance in this pond was initially 17 times greater than in the graded pond (N2). Larval mosquito abundance in the pond (N3) that previously supported 50% vegetation coverage was 5.5 times greater than in the graded pond soon after flooding. Larval mosquito abundance differed significantly among sampling dates ($F_{3,99} = 4.21, p = 0.008$) and declined following *B. sphaericus* treatments. Larval mosquito abundance did not differ significantly among the 3 vegetation management treatments across the entire period of the study ($F_{2,33} = 2.94, p = 0.067$); however, the treatment by date interaction was significant ($F_{6,99} = 2.60, p = 0.022$) with larval mosquito abundance in ponds containing knocked down vegetation supporting significantly larger mosquito populations prior to larvicide applications (Tukey tests for date within treatment comparisons, $p < 0.05$).

During spring 2000, *Culex tarsalis* was the most numerous

Table 1. Number of immatures ·collected during basin-wide dip surveys of the Prado Wetland, 1999.

	Culex	*Anopheles*	pupae	No. dips	Immatures·dip^{-1}
8 May	0	0	1	162	<0.01
29 June	28	13	4	180	0.25
27 July	11	25	1	153	0.24
24 August	0	9	0	92	0.10

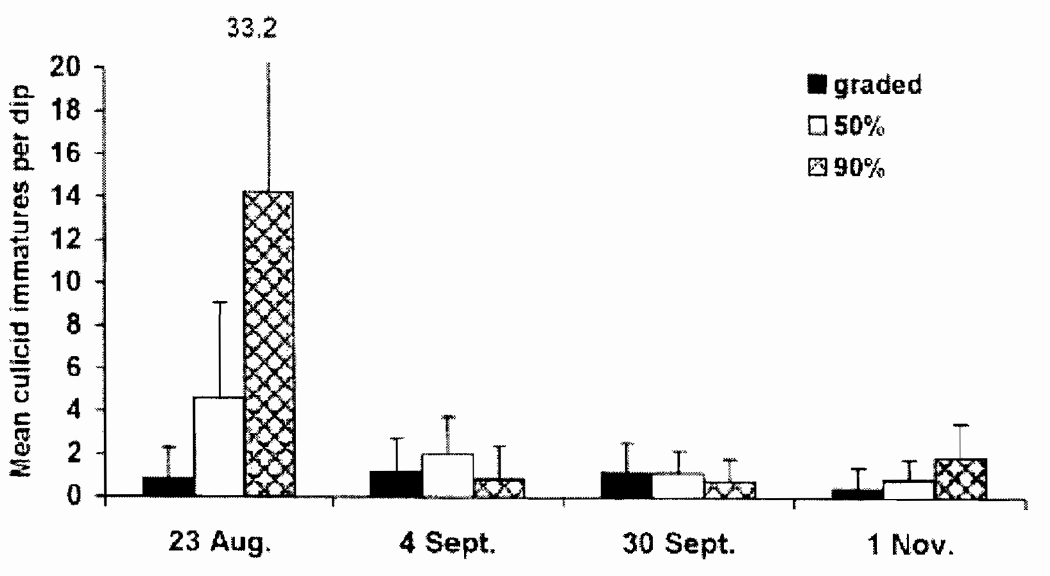


Figure 5. Mean abundance (± SD) of *Culex* larvae in ponds after vegetation maintenance. After draining and drying the ponds, vegetation was either removed by grading or left intact and flooded. Coverage in the two ponds for which the vegetation was left intact was 50% and 90%.

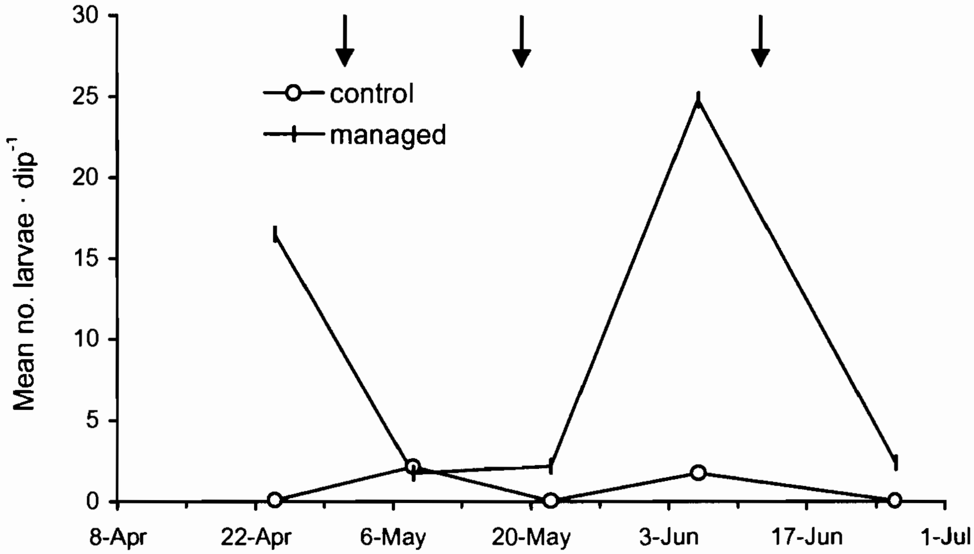


Figure 6. Mean abundance of late-stage *Culex* larvae in marshes following the knock down of vegetation (managed) and older, established marshes that received no vegetation manipulation (controls). Arrows indicate *Bacillus sphaericus* addition.

species obtained during the comparison of newly flooded versus established marshes, with a mean of > 10 3rd- and 4th-stage larvae·dip^{-1} taken in early June. Roughly 35 1st- and 2nd-stage *Culex* larvae·dip^{-1} were collected. Early instars peaked in early June, and significantly more larvae were taken from newly flooded marshes compared to controls ($F_{1,90} = 4.01, p = 0.05$). A similar pattern for late-stage *Cx. tarsalis* larval abundance was found ($H_{90} = 660.0, p < 0.001$). *Culex tarsalis* and early-stage *Culex* numbers were at their greatest in early June, between the second and third application of *B. sphaericus*.

Culex quinquefasciatus, Cx. stigmatosoma, and *Cx. restuans* Theobald larval populations, as well as *Culex* pupae, were at their highest on the first sampling date after reflooding. However, no significant differences were found for any of these taxa due to the large amount of variation among dip samples. *Culex quinquefasciatus* was taken only rarely after the first sampling date. *Cx. stigmatosoma* was rare throughout the study and *Cx. restuans* was taken sporadically during May and early June. The early applications of *B. sphaericus* coincided with the drop in abundance of these species (Fig. 6).

Anopheles hermsi was collected sporadically during the 2-month study and abundance in the newly flooded marshes increased as the ponds aged. Overall, more *An. hermsi* larvae were found in the newly flooded marshes than in the control marshes ($F_{1,90} = 4.83, p = 0.03$). The application of *B. sphaericus* did not coincide with reductions in the *An. hermsi* larval populations.

DISCUSSION

Mosquito production at the Prado Constructed Wetland never reached the numbers observed at other constructed treatment wetlands where excessive mosquito populations developed (Walton and Workman 1998, Walton 2002); however, host-seeking mosquito and larval mosquito numbers showed increasing trends at the Prado Wetland. Keiper et al. (1999) reported that although the production of aquatic insects was generally high within the Prado Constructed Wetland during 1998, mosquito production was negligible, with a maximum of 0.67 emergent adults·m^{-2}. During 2000, mosquito production from undisturbed areas (i.e., marshes that have not undergone a draining and reflooding cycle) remained low (maximum = 1.22 emergent adults·m^{-2}). Host-seeking mosquito catches increased each year following 1998 (cf. Keiper et al. 1999) and nearly 3-fold from 1999 to 2000. A nearly 10-fold increase in larval numbers collected from established marshes occurred between 1998 and 1999.

A shift in the species composition of the host-seeking populations occurred since we initiated studies of the Prado Constructed Wetland in 1998 (Keiper et al. 1999) and is associated with increases in vegetation coverage during aging of the marshes. The emergent vegetation in the wetland was reduced appreciably by scouring during high discharge from severe vernal storms in winter 1998, and these harsh disturbances reverted the Prado Basin to an earlier successional state (Keiper et al. 1999). During the 3 years after severe scouring of the wetland, vegetation coverage increased and *Cx. erythrothorax* increased proportionately in CDC trap collections.

A change in wetland management procedures also caused a change in mosquito species composition in the Prado Basin. Prior to management as a continuously flooded wetland for water quality improvement, the Prado Basin was used for recreational duck hunting during autumn and the seasonal flooding of wetlands in the basin produced equal proportions of *Cx. quinquefasciatus*, *Cx. tarsalis* and *Cx. erythrothorax* (Mian et al. 1990). While movement of *Cx. quinquefasciatus* adults produced in dairy wastewater lagoons on the north side of the wetland might have had a small influence on host-seeking collections at the Prado Wetland, production of this mosquito from within the Prado Wetland is supported by oviposition studies that showed that egg laying *Cx. quinquefasciatus* females were attracted to field-collected water from the Prado Basin (Beehler and Mulla 1993) and by the presence of larvae of this species during the present study. Keiper et al. (1999) found that *Cx. quinquefasciatus* continued to be the dominant host-seeking mosquito during 1998 in collections around the periphery and inflow area of the Prado Wetland. *Culex quinquefasciatus* was an uncommon component of our sampling during the current study and larvae were found only during reflooding of marshes that had undergone vegetation management.

Host-seeking *Cx. tarsalis* catches increased as the wetland aged; however, management practices within the Prado Wetland and at adjacent wetlands were associated with increased abundance of this mosquito. The abundance of host-seeking *Cx. tarsalis* during spring 1999 and summers of 1999 and 2000 was low (< 5 females$\cdot$trap$^{-1}\cdot$night^{-1}). Larval mosquito abundance in basin-wide surveys and vegetation management studies suggests that *Cx. tarsalis* production from the continuously flooded wetland remained low during late summer-early autumn. The spring 2000 and late summer peaks in host-seeking females were associated with vegetation management in the Prado Wetland and water management in adjacent duck hunting clubs.

Peripheral areas of *Typha* growth tend to support very few plant stems per unit area (unpublished data) and are less productive habitats for mosquitoes than are habitats 3-5 m into vegetation stands. No emergent mosquitoes were taken at the periphery of cattail stands and the abundance of other macroinvertebrates was also comparatively low. Buttercups did not produce emergent mosquitoes, but predaceous invertebrates and mosquitofish were most abundant within the tangled mats of this emergent macrophyte. Bulrush tends to grow in uniformly dense stands with little differentiation between peripheral and central areas (personal observation); no significant differences were found for macroinvertebrate, fish, or mosquito abundance in these two microhabitats. Densely vegetated, central areas of plant stands produced 77% of all emergent mosquitoes. Collins and Resh (1989) suggested that immature mosquitoes deep in plant coverage were less susceptible to predation by mosquitofish, and this may explain why more adults were produced in cryptic areas such as deep within *Typha* stands.

Newly flooded habitats have greater mosquito production than do older, established habitats (Fanara and Mulla 1974, Walton et al. 1990). This was true in the Prado Constructed Wetland, and creates potential problems for vegetation management practices that use a draining-vegetation knockdown-reflooding cycle. The current vegetation management protocol does not include vegetation removal because the dead plants provide a carbon source to bacteria necessary for nitrogen conversion upon reflooding (B. Baharie, pers. comm.). Whereas vegetation management practices in this functional treatment wetland were not always amenable to replicated experimental studies, the autumn 1999 study indicated that the extent of inundated, knocked down vegetation was directly related to mosquito production. Because the treatments were not replicated, these results are suggestive of the aforementioned effect but pond-specific effects unrelated to the vegetation treatment cannot be conclusively ruled out.
Marshes undergoing vegetation management in the Prado Wetland will require larvicidal treatment shortly after inundation to suppress early colonizing mosquito species, and multiple abatement treatments during the 3-7 week post-inundation period to target *Cx. tarsalis*. Layers of fallen vegetation shaded pools slowing evaporation and caused mosquito production to occur in concealed areas which are difficult to treat with conventional methods such as bacterial insecticides. When flooding commenced, *Cx. quinquefasciatus, Cx. stigmatosoma,* and *Cx. restuans* represented the first wave of problematic mosquito populations at the Prado Wetland. *Culex tarsalis* production peaked at approximately 7 weeks after inundation. However, this peak occurred during the longest interval between *B. sphaericus* applications in spring 2000 and indicates that the ponds which had undergone vegetation management were still attractive development sites for mosquitoes at least two months after reflooding.

Acknowledgments

This study was funded through the Research Foundation of the Mosquito and Vector Control Association of California. We gratefully acknowledge the OCWD and the NWMVCD for assistance, especially Brian Baharie, Jeff Beehler, and Major Dhillon. Karrie Chan, L. Hannah Gould, Margaret Wirth, and Parker Workman provided help in the laboratory and field. We thank Ferenc de Szalay and Ben Foote for useful discussions and information.

REFERENCES CITED

Beehler, J. W., and M. S. Mulla. 1993. The effect of organic enrichment and flooding duration on the oviposition behavior of *Culex* mosquitoes. Proc. California Mosq. Vector Control Assoc. 61: 121-124.

Collins, J. N., and V. H. Resh. 1989. Guidelines for the Ecological Control of Mosquitoes in Non-tidal Wetlands of the San Francisco Bay Area. California Mosquito and Vector Control Association, Inc., Sacramento, CA.

de Szalay, F. A., D. P. Batzer, E. B. Schlossberg, and V. H. Resh. 1995. A comparison of small and large scale experiments examining the effects of wetland management practices on mosquito densities. Proc. California Mosq. Vector Control Assoc. 63: 86-90.

de Szalay, F. A., N. H. Euliss, and D. P. Batzer. 1999. Invertebrate community ecology and responses to management methods, pp. 829-855. *In* D. P. Batzer, R. B. Rader, and S. A. Wissinger [eds], Invertebrates in Freshwater Wetlands of North America: Ecology and Management. John Wiley and Sons, Inc., New York, N.Y.

Kadlec, R., and R. L. Knight. 1996. Treatment Wetlands. CRC Press, Boca Raton, FL.

Keiper, J. B., J. Jiannino, M. Sanford, and W. E. Walton. 1999. Distribution and abundance of Culicidae and Chironomidae following storm damage in a southern California constructed wetlands. Proc. California Mosq. Vector Control Assoc. 67: 47-54.

Keiper, J. B., M. Sanford, J. Jiannino, and W. E. Walton. 2000. Invertebrates colonizing wetland monocots damaged by lepidopteran herbivory. Entomol. News 111: 348-354.

Merritt, R. W., and K. W. Cummins. 1996. An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing, Dubuque, IA.

Meyer, R. P., and S. L. Durso. 1998. Identification of the Mosquitoes of California. Mosquito and Vector Control Association of California. Sacramento, CA.

Mian, L. S., M. S. Mulla, H. Axelrod, J. H. Chaney, and M. S. Dhillon. 1990. Studies on the bioecological aspects of adult mosquitoes in the Prado Basin of southern California. J. Amer. Mosquito Control Assoc. 6: 64-71.

Orange County Water District (OCWD). 2001. Prado Wetland. OCWD Online. http://www.ocwd.com/_html/prado.htm (updated: May 31, 2001).

Sartoris, J. J., J. S. Thullen, L. B. Barber, and D. E. Salas. 2000. Investigation of nitrogen transformations in a southern California constructed wastewater treatment wetlands. Ecol. Eng. 14: 49-65.

Schlossberg, E. B., and V. H. Resh. 1997. Mosquito control and waterfowl habitat enhancement by vegetation manipulation and water management: a two year study. Proc. California Mosq. Vector Control Assoc. 65: 11-15.

Walton, W. E. 2002. Multipurpose constructed treatment wetlands in the arid southwestern United States: Are the benefits worth the risks? pp. 115-123. *In*: J. Pries (ed.) Constructed Wetlands for Water Quality Improvement: Quebec 2000 Conference Proceedings. CH2MHILL Canada Limited, Pandora Press, Waterloo, ON.

Walton, W. E., K. Chan, L. H. Gould, and J. B. Keiper. 1999a. Mosquito production from three wetland management practices for constructed treatment wetlands: preliminary findings. Proc. California Mosq. Vector Control Assoc. 67: 18-21.

Walton, W. E., N. S. Tietze, and M. S. Mulla. 1990. Ecology of *Culex tarsalis* (Diptera: Culicidae): factors influencing larval abundance in mesocosms in southern California. J. Med. Entomol. 27: 57-67.

Walton, W. E., and P. D. Workman. 1998. Effect of marsh design on the abundance of mosquitoes in experimental constructed wetlands in southern California. J. Amer. Mosquito Control Assoc. 14: 95-107.

Walton, W. E., P. D. Workman, and J. B. Keiper. 1999b. An inexpensive collapsible pyramidal emergence trap for the assessment of wetland insect populations. Proc. California Mosq. Vector Control Assoc. 67: 15-17.

Workman, P. D., and W. E. Walton. 2000. Emergence patterns of *Culex* mosquitoes at an experimental constructed treatment wetland in southern California. J. Amer. Mosquito Control Assoc. 16: 124-130.

Surveillance for Mosquito-Borne Encephalitis Virus Activity and Human Disease in California, 2001

Stan Husted, Ashley B. Houchin, Vicki L. Kramer, Robert E. Chiles[1], Michele Jay, William K. Reisen[1], Bruce F. Eldridge[2], Carol Glaser, Cindi Cossen, Evelyn Tu, William C. Reeves[3], Thomas W. Scott[1], Martin Castro, Aline Cornelius, and Malcolm Thompson

Division of Communicable Disease Control, California Department of Health Services, 601 North 7th Street, M/S 486, P. O. Box 942732, Sacramento, CA 94234-7320
[1]Davis Arbovirus Research Unit, Center for Vectorborne Diseases, University of California, Davis, CA 95616
[2]Department of Entomology, University of California, Davis, CA 95616
[3]School of Public Health, University of California, Berkeley, CA 94720

ABSTRACT: The California surveillance program for mosquito-borne encephalitis virus activity tested humans, equids, mosquitoes, sentinel chickens and dead birds to detect virus activity. Adult mosquito abundance monitored by New Jersey light traps was reported to CDHS. In 2001, 49 local agencies in California maintained 194 sentinel chicken flocks. There were 62 seroconversions to St. Louis encephalitis virus (SLE) and three seroconversions to western equine encephalomyelitis virus (WEE). Twenty-six agencies submitted 3,501 mosquito pools; 70 pools were positive for SLE and 9 pools positive for California encephalitis virus (CE). There were 210 suspected human cases of aseptic meningitis/encephalitis tested for SLE and/or WEE with 167 also tested for West Nile virus (WN) infection; all tests were negative. Thirteen equids with suspect viral encephalitis were tested for mosquito-borne encephalitis virus; all were negative. Of the 68 dead birds reported to CDHS, 18 were tested for WN, and all were negative.

The California Mosquito-Borne Encephalitis Surveillance Program is a cooperative effort of the California Department of Health Services Division of Communicable Disease Control (CDHS), the University of California at Davis and Berkeley, the Mosquito and Vector Control Association of California, local mosquito and vector control agencies, local health departments, physicians, veterinarians, and other interested parties. Collaborating agencies in the West Nile virus (WN) surveillance program include the California Department of Food and Agriculture (CDFA), California Animal Health and Food Safety Laboratory (CAHFS), California Department of Fish and Game (CDFG), the U.S. Fish and Wildlife Service (USFWS), and the Centers for Disease Control and Prevention (CDC).

The program included the following components:

1) Diagnostic testing of specimens from human patients exhibiting symptoms of viral meningitis or encephalitis.
2) Enrollment of patients diagnosed with encephalitis into the California Encephalitis Project (CEP) which evaluates demographics, exposure to arthropods, and laboratory analyses to determine etiology.
3) Diagnostic testing of specimens from equids that exhibited clinical signs of viral neurologic disease compatible with arboviral infection (western equine encephalomyelitis virus (WEE), WN, and other arboviruses as appropriate).
4) Monitoring and testing of mosquitoes for the presence of St. Louis encephalitis virus (SLE) and WEE. Pools presumptively positive for SLE were confirmed by additional serological and molecular methods. A limited number of pools were tested for California serogroups, dengue, and other arboviruses.
5) Serological monitoring of sentinel chickens for SLE and WEE antibodies in areas of California where evidence of encephalitis virus activity has occurred historically. Chicken sera from geographic areas where SLE seroconversions occurred and specimens from other regions were also tested for WN.
6) Surveillance and diagnostic testing of dead birds, especially crows, for WN.
7) Weekly reporting in the CDHS arbovirus surveillance bulletin of the arbovirus testing results in California and arbovirus activity throughout the United States.

HUMAN DISEASE SURVEILLANCE

The CDHS Viral and Rickettsial Disease Laboratory (VRDL) tested sera and/or cerebrospinal fluid specimens from 210 patients exhibiting symptoms of viral meningitis or encephalitis for antibodies to SLE and WEE. Neither elevated IgM antibody nor a 4-fold rise in total antibody titer between paired sera was observed in any of the suspect cases.

Of the 210 patients tested, 167 were enrolled in the CEP. For each patient enrolled, a battery of tests was conducted, including reverse transcriptase-polymerase chain reaction (RT-PCR), serology, and viral isolation for 15 agents. Testing for additional etiologic agents was pursued as clinical symptomatology and exposure history warranted it; extensive testing for arboviruses was conducted for cases with known mosquito exposure and those with a travel history to an area of WN activity. No cases of St.

Table 1. Participation by local agencies in the mosquito-borne encephalitis surveillance program, 2001.

County	Agency	Agency Code	New Jersey Light Trap	Number of Light Traps	Mosquito Pools	No. Flocks	No. Chickens	No. Sera Samples Tested
Alameda	Alameda Co. MAD	ALCO	X	7		3	19	273
Butte	Butte Co. MVCD	BUCO	X	25	X	7	91	1187
Colusa	Colusa MAD	CLSA	X	3		1	9	130
Contra Costa	Contra Costa MVCD	CNTR	X	18	X	3	30	390
Fresno	Consolidated MAD	CNSL	X	12		4	40	480
Fresno	Fresno MVCD	FRNO	X	9	X	2	20	260
Fresno	Fresno Westside MAD	FRWS	X	10	X	2	15	260
Glenn	Glenn Co. MVCD	GLEN	X	4	X	1	13	156
Imperial	Imperial Co. Environmental Health	IMPR				2	24	157
Inyo	Owens Valley MAP	OWVY	X	10		0	0	0
Kern	Delano MAD	DLNO	X	8		2	20	200
Kern	Kern MVCD	KERN	X	20	X	9	90	1260
Kern	Westside MVCD	WEST	X	12		3	30	330
Kings	Kings MAD	KNGS	X	9	X	3	29	390
Lake	Lake Co. VCD	LAKE			X	2	19	200
Los Angeles	Antelope Valley MVCD	ANTV	X	9		5	35	455
Los Angeles	Greater Los Angeles Co. VCD	GRLA	X	14	X	4	38	666
Los Angeles	Long Beach Environmental Health	LONG			X	2	20	280
Los Angeles	Los Angeles Co. West VCD	LACW				18	99	432
Los Angeles	San Gabriel Valley MVCD	SGVA				10	60	820
Madera	Madera Co. MVCD	MADR	X	5	X	2	20	240
Marin/Sonoma	Marin-Sonoma MVCD	MARN	X	33		7	75	1078
Merced	Merced Co. MAD	MERC				6	36	390
Monterey	North Salinas MAD	NSAL	X	18		1	10	120
Napa	Napa MAD	NAPA	X	12		2	10	195
Orange	Orange Co. VCD	ORCO			X	1	7	170
Placer	Placer Co. VCD	PLCR	X	4	X	1	10	120
Riverside	Coachella Valley MVCD	COAV	X	24	X	9	90	1260
Riverside	Northwest MVCD	NWST	X	12	X	6	48	624
Riverside	Riverside Co. Environmental Health	RIVR	X	13		6	68	924
Sacramento/Yolo	Sacramento-Yolo MVCD	SAYO	X	54	X	9	80	1077
San Bernardino	San Bernardino Co. VCP	SANB	X	19	X	7	70	1050
San Bernardino	West Valley MVCD	WVAL				3	29	420
San Diego	San Diego Co. Dept of Health	SAND	X	10	X	3	30	520
San Joaquin	San Joaquin Co. MVCD	SJCM	X	44	X	3	36	630
San Mateo	San Mateo Co. MAD	SANM	X	20		2	20	390
Santa Barbara	Santa Barbara Coastal VCD	SBCO			X	4	36	300
Santa Clara	Santa Clara Co. VCD	STCL	X	14	X	2	19	260
Santa Cruz	Santa Cruz Co. MVCD	SCRZ	X	7		1	9	130
Shasta	Burney Basin MAD	BURN	X	6		2	17	200
Shasta	Shasta MVCD	SHAS	X	18	X	5	54	605
Solano	Solano Co. MAD	SOLA	X	23		2	21	264
Stanislaus	East Side MAD	EAST				1	11	140
Stanislaus	Turlock MAD	TRLK	X	21	X	4	47	528
Sutter/Yuba	Sutter-Yuba MVCD	SUYA	X	38	X	7	70	960
Tehama	Tehama Co. MVCD	TEHA	X	9		2	20	240
Tulare	Delta VCD	DLTA	X	12	X	6	62	780
Tulare	Tulare MAD	TRLE	X	10		2	20	260
Ventura	City of Moorpark	MOOR	X	4		1	10	150
Ventura	Ventura Co. Environmental Health	VENT	X	18	X	4	40	560
Total			40	618	26	194	1,776	22,911

Source: California Department of Health Services

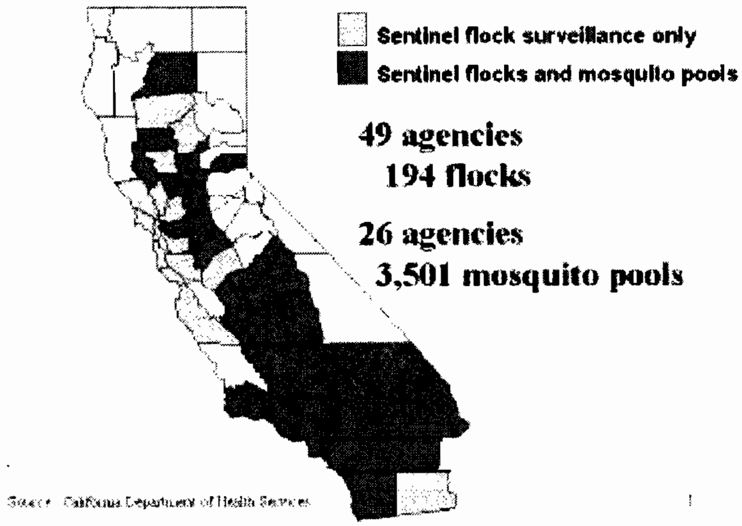


Figure 1. Counties which submitted chicken sera and/or mosquito pools for SLE, WEE, WN, and CE testing, 2001.

Louis encephalitis or western equine encephalomyelitis were identified through the CEP. Assays for antibody to WN were performed for all patients enrolled in 2001 which included testing by the UC Davis Arbovirus Research Unit (DARU) by plaque reduction neutralization (PRNT). Of these patients, 28 had recent mosquito exposure and six had traveled to the eastern United States within the incubation time of WNV consistent with arboviruses. No cases of WNV were identified.

EQUINE SURVEILLANCE

Serum and brain tissue specimens from 13 horses displaying neurological signs were submitted for arboviral testing to DARU. Testing failed to detect antigen or antibody for WEE or WN.

ADULT MOSQUITO SURVEILLANCE

Forty local agencies from 32 counties initiated weekly adult mosquito collection reports in April 2001 using a total of 618 New Jersey light traps (NJLT) statewide (Table 1). Data from these sources were forwarded to CDHS and collated weekly into the Adult Mosquito Occurrence Summary Report (AMOR) from April 4 - October 31.

MOSQUITO TESTING

Twenty-six local mosquito control agencies in California (Table 1) submitted a total of 145,338 mosquitoes (3,501 pools) (Tables 2 and 3 and Fig. 1) to be tested for arboviruses at DARU. The test was an *in situ* enzyme immunoassay using Vero cell culture. Sixty-seven pools of *Culex tarsalis* and three pools of *Culex quinquefasciatus* were positive for SLE (Table 4 and Figures 2 and 3); but none was positive for WEE. Nine pools of *Ochlerotatus melanimon* were positive for California encephalitis virus (CE) (Table 4 and Fig. 2). All SLE- and CE-positive mosquito pools were also tested for WN; none was positive. WEE and SLE isolates from pooled mosquitoes over the past ten years are summarized in Fig. 3.

The 70 SLE-positive pools were tested for WN by *in situ* EIA with negative results. Of these, 5 pools were confirmed as SLE by RT-PCR and were sequenced. Data indicated that the SLE genotype active in southeastern California during 2001 was identical to the strain isolated during 2000, indicating that this viral strain apparently had successfully overwintered in the Coachella Valley.

CHICKEN SEROSURVEILLANCE

Forty-nine local mosquito and vector control agencies maintained 194 sentinel chicken flocks (Table 1 and Fig. 1). Blood specimens from each flock were collected and tested biweekly. Chicken sera were screened using an enzyme immunoassay (EIA) and confirmed with immunofluorescent antibody (IFA). Overall, 20,087 chicken sera from 46 of the 49 agencies in California and an additional 750 sera samples from Nevada, Oregon, and Utah were tested for antibodies to SLE and WEE by VRDL.

Fifty-one of the 62 seroconversions to SLE occurred in Riverside County; the 11 other seroconversions were in Imperial County (Table 5 and Fig. 2 and 4). The first SLE seroconversions were in four chickens bled on June 18 in Riverside County in samples sent to VRDL for testing. The winter testing program done by DARU indicated one SLE seroconversion in February, 2001. The last SLE seroconversions for 2001 were in Imperial County on September 5. There were 3 seroconversions to WEE in Riverside County. Seroconversions to SLE and WEE in sentinel chickens from 1992-2001 are summarized in Figure 4.

Table 2. Mosquitoes (*Culex* spp. and *Ochlerotatus melanimon*) tested for WEE and SLE by submitting county and agency, 2001

		Cx pipiens		*Cx quinquefasciatus*		*Cx stigmatosoma*		*Cx tarsalis*		*Oc melanimon*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Butte	BUCO							9	464	6	307	15	**771**
Contra Costa	CNTR							235	11,701			235	**11,701**
Fresno	FRNO							16	679			16	**679**
Fresno	FRWS							12	549			12	**549**
Glenn	GLEN							27	1,350			27	**1,350**
Kern	KERN							335	9,568	108	4,620	443	**14,188**
Kings	KNGS							10	491			10	**491**
Lake	LAKE					11	406	117	5,692	30	1,418	158	**7,516**
Los Angeles	GRLA			181	7,448			69	2,852			250	**10,300**
Los Angeles	LONG			69	2,254			2	46			71	**2,300**
Madera	MADR	9	450					1	50			10	**500**
Merced	TRLK							44	2,160	5	250	49	**2,410**
Orange	ORCO			73	1,937	1	11	31	918			105	**2,866**
Placer	PLCR							8	348			8	**348**
Riverside	COAV			37	936			579	24,635			616	**25,571**
Riverside	NWST			184	8,155	69	2,416	39	1,138			292	**11,709**
Sacramento	SAYO							294	13,272	174	7,896	468	**21,168**
San Bernardino	SANB			15	377	7	132	25	992			47	**1,501**
San Diego	SAND	1	19					2	98			3	**117**
San Joaquin	SJCM	29	945					43	1,563	10	236	82	**2,744**
Santa Barbara	SBCO			25	1,001	4	119	47	2,216			76	**3,336**
Santa Clara	STCL							2	90			2	**90**
Shasta	SHAS							22	1,044			22	**1,044**
Stanislaus	TRLK							44	2,019	1	50	45	**2,069**
Sutter	SUYA							106	4,924	3	125	109	**5,049**
Tulare	DLTA							29	1,285			29	**1,285**
Ventura	VENT							15	750			15	**750**
Yolo	SAYO							193	9,192	16	694	209	**9,886**
Yuba	SUYA							12	528			12	**528**
Grand Total		**39**	**1,414**	**584**	**22,108**	**92**	**3,084**	**2,368**	**100,614**	**353**	**15,596**	**3,436**	**142,816**

Source: California Department of Health Services

Table 3. Other mosquito species tested for WEE and SLE by submitting county and agency, 2001.

		An franciscanus		*An hermsi*		*Oc taeniorhynchus*		*Oc washinoi*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Riverside	NWST			1	20					1	**20**
Santa Barbara	SBCO	1	20	19	790	5	250	11	430	36	**1490**
Grand Total		**1**	**20**	**20**	**810**	**5**	**250**	**11**	**430**	**37**	**1510**

		Cs incidens		*Cs inornata*		*Cx erythrothorax*		*Cq perturbans*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Sacramento	SAYO			1	2					1	**2**
San Bernardino	SANB					1	12			1	**12**
San Joaquin	SJCM					4	94			4	**94**
Santa Barbara	SBCO	3	49	2	21	16	784			21	**854**
Shasta	SHAS							1	50	1	**50**
Grand Total		**3**	**49**	**3**	**23**	**21**	**890**	**1**	**50**	**28**	**1012**

Source: California Department of Health Services

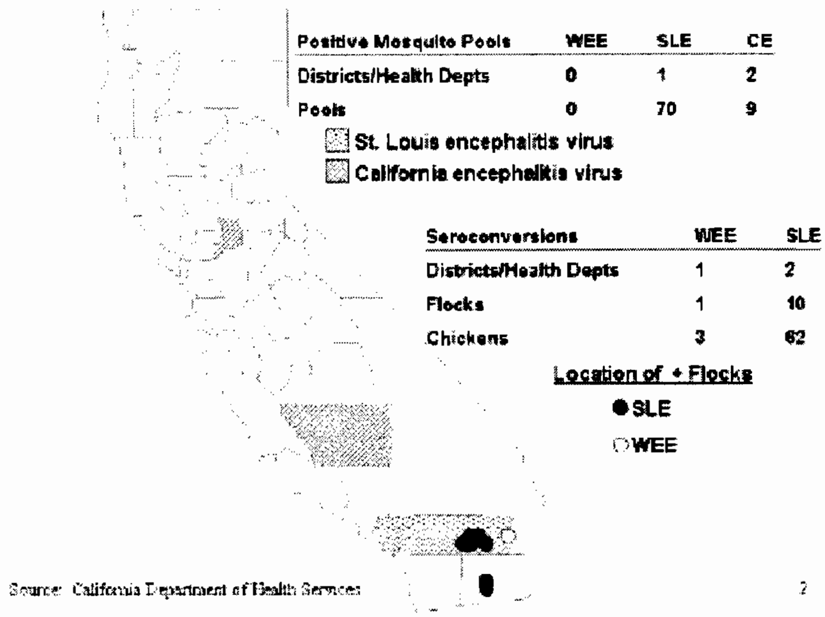


Figure 2. Collection sites of mosquito pools positive for SLE or CE, and location of sentinel chicken flocks with at least one seroconversion to SLE or WEE, California, 2001.

Because chicken IgG antibody to SLE cross-reacts with WN, a subsample of SLE-positive chickens, and specimens from other regions, were subsequently tested for WN. None of the 69 sera from nine counties was positive for WN by DARU.

DEAD BIRD SURVEILLANCE FOR WEST NILE VIRUS

The dead bird surveillance program initiated in 2000 continued in 2001 and was supported through a CDC grant. Notifications were sent twice during the year to approximately 600 agencies and groups explaining the program and requesting they contact CDHS when dead birds were found, especially crows. Recipients of the mailing included CDFA, CDFG, USFWS, wildlife rehabilitation and refuge centers, National Audubon Society, local health departments, mosquito and vector control districts, veterinarians, animal control and environmental health officers. Necropsies of submitted carcasses were performed by CAHFS. Kidney, brain, and heart tissues were forwarded to DARU for testing via cell culture and RT-PCR.

A total of 68 dead birds were reported to CDHS from 19 counties: Butte, Contra Costa, Kern, Los Angeles, Marin, Mendocino, Monterey, Orange, Riverside, Sacramento, San Bernardino, San Diego, San Joaquin, San Luis Obispo, Shasta, Trinity, Tuolumne, Ventura and Yolo (Figure 5). Eighteen birds met the criteria for testing; all specimens were negative for WN, SLE and WEE. The criteria included two conditions that the bird satisfy before being considered for testing: 1) the bird has been dead for less than 24 hours and 2) the bird is one of the Family Corvidae.

WEEKLY ARBOVIRUS SURVEILLANCE BULLETIN

CDHS has historically published a weekly bulletin reporting arbovirus test results of humans, equids, mosquitoes, and sentinel chickens in California. Last year, dead bird reports and other WN surveillance information in California and throughout the United States were added to the weekly report and continued in 2001. The bulletin provided weekly updates concerning the spread of WNV in the eastern half of the United States. The bulletin was distributed from May 4 to December 20 to local, state, and federal public health agencies, universities in California, other state health departments, and the CDC.

CALIFORNIA STATE MOSQUITO-BORNE VIRUS SURVEILLANCE AND RESPONSE PLAN

The Response Plan written by CDHS in collaboration with the Mosquito and Vector Control Association of California, and the University of California at Davis and Berkeley was completed in 2001 and distributed to all local mosquito control agencies. The document describes an enhanced surveillance and response program for California to ensure that local and state agencies are prepared to detect and respond in a concerted effort to protect humans and animals from mosquito-borne diseases. Unique to this document is the mosquito-borne virus risk assessment model which delineates 3 levels of alert conditions and associated intervention responses: normal season, emergency planning, and epidemic conditions.

Table 4. SLE and CE viral isolates from mosquito pools during 2001.

Mosquito species	Dates collected	County	Agency	Virus Isolated					
				SLE		CE		Totals	
				Pools	Mosquitoes	Pools	Mosquitoes	Pools	Mosquitoes
Culex tarsalis	6/13-7/5	Riverside	COAV	43	2179	-	-	43	2179
	7/10-8/02	Riverside	COAV	21	895	-	-	21	895
	9/4	Riverside	COAV	1	10	-	-	1	10
	7/10-7/11	Riverside	COAV	2	75	-	-	2	75
Culex quinquefasciatus	9/4	Riverside	COAV	1	21	-	-	1	21
	7/24-8/02	Riverside	COAV	2	74	-	-	2	74
Ochleratus melanimon	6/22	Kern	KERN	-	-	3	130	3	130
	5/26 – 6/8	Kern	KERN	-	-	5	242	5	242
	9/14	Sacramento	SAYO	-	-	1	14	1	14
Totals				70	3180	9	386	**79**	**3566**

Source: California Department of Health Services

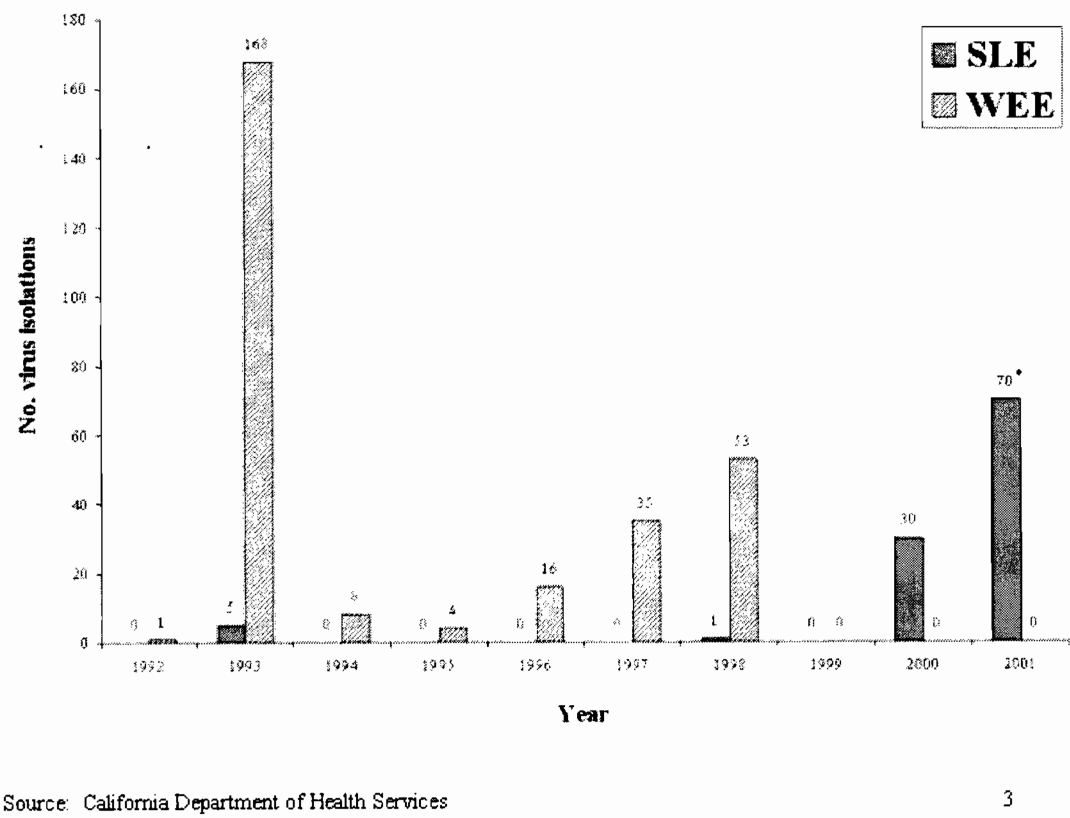
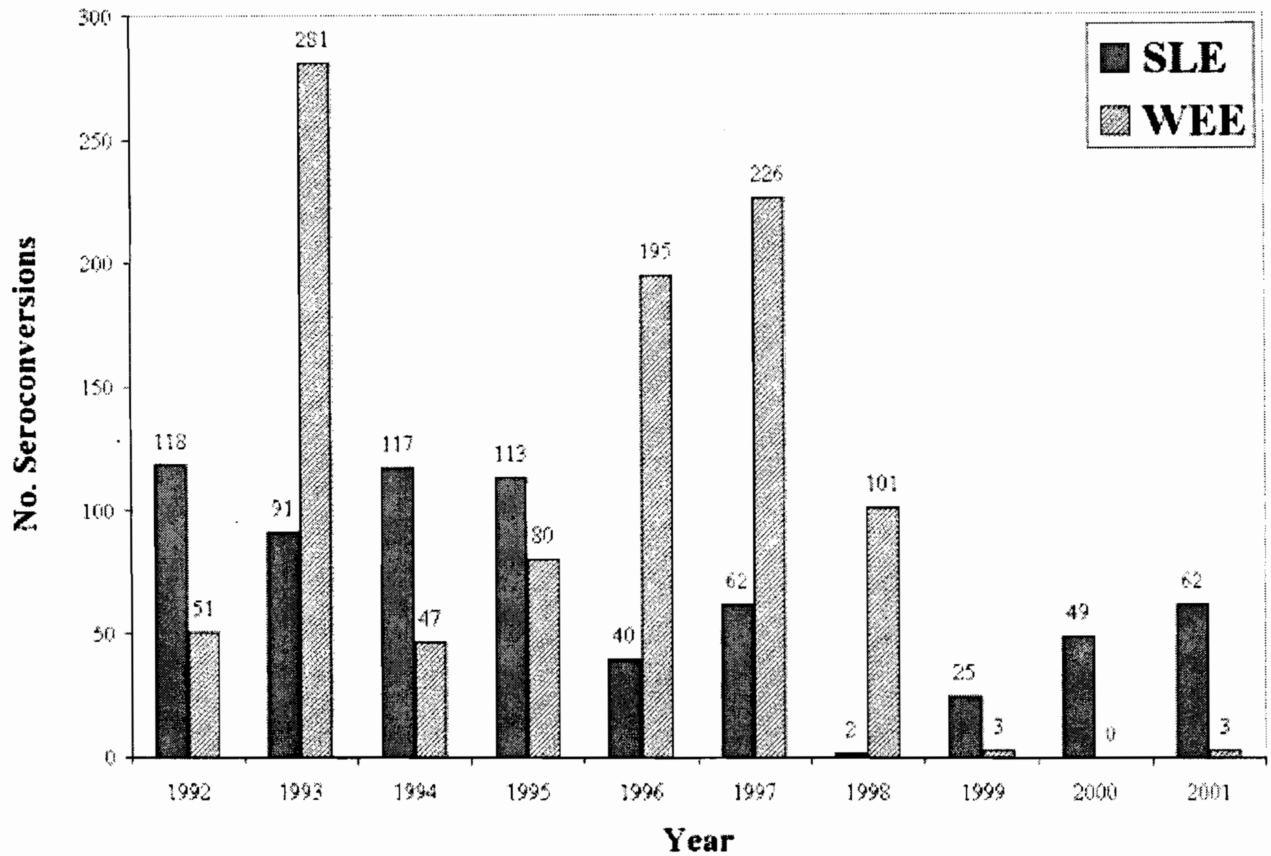


Figure 3. Isolations of SLE and WEE from pooled *Culex tarsalis* in California, 1992-2001. *This includes three positive pools of *Culex quinquefasciatus* in 2001.



Source: California Department of Health Services 4

Figure 4. Seroconversions to SLE and WEE in sentinel chicken flocks in California, 1992-2001.

Table 5. Chicken seroconversions to SLE and *WEE* by location and date bled, 2001

County	Agency	City	18-Jun	2-Jul	16-Jul	30-Jul	2-Aug	7-Aug	16-Aug	13-Aug	27-Aug	5-Sep	Total
Imperial	IMPR	El Centro						1				4	**5**
Imperial	IMPR	Seeley						2				4	**6**
Riverside	COAV	Blythe					*1*		*2*				***3***
Riverside	COAV	Thermal				1							**1**
Riverside	COAV	Oasis			1	2							**3**
Riverside	COAV	Mecca (A)	4	4	2	1					1		**12**
Riverside	COAV	North Shore (D)			5					1	1		**7**
Riverside	COAV	Mecca (G)		5	4	1							**10**
Riverside	COAV	Mecca (M)		1	8	1				1	2		**13**
Riverside	COAV	North Shore (SP)			4								**4**
Riverside	COAV	Indio								1			**1**
SLE Totals			**4**	**10**	**24**	**6**		**3**		**3**	**4**	**8**	**62**
WEE Totals							***1***		***2***				***3***

Source: California Department of Health Services

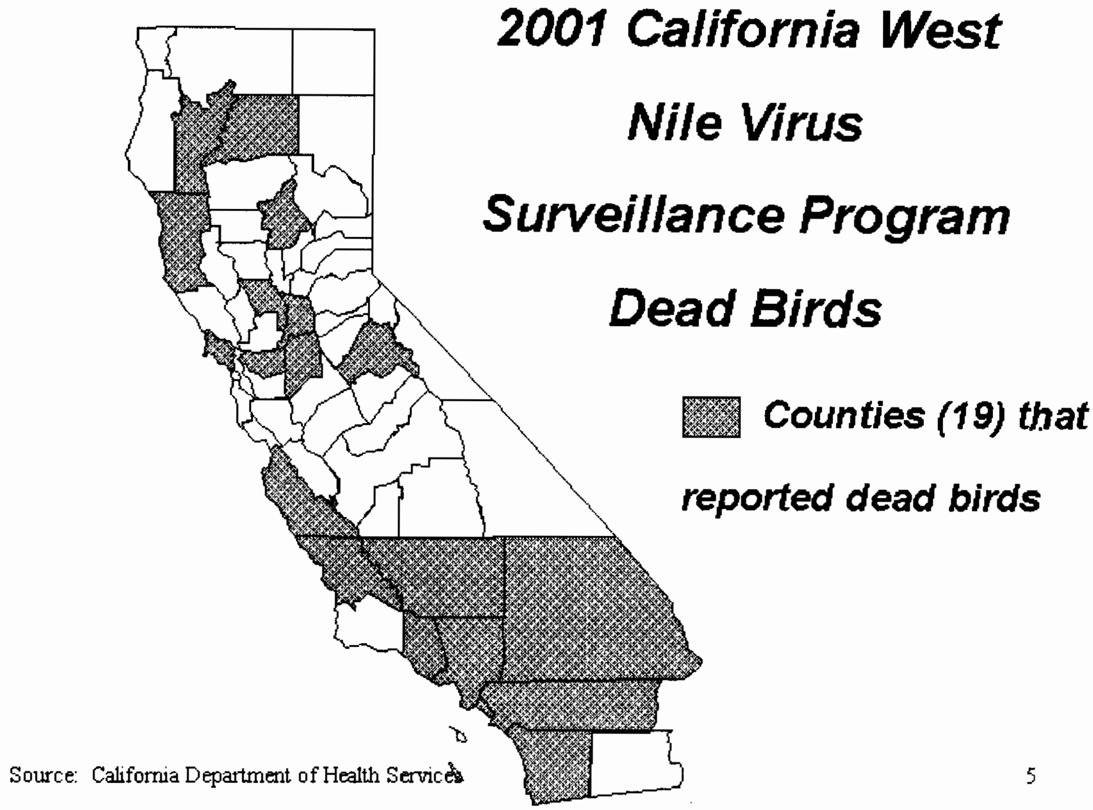


Figure 5. Counties which reported dead birds to CDHS in 2001 for WN testing.

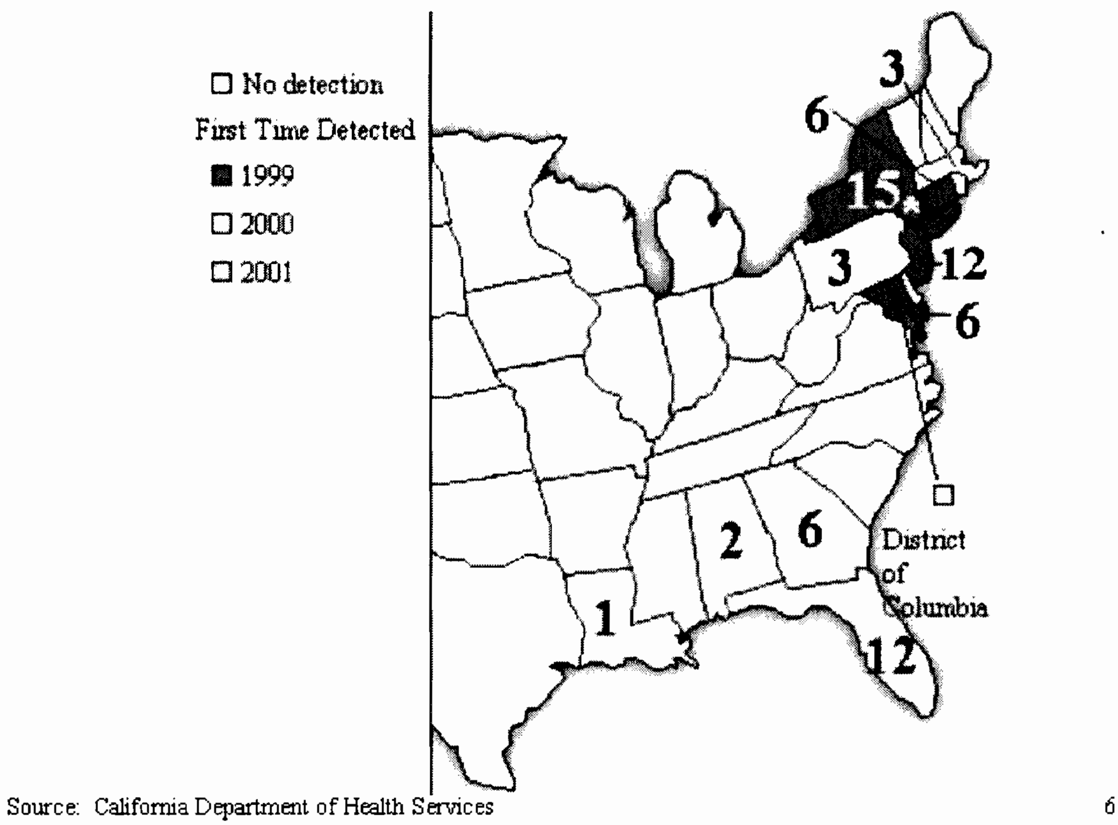


Figure 6. States which reported WN activity, (bold number is the number of human cases for that state in 2001).
* includes 2 non-hospitalized mild cases

WEST NILE VIRUS IN THE UNITED STATES

In 2001, WN activity was reported in 27 states and the District of Columbia, including 16 states where the virus had not been detected previously. The westernmost expansion of WN was western Arkansas. In 2001, there were 66 human cases (64 hospitalized) from 10 states with nine fatalities (21 human cases with 2 fatalities were reported in 2000) caused by this virus (Fig. 6). Also, in 2001, there were 731 equine cases from 19 states, 7,114 infected dead birds from 27 states and the District of Columbia, and 919 positive mosquito pools (26 species) from 16 states and the District of Columbia. Florida, New York, and North Carolina reported seroconversions to WN in sentinel chicken flocks.

Acknowledgements

The authors gratefully acknowledge the cooperation and assistance of the local mosquito and vector control agencies collecting and submitting samples for testing; the valuable contributions of Ying Fang, Emily Green, and David Gutierrez (DARU); Curtis Fritz, Denise Steinlein, Kris Carter, Martha Needham, Pamela Brown and Al Hom (CDHS Disease Investigations and Surveillance Branch); Natasha Huntziker, Gordon Shell, Somayeh Honarmand, Jennifer Hummel (CDHS VRDL).

Mosquitofish in the High Desert

Keith White, Karen S. Mellor

Antelope Valley Mosquito & Vector Control District, P.O. Box 1192, Lancaster, CA 93584, Phone: (661) 942-2917, mosq2@earthlink.net

ABSTRACT: The moderate climate in most of southern California provides an ideal environment for mosquitofish (*Gambusia affinis*). But what about the High Desert with it's extreme temperatures? How do these fish survive the severe heat in the summer and the freezing temperatures in the winter? This paper will attempt to show that mosquitofish are very well adapted to these conditions and don't seem to have too many problems dealing with the extremes.

This presentation will address the places we keep fish for "storage", what kinds of habitats are used as "breeding reservoirs" and ways we obtain mosquitofish for distribution throughout the District. Part of the paper will deal with the setup, filtration system and the cleaning of our pond at the District facility. It will also provide a summary of sources the District will stock with fish for biological mosquito control.

At the Antelope Valley Mosquito & Vector Control District we stock fish in retention basins for two reasons: one is to control mosquito larvae and the second reason is to provide a breeding reservoir for the fish. It is very important to keep the basins from weed overgrowth and have access in order to retrieve fish. The fish are gathered with seines, hand nets or minnow traps. When the sides of the basins are too overgrown, we are able to use waders to take the nets out, if the basin is shallow enough. The fish then are transferred to a transport tank in the back of a truck. We usually put the water in this transport tank a day before we go fishing in order for any chemicals (especially chlorine) to evaporate. To reduce the stress for the fish we add un-iodized salt to the water and aerate the tank during transport.

The fish are stored in a shaded 800 gallon holding tank (16 ft. x 5 ft. x 2.5 ft.) at the District yard, which is constructed of brick and sealed with swimming pool paint. From this pond, technicians can distribute the fish to other sources within the District and residents can pick them up for backyard sources like ornamental ponds, horse troughs, and aquatic plants. A water trough float maintains the water level. Gravel, bricks and 4" PVC pipes are provided as shelter for the fish. The fish are fed at a minimum with dry dog food to keep the nitrates from building up.

The pond is cleaned approximately every 3 weeks in summer and less frequently in the wintertime. The biological filtration system is a modified version of one designed by a person who builds Koi ponds. It is simple and very effective and therefore easy to install in any situation.

The filter consists of a 55-gal drum, PVC pipes, an electric pool pump (low amperage), and some ¾ - 1 inch round rocks, pea gravel and # 12 silica sand (see Figure 1). It was important to

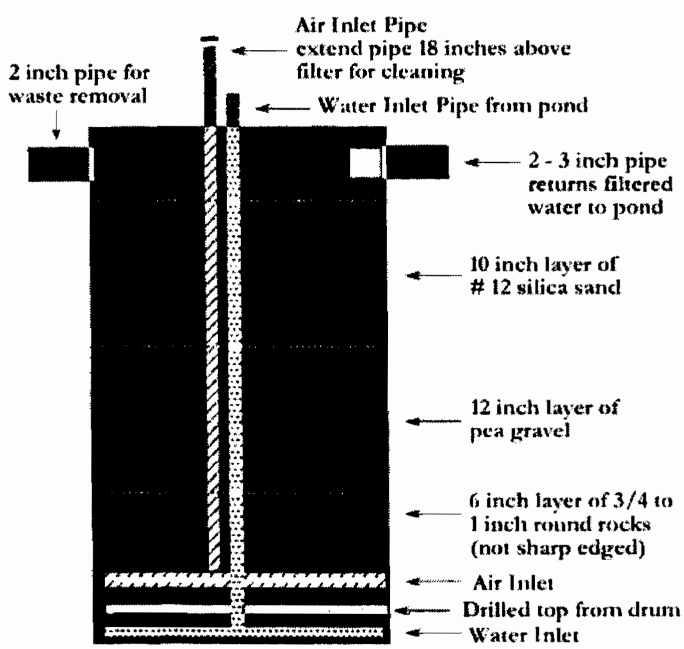


Figure 1: Diagram of the filter

make sure the 55-gal drum did not contain any toxic materials before using it for the filtration system. It is also necessary to use the larger grains of sand (#12) to prevent it from washing away when the filter is backwashed. To clean out the filter, compressed air is blown through the air inlet pipe of the filter in order to loosen dirt that may have accumulated on the sand and gravel. During this procedure the water return pipe to the pond is plugged and the waste removal pipe is opened. The wastewater is used to irrigate some bushes along the property line. The filter is backflushed until the water appears clear.

Some algal growth is allowed on the sides and bottom of the pond. If it becomes too overgrown, the sides are scrubbed with a pool brush prior to cleaning the filter to get the particles out of the water.

A FINAL THOUGHT – THE FROG PHENOMENON

As mentioned above, the breeding grounds for our mosquitofish are generally retention basins. In early spring we have noticed a phenomenon that, to our knowledge, has not been researched, but seems to be very consistent. In the first few weeks of spring the mosquitofish come to the surface and we are able to catch some easily. Then, when frog and toad tadpoles hatch out of eggs laid in those basins, the fish disappear and it is almost impossible to catch any until the adult frogs leave the water. After they are gone, the fish mysteriously re-appear in the basins. We're not sure exactly what happens during that time, but since the fish can't leave, we believe that they might be hiding either in the inlet pipes or on the bottom of the basins while the tadpoles are present.

With all the bad reputation mosquitofish have received in the past about eating and damaging tadpoles, I believe this phenomenon is worth mentioning and maybe even worth further research.

Factors Affecting the Outcome of *Culex tarsalis* Mark-Release-Recapture Experiments in the Coachella Valley of California

Hugh D. Lothrop and William K. Reisen

Arbovirus Research Unit, Center for Vector-borne Disease Research, School of Veterinary Medicine, University of California, Davis, CA 95616

ABSTRACT: Dispersal patterns and recapture rates of *Culex tarsalis* Coquillett females used in mark-release-recapture (mrr) experiments were influenced by the female site of collection prior to release and reproductive and calendar age at release. Four experiments produced inconsistent results: females originating at sites distant to the release site had different recapture rates than females collected locally. Reared mosquitoes were not recaptured in substantial numbers, but gravid mosquitoes had a promising recapture rate as parous host-seeking females indicating that they might be a useful substitute for those reared from pupae.

INTRODUCTION

Previous mrr studies in Kern County (Reeves et al. 1948, Dow et al. 1965, Reisen et al. 1992) and the Coachella Valley (Reisen and Lothrop 1995) indicated that the mean daily population dispersal distance of *Culex tarsalis* females from the release site averaged about 1 km/day. Overall, mosquitoes reared from field-collected immatures in Kern County had a recapture rate of 2.4%, whereas in Coachella, females that were reared and or collected host-seeking at CO_2 baited CDC style traps had negligible and 6.7% recapture rates, respectively. These studies focused on large scale dispersal and estimates of demographic parameters such as absolute population size, addition and deletion rates, host-seeking frequency, age structure and survival.

Our current studies seek to determine the microhabitat distribution of host-seeking and other flying components of the female population and their patterns of movement from breeding sources into upland habitats (Lothrop and Reisen 2001, Lothrop et al. 2002). These data will be incorporated into our experiments on targeted adult mosquito control at microhabitat features within the landscape.

MATERIALS AND METHODS

Immature mosquitoes were collected by dipper and adults by CO_2 traps and transported to the laboratory. Reared adults were held in 5-gallon plastic buckets and offered 10% sucrose on cotton wicks. CO_2 collected mosquitoes were held in the laboratory between trapping and release in the same type of buckets and offered H_2O on wicks. Adults were counted by the strip method (Dow et al. 1965) and marked with ultraviolet fluorescing dust. Recapture was attempted on 3 consecutive nights, beginning on the evening of release. Four sites with different sampling patterns were located along the northwestern margin of the Salton Sea, Riverside County. Sites are shown in the figure and were numbered by experiment (1-4) with an additional site, number 5, where adults were collected for release at sites 1 and 3.

Experiment 1, April 23 2001, was conducted at shoreline and citrus habitats. For this experiment we used reared and adult collected mosquitoes. Reared mosquitoes were collected from seepage at a duck club (site 3). Adults were combined from collections at the release area and site 5. Both unbaited 8 inch suction and CO_2 traps were used for recapture, because CO_2 traps previously had shown poor recapture of reared mosquitoes. Traps were positioned along the shoreline and inside and around the perimeter of a citrus orchard. Mosquitoes were marked with 6 unique dust colors, one each for point of release and collection method. Release was done before sunset at three release points located at the shore, a fish farm, and inside the citrus (Table 1). Unmarked adults captured in CO_2 traps during the recapture period were dissected to determine age and insemination status.

Experiment 2, May 17 2001, was conducted at the duck club site where larvae and pupae were collected for the previous experiment. For this experiment we again used reared and adult collected mosquitoes. Reared mosquitoes were collected onsite and handled as in the previous trial. Adult mosquitoes were collected locally and from the previous site and were labeled as "local" and "foreign". Recapture was attempted using suction and CO_2 traps. Traps were located in a cluster near the release site and at positions to the north and south. Six unique colors were used for treatments comparing local vs. foreign female source, reared vs. CO_2 trap collected females, and morning vs. evening release.

Experiment 3, June 18 2001, was conducted within an area that overlapped parts of the first site. Local host-seeking mosquitoes were engorged on young chickens the night of 14 June, 4 days before their release on the evening of June 18. Adult mosquitoes were collected locally and at North Shore on June 18 and released before sunset on June 19 when recapture trapping was begun.

Experiment 4, 25 September 2001, was conducted with CO_2 traps in the duck club region. Adults for release were collected

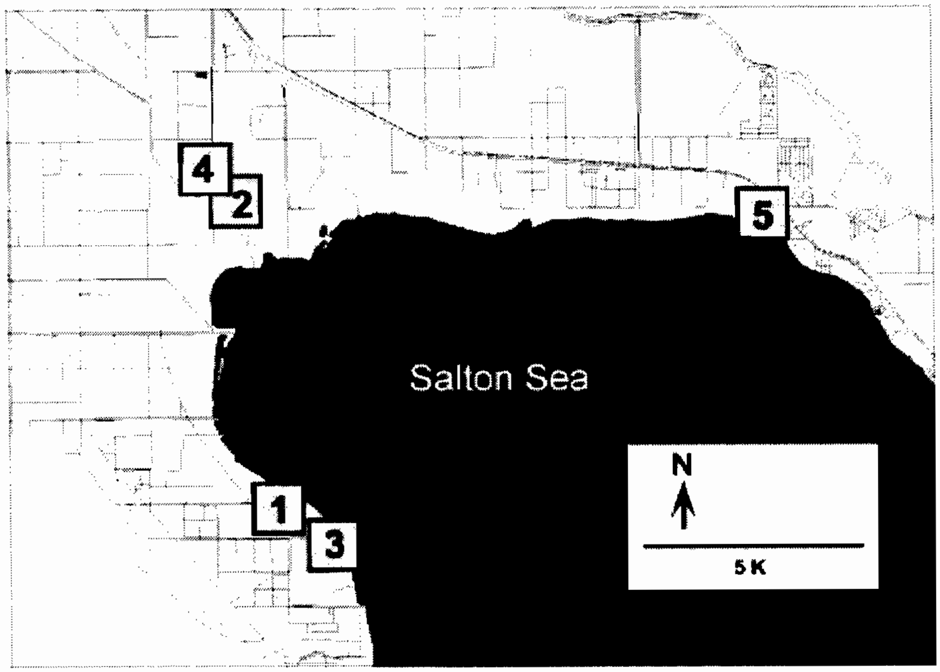


Figure 1. Sites of mosquito origin and release

from all the traps within the sample grid and released before sunset to study their pattern of movement.

RESULTS AND DISCUSSION

Experiment 1 results did not indicate that citrus habitat acted as a magnet to accumulate dispersing mosquitoes, because marked mosquitoes readily dispersed from citrus to shoreline habitats. There was a weak correlation (0.54) between the numbers of marked and unmarked females captured at each trap, after removing one trap placed too close to one release point. The greater recapture of mosquitoes released in citrus [Table 1] was likely skewed by this same trap. CO_2 traps and unbaited suction traps showed interesting differences in relative recapture rates, with suction traps showing low but similar recapture rates between the 2 types of released mosquitoes, whereas mostly females collected host-seeking originally were collected at CO2 traps.

Experiment 2 was conducted to elucidate the effects of release time and cohort origin on dispersal and recapture success. Our purpose for the morning release was to see if mosquitoes would seek shelter rather than disperse immediately and would be more naturally accommodated by the environment when making their evening flight. The other treatments were intended to separate the additional variables of local vs. foreign origin of adult collected females and age (reared vs. CO_2 trap collected). Release time did not effect recapture success. Locally collected mosquitoes were recaptured at a significantly higher rate than foreign mosquitoes [6.56% vs. 1.24%, χ^2= 132, P<0.001]. The recapture rates of reared mosquitoes in experiments 1 and 2 were $\leq$0.5%. These results were similar to our previous experiments (Reisen and Lothrop 1995) and reinforced our conclusion that adults reared from field-collected immatures were not useful for mark-release-recapture studies in the Coachella Valley. This has created a problem for simulating and tracking dispersal of tenerals from larval habitats that we attempted to resolve in Experiment 3 by replacing the reared cohort with gravid females. Our rationale was that these females would oviposit at the same larval habitats and then disperse similarly to the natural population.

Experiment 3 compared local vs. foreign host-seeking females collected by CO_2 trap and locally gravid females. Foreign mosquitoes were recaptured significantly more frequently than local mosquitoes [8.88% vs. 3.45%, χ^2= 40.3, P<0.001], contradicting the results in experiment 2 and indicating that other handling variables may influence recapture success. Dispersal for marked mosquitoes was weakly correlated (0.54) with abundance of unmarked females. The low number of gravid mosquitoes released resulted in few recaptures, but the recapture rate of 2.1% indicated that this method may be useful for measuring dispersal from an oviposition site.

Experiment 4 was part of a study designed to determine the contribution of production from immature habitats to counts in CO_2 traps deployed in a regular grid throughout an area dominated by duck clubs. Mosquitoes were released adjacent to the most productive larval source with the hypothesis that recapture patterns would parallel local abundance. The correlation of 0.67 was the best of the four experiments and indicated that certain highly productive sources were likely responsible for host-seeking abundance values within the 3 km radius study area.

Our data indicated handling of mosquitoes collected for mark-release-recapture influenced recapture success and dispersal behavior. Factors such as local vs. foreign collection of host-seeking females and crowding in traps and holding cages need further examination. In our future experiments we will seek to reduce the holding time by marking and releasing after a few hours

Table 1. Numbers released and recaptured for experiments 1 through 4.

EXP. #	TREATMENT	# RELEASED	# RECAPTURED	% RECAPTURE
1	Shore/Reared	967	5	0.5
	Shore/CO2	3293	82	2.5
	Fish farm/Reared	877	2	0.2
	Fish farm/CO2	3080	76	2.7
	Citrus/Reared	970	3	0.3
	Citrus/CO2	3337	115	3.4
2	Local/AM	1230	80	6.5
	Local/PM	820	64	7.8
	Foreign/AM	2330	24	1
	Foreign/PM	1660	26	1.5
	Reared/AM	267	8	0.3
	Reared/PM	272	5	0.2
3	Local	2347	112	4.8
	Foreign	890	79	8.9
	Gravid	280	6	2.1
4	Local/PM	1720	60	3.5

of collection or the morning after collection. In the Coachella Valley we will use gravid rather than newly emerged mosquitoes for describing dispersal from breeding foci.

REFERENCES

Dow, R.P., W.C. Reeves and R.E. Bellamy. 1965. Dispersal of female Culex tarsalis into a larvicided area. Am. J. Trop. Med. Hyg. 14: 656-670.

Lothrop, H.D., B. Lothrop and W.K. Reisen. 2002. Nocturnal microhabitat distribution of adult *Culex tarsalis* (Diptera: Culicidae) impacts control effectiveness. J. Med. Entomol. [in press]

Lothrop, H.D. and W.K. Reisen. 2001. Landscape affects the host-seeking patterns of *Culex tarsalis* (Diptera: Culicidae) in the Coachella Valley of California. J. Med. Entomol. 38: 325-332.

Reeves, W.C., B. Brookman and W.M. Hammon. 1948. Studies on the flight range of certain *Culex* mosquitoes, using a fluorescent-dye marker, with notes on *Culiseta* and *Anopheles*. Mosq. News 8: 61-69.

Reisen, W.K. and H.D. Lothrop. 1995. Population ecology and dispersal of *Culex tarsalis* (Diptera: Culicidae) in the Coachella Valley of California. J. Med. Entomol. 32: 490-502.

Reisen, W.K., M.M. Milby and R.P. Meyer. 1992. Population dynamics of adult *Culex* mosquitoes (Diptera: Culicidae) along the Kern River, Kern County, California, 1990. J. Med. Entomol. 29: 531-543.

Description of a Nurse Rig Trailer Used as Support for Larviciding Operations by the San Mateo County Mosquito Abatement District

Stanley Kamiya and Chindi Peavey

San Mateo County Mosquito Abatement District, 1351 Rollins Road, Burlingame, CA 94010

ABSTRACT: Mosquito control operations in San Mateo County require the ability to treat large areas in remote and environmentally sensitive sites. Materials are applied to these areas via ARGO® all-terrain vehicle or boat. Such vehicles have low carrying capacity (typically 25 gallons) and require frequent reloading. The "nurse rig trailer" described here was designed to serve as a mobile supply unit in areas without access to electricity or running water. The trailer is 14 ft long and holds two 55-gal fiberglass tanks and 2 gas-powered pumps. Plumbing connections between the tanks and pumps allow them to be used separately, with different materials, or as a single unit. This unit allows for transport, staging and mixing of up to 100 gal of material and has greatly decreased the time required for large-scale control operations.

INTRODUCTION

The San Mateo County Mosquito Abatement District (SMCMAD) covers 166 sq mi of urbanized area on the San Francisco Peninsula, directly south of the city of San Francisco. The District controls mosquito-breeding sources on several hundred acres of salt marsh along the bay. These sites are inspected on a weekly basis during winter and spring and may require treatment every few weeks. Depending on the stage of mosquitoes present, marsh sources may be treated with liquid formulations of *Bacillus thuringiensis israelensis* (Bti), methoprene, or a combination of the two (duplex). These materials must be mixed with fresh water that is free of sediment, a resource that is not readily available in salt marshes. The District operates with 6 full-time personnel responsible for mosquito control, assigned to 6 half-ton pickup trucks. Roadside sources are treated with power sprayers supplied by 50 gal tanks mounted in the back of the trucks. Less accessible sources are treated using 2 Points West ARGO®s equipped with 25-gal tanks. The small size and tracked design of this vehicle minimizes its impact on vegetation in sensitive wetland habitats. Unfortunately, this also limits its carrying capacity and necessitates frequent reloading. Initially, the ARGO®s were resupplied from tanks on the technician's vehicles. However, this strategy ties up both technicians and their vehicles and prevents them from treating other sources. Returning to the district yard for clean water and material was also time-consuming. Traffic congestion on the peninsula has increased dramatically in recent years and travel time to salt marsh treatment sites can take over 45 minutes. A mobile unit was needed that could be stationed to the treatment site and used to resupply application equipment.

The "nurse rig" described here was designed and built by District staff to fill this need (Figure 1 and 2). It consists of a 10' X 5' utility trailer carrying 2 power spray units and two 55-gallon tanks. Dual tanks and pumps increase the flexibility and

Figure 1

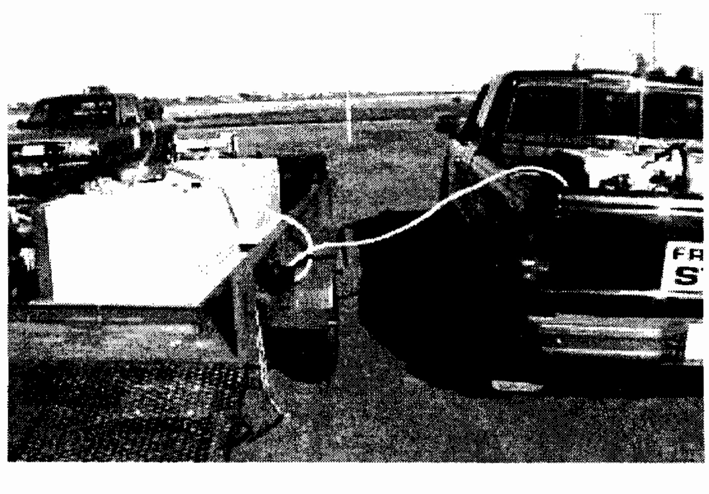


Figure 2

dependability of the unit. Hoses and valves are configured (Figure 3) so that the two tanks can be operated as a single unit or independently. One valve configuration allows the tanks to be emptied in series. Another configuration allows crossmixing between the two tanks. The tanks can be filled with different products (such as Bti and methoprene) and emptied simultaneously from a single hose. The valves can also be configured to allow each tank and its pump to operate independently to supply 2 vehicles simultaneously. The trailer is equipped with two 50-foot hoses that can be extended on both sides. The dual pumps also provide protection against equipment failure (either pump can empty both tanks should one pump fail). A standard lockbox, such as those used in pickup trucks, provides space for pesticide concentrate.

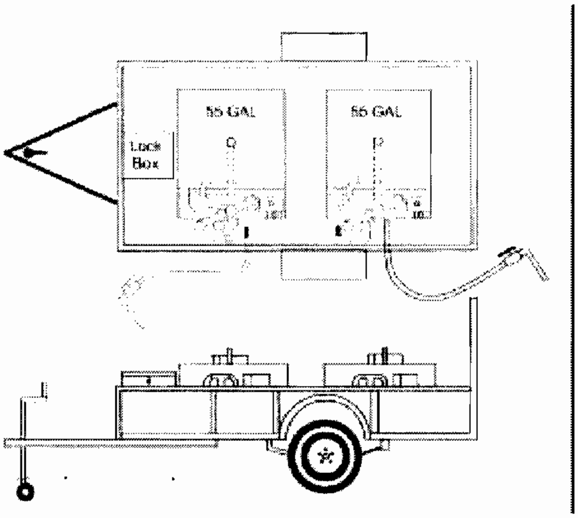


Figure 3

Construction of this trailer has saved the district both time and money. It was constructed in 1977 by staff personnel using readily available materials (Table 1). Use of the nurse rig has reduced the amount of time spent traveling to and from District, allowing for more efficient deployment of personnel and equipment. Large sources can be treated by fewer staff members. The nurse rig is employed frequently during winter months for treatment of *Ochleratatus squamiger* (Coquillett) in the salt marsh. During a recent helicopter treatment of Bair Island, it was used to augment the contractor's own nurse truck. In addition, this equipment has also been useful in a program to eradicate imported cordgrass under subcontract to the US Fish and Wildlife Service.

Acknowledgements

The authors wish to acknowledge assistance provided by members of the staff of the SMCMAD. The equipment described here was designed and built by James Counts and Stanley Kamiya. Alexandra Porshnikoff provided editorial assistance and produced the drawings for Figure 1.

Table 1. Specifications for construction of mobile supply trailer.

Trailer	
Bed	10 ft by 5 ft, constructed from 2"x 2" and 3" X 3" "L" angle steel
Siderails	18 inch steel supports
Rear ramp	4 ft constructed from 1/8" expanded steel
Wheels	14 x 5" steel trailer wheels on 1 ton trailer spindles
Suspension	1 ton leaf spring (2), 2200 lb
Hitch	Receiver tongue with 2" ball, class III Retractable tongue wheel Removable caster
Spray Equipment	
Tanks (2)	55 gallon capacity, fiberglass
Motors (2)	5hp, 4stroke, Briggs & Stratton
Pumps (2)	400 psi piston pump, Hydro Univar
Valves (4)	¾" ball type
Hoses	Reinforced neoprene, 220 psi rated

Total cost of materials: approximately $1500.00

Description of Equipment Used for Application of Pesticides from an Argo® All-Terrain Vehicle

Christopher Edwards and Chindi A. Peavey

San Mateo County Mosquito Abatement District, 1351 Rollins Road, Burlingame, CA 94010

ABSTRACT: This paper describes equipment used to apply pesticides from an ARGO® all-terrain vehicle. The vehicle features three pesticide dispersal devices, and is designed to allow a single operator to apply pesticides while maneuvering the vehicle. A cluster nozzle is the primary dispersal unit. It applies a 30 ft swath of material in its wake and is used for large-scale treatment work. A telescoping side boom sprayer is used for applications to ditches or spraying from the tops of dikes. Granular or pelleted materials are applied with a Herd® seeder, which can be operated independently or in conjunction with liquid applications. The combination of these devices allows a single vehicle to apply pesticides under a wide variety of conditions. This has increased the efficiency, accuracy and safety of pesticide applications to both the applicator and the environment.

INTRODUCTION

In San Mateo County, millions of tons of asphalt and concrete share the terrain with thousands of acres of salt marsh and mud. This arrangement provides the city of San Mateo and surrounding area with a periphery of seasonal and tidal wetlands, which are habitat for developing mosquitoes. The character of these wetlands has changed over the history of the District, as has the methods and equipment used to control mosquitoes produced there. Prior to 1900, approximately 14,000 acres of salt marsh occupied the shores of central San Francisco Bay (Goals Project 1999). In the early 1900's much of this area was diked and "reclaimed". When neglected, these diked areas became vast seasonal wetlands with little water circulation, producing mosquito populations so thick they blackened the sky. As early as 1906, efforts were made to stem the annual fly-off of salt marsh mosquitoes. Ditches were constructed to enchance tidal circulation, while diesel, kerosene, and nicotine were applied as larvicidal agents. Occasionally, adulticides were applied in populated areas.

From 1960 through the early 1980's, many of the diked areas in the county were filled and developed. The 5,000 acres of salt marsh remaining has become fragmented into patches and strips lying within utility right-of-ways, airport flyways, community open space preserves, and wildlife refuges. In winter and spring, rainwater collects in depressions, borrow ditches along dikes, cracks in the floor of abandoned salt pans, and remnants of sloughs. These areas provide ideal habitat for *Ochleratatus squamiger*, *Culiseta inornata* and *Culex tarsalis*. Many of these habitats are adjacent to residential neighborhoods.

The diversity of source types and fragmentation of mosquito breeding habitat within the San Mateo County requires the use of a wide range of application equipment. Rough terrain and the presence of widely dispersed breeding sites make treatment by backpack sprayers or hand cans impractical. The equipment described here is designed for treatment diverse and scattered sources by minimal equipment and staff.

APPLICATION EQUIPMENT

The SMCMAD has used a number of different vehicles for salt marsh treatment over the years (Counts et al 2000). It currently employs two Points West ARGO® Conquest All-Terrain vehicles. The ARGO® is a tracked, amphibious vehicle, capable of carrying two operators, their equipment and extra materials into otherwise inaccessible parts of the marsh. This vehicle is gasoline powered and has eight wheels in banks of four, with a wide plastic track stretched around pneumatic tires. The hull is thick plastic with an extensive internal steel framework. With its lightweight and tracked design, the ARGO® will travel over the most challenging terrain and is capable of short water crossings. When fully loaded a ¾ ton ARGO® exerts ground pressure of less than two lbs per square inch, with minimal impact on marsh substrate and flora. The rear bay is large enough to carry a 50-gallon spray tank. The District uses a 25-gallon polyethylene tank to save on weight. The tank can be refilled in the field from a "nurse rig" during large-scale spray operations (Kamiya and Peavey 2002).

Mosquito control operations at the SMCMAD are carried out by 7 full time personnel. The diversity of source types and limited manpower places a premium on flexibility in treatment equipment. To meet this need, each ARGO® is equipped with a wide range of application equipment. A ShurFlo® pump motor mated to a Pump Tec® pump head tops the tank. It puts out up to 220 psi to an array of nozzles via a set of flow selector valves (Figure 1). The selector valves send materials to a cluster nozzle, a side boom, or a hand-held nozzle.

The cluster nozzle (Figure 2) was developed and installed by the Marin-Sonoma Mosquito and Vector Control District. It supplies a 30-35 ft swath at 1.3 gal/min through a set of five nozzles arrayed to make an arc of 240° through two 10° flat fans, two 60° flat fans and a single pinstream. The cluster nozzle is used for grid work in large areas. The side boom (Figure 3) extends three feet and employs an adjustable pinstream nozzle on an adjustable elbow capable of delivering 1 gal/min with a swath width of up to 40 ft. The side boom is especially useful for spraying borrow ditches from atop dikes, where even greater distance and coverage can be attained. A hand-held nozzle with an adjustable tip is used for spot treatment of small impounds or areas inaccessible to the ARGO®.

Granular applications are made with a Herd® seeder (Figure 2). It is mounted on the outboard motor transom on the ARGO®, and controlled via dashboard mounted switches. The flow gate is controlled by the motor from an automobile power window

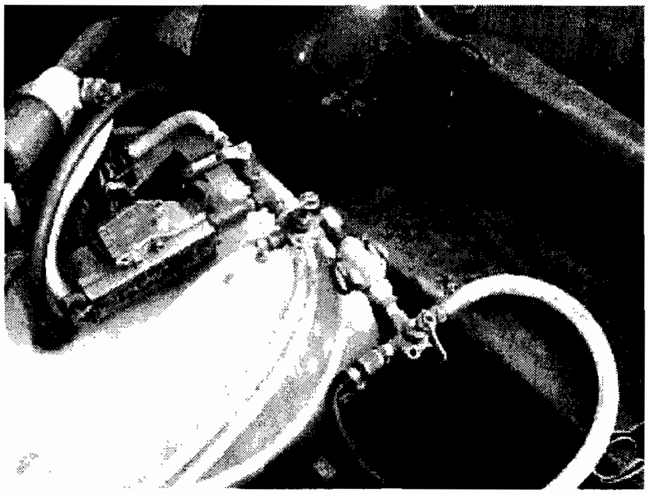


Figure 1. Selector valves

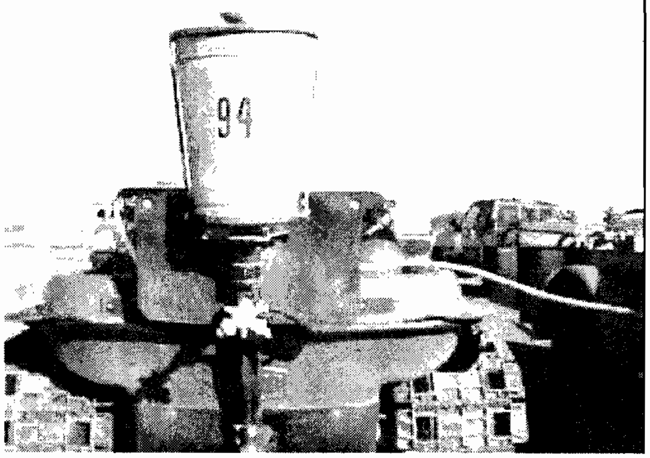


Figure 2. Cluster nozzle and Herd seeder

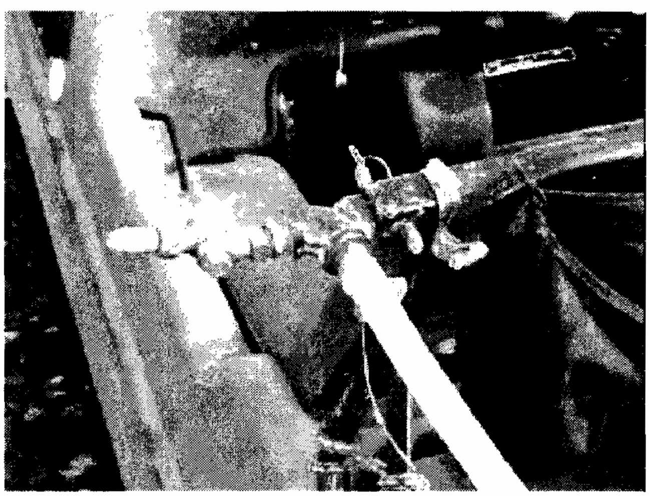


Figure 3. Telescoping side boom sprayer

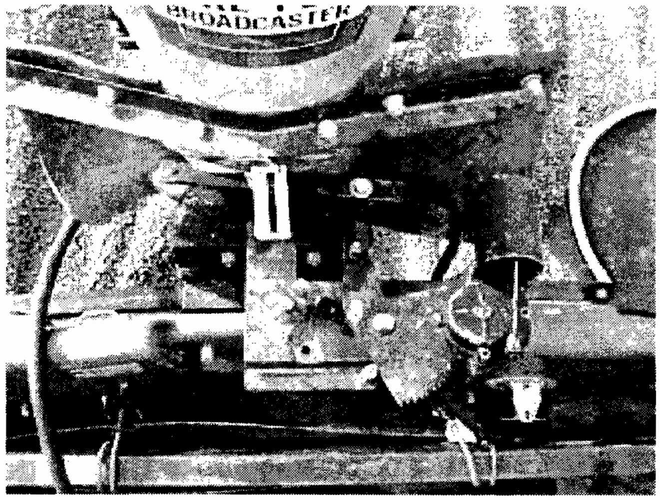


Figure 4. Flow gate for Herd seeder is controlled by an automobile power window regulator.

regulator (Figure 4). The seeder gives a swath width of about 50 ft and can empty a full hopper (about 40 lbs) in minutes (3 lbs / min is its usual output).

Salt marshes of San Mateo County are notable in their fragmentation. The diversity of mosquito development sites in these marshes requires technicians to have access to a wide array of different treatment equipment. The arrangement of spray equipment on the District's ARGOs was designed to allow several different modes of treatment from a single vehicle. This efficient configuration saves the District valuable time and minimizes travel through environmentally sensitive areas.

Acknowledgements

The equipment described here was designed and installed by the staff of the Marin-Sonoma Mosquito and Vector Control District in conjunction with members of the San Mateo County Mosquito Abatement.

REFERENCES

Counts, J.H., D. Jewell and C.A. Peavey, 2000. History of Pesticide Dispersal Equipment Used to Control *Aedes squamiger* on Bair Island San Mateo County, California. Proceedings and Papers of the Mosquito and Vector Control Association of California. 68: 61-63.

Kamiya, S. and C.A. Peavey 2002 (in press). Description of a nurse rig trailer used as support for Larviciding operations by the San Mateo County Mosquito Abatement Dsitrict. Proc. of the MVCAC. Proceedings and Papers of the Mosquito and Vector Control Association of California.

Goals Project, 1999. Baylands Ecosystem Habitat Goals. United States Environmental Protection Agency, San Francisco Calif / San Francisco Bay Regional Water Quality Control Board, Oakland, Calif.

Field Evaluation of Aqua-Reslin Against *Culex quinquefasciatus* Using Standard and Optimized Aerial Application Techniques

Allan Inman[1], David Sykes[2], Janice Stroud[2] and Bill Reynolds[3]

[1]*Merced County Mosquito Abatement District, P.O. Box 909, Merced, CA 95341*
[2]*Bayer Environmental Science, 95 Chestnut Road, Montvale, NJ 07645*
[3]*Adapco, Inc., 2800 South Financial Ct., Sanford, FL 32773*

ABSTRACT: Aqua-Reslin was applied against lab reared *Culex quinquefasciatus* utilizing standard and computer modeled optimized aerial ultra low volume application techniques. Both application methods recorded nearly identical 1-hour knockdown values (87%). The standard application provided 90.92% mortality at 12-hours post treatment while the optimized method resulted in 85.64% mortality of caged *Culex quinquefasciatus*.

INTRODUCTION

Aerial ultra low volume (ULV) adulticiding is a major component of the Merced County Mosquito Abatement District (MCMAD) disease and nuisance mosquito control strategy. Global positioning systems, improved droplet analysis, accurate meteorology, and the development of droplet fate predictive models has improved the safety and efficacy of aerial ULV applications. This study, utilizing Aqua- Reslin, compares the MCMAD's standard aerial application technique with a computer modeled optimized application strategy.

MATERIALS AND METHODS

Lab reared adult *Culex quinquefasciatus* mosquitoes were used for the trial. The mosquitoes originated from a susceptible colony maintained by the University of California Mosquito Research Laboratory in Parlier. The mosquitoes were blood fed three days prior to testing and transported to the laboratory facility of the MCMAD on the morning of the evaluation. The mosquitoes were transferred to screened adulticide test cages using hand-held insect vacuums. The test cages were stored in ice chests and held in the lab until treatment. The total number of mosquitoes in each cage ranged from 23 to 72.

Testing was conducted on April 30, 2001 at two sites. Site #1 was a 19-acre fallow parcel of property adjacent to the runway of the District while Site #2 was a 20-acre parcel of recently harvested oats located approximately 2 mi northeast of Site #1. Aqua-Reslin was aerially applied using standard spray techniques to Site #1 at 7:47 PM. A computer modeled optimized application of Aqua-Reslin was initiated at Site #2 at 8:06 PM. Weather data were recorded at various altitudes (15, 50, 100, and 200 ft) using the Adapco Kitoon System. During the application the Kitoon was positioned southeast of the standard and optimized sites. Air temperatures ranged from 66.1° at 200 ft to 61.2° at 15 ft. Prevailing northwest wind speeds of 13.3 knots were recorded at 200 ft. At 15 ft the winds were a calmer 4 knots. The weather data remained consistent during the applications.

Seven gallons of Aqua-Reslin was mixed with 32 gal of water to achieve a dosage rate of .007 lb. of permethrin per acre. Spray system calibration, 1.87 gal/min. was performed prior to treatment to insure a 3 oz/ac. application rate. The application was completed with the District's Bellanca Scout 8GCBC. The Scout is equipped with twin Micronair AU 5000 rotary atomizers. Following manufacturer specifications, the atomizers were set to rotate at 9,000 rpm to produce droplets in the 40-60 micron range. Rotary impingers with 1-inch magnesium oxide slides were placed at the 200, 600, 1,000, and 1,400 ft mark within each site. Droplet size ranged from 48 to 62 microns volume median diameter (VMD) in the standard application (Site #1) and 14 to 23 microns VMD in the optimized plot (Site #2). During both treatments the aircraft operated at a speed of 100 mph from an altitude of 200 ft. At site #1 (standard application) the initial pass was offset 500 ft upwind of the target area with three successive upwind passes at 400-ft intervals. Each pass was ¾ mi long and flown from east to west. At Site #2 (optimized application) the first pass was 6,000 ft upwind of the target area. Three more passes (1-mi long) were flown from east to west along the same path as the initial run without any offset.

The test plots consisted of a straight line of 8 stakes oriented perpendicular to the wind with 200 ft between each stake. Caged mosquitoes were placed in the plot just before each test. Two cages were attached to each stake in the test plot. The cages were collected 30 min after treatment and transported back to the laboratory. The mosquitoes were anesthetized with CO_2 and transferred to clean 1-pint plastic containers with screened lids. Raisins were wrapped in water soaked cotton balls and placed on top of the screen lids. Untreated control cages were handled in a similar fashion. Knockdown was observed at 1 hr while mortality was recorded at 12 hr post-treatment. Data were corrected for control mortality using Abbott's Formula.

RESULTS AND DISCUSSION

Average 1-hr knockdown and 12 hr mortality for standard and optimized aerial applications of Aqua-Reslin are presented in Table I. Untreated control knockdown and mortality was 10.51% and 12.94%, respectively. Average 1 hr knockdown figures for both application techniques were nearly identical. The 5% difference in 12 hr mortality figures between application

Table I. Efficacy of aerially applied Aqua-Reslin against caged *Culex quinquefasciatus*

Application	Insecticide	Rate	Post-Treatment 1-hour	12-hour
Standard	Aqua-Reslin	3 oz/acre	87.30%	90.92%
Optimzed	Aqua-Reslin	3 oz/acre	87.49%	85.64%
	Untreated		10.51%	12.94%

Mean percent knockdown at 1-hour and mortality at 12-hours

methods needs to be statistically analyzed for significance. Examining the reductions for each stake implies the standard application was not sufficiently offset since the lowest reductions (50% mortality) occurred in the 1st stake downwind of the application. The optimized flight recorded excellent reductions except at stake #2 (approximately 6,200 ft downwind). There may have been an obstruction at the surface (tree line, house, etc) or a weather anomaly that prevented sufficient droplet deposition at stake #2. Impressively, over 90% mortality was recorded 7,400 ft downwind from the release point in Site #2.

The results of the study suggest: 1) weather conditions aloft can be quite different from weather conditions at the surface, 2) the District's standard offsets are probably not sufficient to hit the leading edge of the target area, 3) larger droplets (48-62 microns) settled more quickly and provided comparable control to smaller droplets (14-23 microns), and 4) excepting the 6,200-ft mark, over 90% mortality was achieved from 1,900 to 7,400 ft downwind from the flight path of the aircraft indicating a killing zone over 1-mi wide. Consequently, the effective swath is larger than the 400-ft calibrated swath width.

Acknowledgements

The authors wish to thank Anton Cornel, Mosquito Research Lab., UC-Davis for providing the mosquitoes for this trial and personnel of the MCMAD for providing the manpower to complete the evaluation.

Notes on the Aerial Application of Trumpet In the Butte Sink

Kenneth Townzen[1], Michael Kimball[2], Ronald McBride[2], and David Whitesell[3]

[1] *California Department of Health Services, Vector-Borne Disease Section, 8633 Bond Road, Elk Grove, CA 95624*
[2] *Sutter-Yuba Mosquito and Vector Control District, Post Office Box 726, Yuba City, CA 95992*
[3] *Colusa Mosquito Abatement District, Post Office Box 208, Colusa, CA 95932*

ABSTRACT: This report presents information obtained during aerial applications of Nailed (TRUMPET® EC) in the Butte Sink area of Sutter and Colusa counties, California. Bioassays of caged adult mosquitoes indicated Naled susceptibility in *Ochlerotatus melanimon* Dyar at up to 2.5 miles downwind from the application and suggested resistance in *Culex tarsalis* Coquillett. Information on weather parameters was gathered several days before the application utilizing a Kitoon®. Wind speeds at 200 feet were considerably higher than those recorded near ground level.

INTRODUCTION

The Butte Sink includes over forty duck clubs, an Audubon bird sanctuary and U.S. Fish and Wildlife Service waterfowl habitat in Colusa and Sutter Counties. These duck hunting grounds total nearly 15,000 acres. Some of the clubs are summer flooded to produce bird feed and all of the clubs are flooded in the fall prior to duck season. Water becomes available and the duck club flooding begins around mid-August. In addition, several thousand acres of rice are produced in the area. After rice harvest, the fields are re-flooded for decomposition of rice straw and additional duck hunting habitat. Adjacent to this area to the north, and in Butte County, are an additional 7,000 acres of the Gray Lodge Waterfowl Management Area and many more duck clubs, equaling the acreage in Colusa and Sutter counties. The potential for *Oc. melanimon* production in this 50,000 acre plus habitat can become a district manager's worst nightmare. In the year 2001, it was!

A field trial was set up for the North Butte-Gray Lodge area adjacent to the Butte Sink in order to gain a better understanding of weather parameters at altitudes of 200 feet or more and to evaluate mosquito control potential at considerable distances downwind from the aircraft application. The experimental protocol called for two days of ground and aerial weather monitoring followed by the trial. On August 28, mosquito production in the Butte Sink was in full swing. As often happens, weather conditions during pre-monitoring were excellent and at trial time were unacceptable. The trial was cancelled due to wind speeds above 20 mph at ground level and over 30 mph at 100 ft.

Because of the high levels of mosquito production, it was determined that operational control would be more appropriate than evaluation on test lines. Two operational applications, August 30 and September 4 were evaluated and the results are presented in this report.

MATERIALS AND METHODS

In both applications, 78% Nailed (TRUMPET® EC, undiluted) was applied by air, using a Cessna Ag Wagon at an air speed of 120 mph and a height of 100 feet. The Cessna was equipped with two mid-wing mounted Micronair AU-5000 rotary atomizers with orifice setting #5 and 24 pounds pressure. Eleven swaths were applied at 500 foot downwind intervals and each swath was two miles long. With these flight parameters, the rate of coverage was 121 acres per minute. Application rate was 1 fl. oz. per acre. In both applications the area covered was 1 x 2 miles, approximately 1300 acres, and took 11 minutes of spray time. The applications, including turning time, took about 20 minutes. Initial offset was 1500 feet upwind.

Elevated atmospheric conditions were monitored with an ADAPCO Kitoon® system with wind speed sensor, wind direction indicator, and temperature sensor. Aerial instrumentation and ground instruments were connected with fiber optic cable. The location of the weather station was 3 miles northeast of the application.

Slide rotators were utilized to collect aerosol droplets at the Butte Creek and Crocker Levee sites during the August 30 application. Three by one inch Teflon coated slides were used with a BioQuip Aerosol Droplet Sampler.

Mortality of caged mosquitoes was used to evaluate the application. Twenty to thirty adult mosquitoes were aspirated into disposable paper and nylon net cages (Townzen and Natvig 1973). Cages were collected approximately one hour after the last swath and the mosquitoes were supplied with sugar water. Cages were then placed individually in plastic bags and returned to the lab in ice chests for mortality evaluation.

On run 1, August 30, caged adult mosquitoes were placed in the open at selected sites (Figure 1) in and downwind of the target

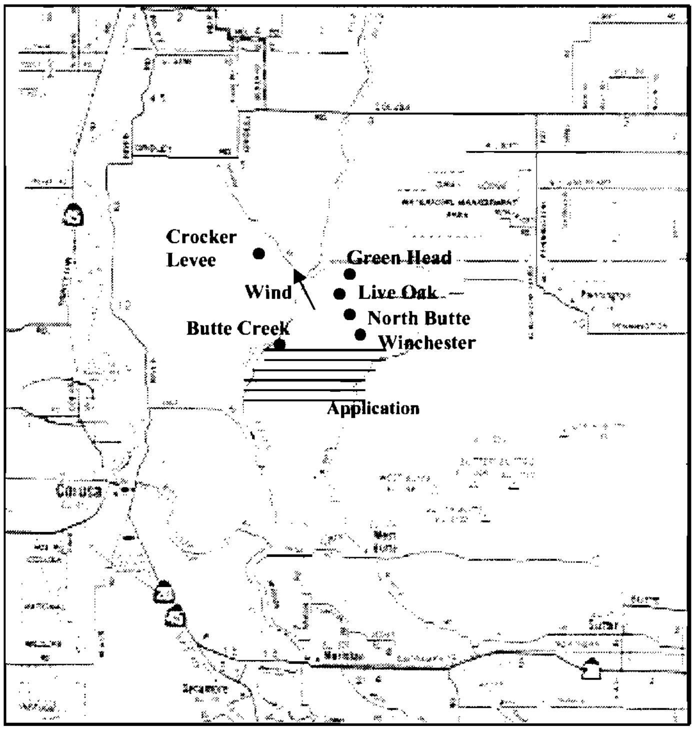


Figure 1. Location of caged mosquitoes during application of TRUMPET® EC by air, Butte Sink area of Colusa and Sutter Counties, California, August 30, 2001.

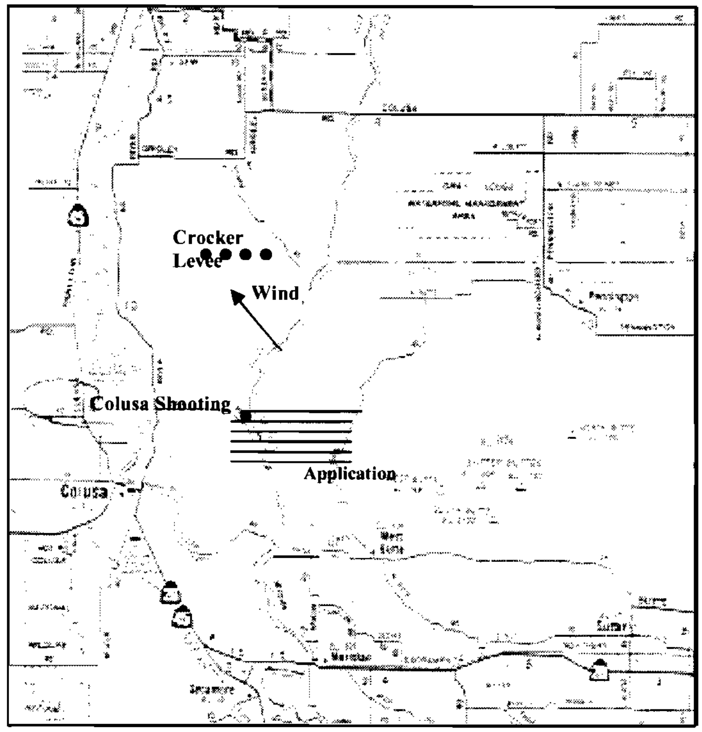


Figure 2. Location of caged mosquitoes during application of TRUMPET® EC by air, Butte Sink area of Colusa and Sutter Counties, California, September 4, 2001.

area. Sites were chosen for accessibility and evaluation purposes. Each site had 3 cages each of wild caught *Ochlerotatus melanimon,* wild caught *Culex tarsalis,* and laboratory-reared, susceptible *Cx. tarsalis.* One cage of each was affixed atop a 3 foot surveyors lath and three laths were staked to the ground 30 feet apart. Wild caught mosquitoes were of indeterminate age and the laboratory reared *Cx. tarsalis* were 4 days old.

On run 2, September 4, caged wild caught *Oc. melanimon* were placed at selected sites (Figure 2) for accessibility and evaluation purposes. At the Colusa Shooting site, 2 cages were placed in the open, but under a heavy canopy of cottonwood trees. Two additional cages were placed nearby under the heavy canopy of trees and inside heavy cover of grapevine and poison oak. At the Crocker Levee site, caged mosquitoes were placed in the open at 4 sites, ¼ mile apart in an east-west line as far to the west as terrain permitted (approximately 1.5 miles). Controls were placed 5 miles to the south west of the target area.

RESULTS

Monitored atmospheric conditions showed wind speeds of 6, 11, 15, and 17 mph at elevations of 6, 100, 150 and 200 feet respectively. Temperature measurements were 72.1°F, 72.3°F, and 72.1°F at 100, 150, and 200 feet respectively.

Drop analysis at the Butte Creek site indicated drops of 14.0 microns MMD. One drop was collected at the Crocker Levee and it measured 7 microns MMD.

On August 30, one hundred percent mortality was observed in 5 of 6 cages of *Oc. melanimon (Table 1).* Caged laboratory reared *Cx. tarsalis* had 100% mortality at a distance of .75 miles. Wild caught *Cx. tarsalis* had 91% mortality in an open area within the application area and 11% or less mortality in the remaining stations downwind.

On September 4, at Colusa Shooting, one hundred percent mortality of *Oc. melanimon* was observed in the cages under the canopy and 60 % in thick brush (Table 2). At the Crocker Levee site, 5 miles downwind, mosquito mortality increased from 17% to 88%, from east to west. No *Cx. tarsalis* were available for this application.

DISCUSSION

In planning and implementing a sizable field test, procedures may not always go as planned. Weather conditions can not be predicted on a day-to-day basis. Wind speeds at ground level are not necessarily the same at higher elevations, having a tendency to increase with height. Cage placement can be on the edge or out of the downwind effective control zone; as in the Green Head site on the first run or the effect on the western cage on the Crocker Levee, run 2. The increased mortality, east to west, on the Crocker Levee was likely due to increased cloud density.

On evaluation of the operational field applications, with a relatively small amount of data collected, several conclusions or indications can be made:

Table 1. Percent mortality of caged mosquitoes at 12 hours, following aerial application of TRUMPET® EC in the Butte Sink, Colusa Mosquito Abatement district, August 30, 2001.

Distance (miles)	*Oc. melanmon*	Lab *Cx. tarsalis*	Wild *Cx. tarsalis*
0.0	100	100	91
.25	100	100	6
75	100	100	3
1.75	100	95	4
2.25	58	39	11
2.50	100	56	8
Control	5	3	12

Table 2. Percent mortality of caged *Oc. melanimon* at 12 hours, following aerial application of TRUMPET® EC in the Butte Sink, Colusa Mosquito Abatement district, September 4, 2001.

Colusa Shooting			
Under Canopy		Under Canopy and Thick Brush	
100		60	
Crocker Levee			
West cage	West Center	East Center	East Cage
88	38	18	17

1) The aircraft configuration and nozzles produced a relatively small droplet, producing an aerosol-like effect.
2) The aerosol cloud build-up had considerable effectiveness in adult mosquito control.
3) Bioassays are a useful and sensitive indicator of insecticide dispersal.
4) *Oc. melanimon* are highly susceptible to Naled.
5) Wild caught *Cx. tarsalis* from the Butte Sink area show less sensitivity than laboratory-reared *Cx. tarsalis* to Naled.

Following field trials, the Sutter-Yuba Mosquito and Vector Control District conducted a laboratory filter paper bioassay to determine susceptibility levels of colonized *Cx. tarsalis* to Naled. Preliminary results showed the colonized wild *Cx. tarsalis* have moderate levels of resistance to the material (Debra Lemenager, personal communication). Future plans are to conduct further laboratory testing on both the colonized and wild *Cx. tarsalis*.

Acknowledgements

The authors wish to thank staff and biologists from the Colusa Mosquito Abatement District, Sutter-Yuba Mosquito and Vector Control District, Butte County Mosquito and Vector Control District, Sacramento-Yolo Mosquito and Vector Control District and staff from the California Department of Health Services, Vector-Borne Disease Section. Special thanks go to Fennimore Chemicals and Adapco for providing the Kitoon® weather system and assistance in the field.

REFERENCES

Townzen, K.R. and H.L. Natvig. 1973. A disposable adult mosquito bioassay cage. Mosq. News. 33(1): 113-114.

Larval Control in Rice Fields Utilizing the Icy Pearl Method

D.B. Jacks[1], P. DeChant[2], G. Yoshimura[1]

[1]Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Road, Elk Grove, CA 95624
[2]Valent BioSciences, Inc., 16443 SE Meadowland Court, Portland, OR 97236

ABSTRACT. Field test trials consisting of Icy Pearls, *Bacillus thuringiensis var. israelensis* (B.t.i.) suspended in ice pellets were applied by helicopter using an insulated sling hopper. Rice field acreage was measured by GPS to determine the sampling regimen and the amount of Icy Pearls required to treat the fields. The 48 h post-treatment sampling showed a reduction of *Culex tarsalis* larvae by 96.5% in the field treated with 3.33% Icy Pearls and a reduction of 78% in the field treated with 2.2% Icy Pearls. *Anopheles freeborni* reduction was 97.5% with the 3.33% formulation and 100% with the 2.2% formulation.

Key words: *Culex tarsalis, Anopheles freeborni*, Icy Pearl, *Bacillus thuringiensis var. israelensis* (B.t.i.), aerial application, insulated sling hopper, mosquito control.

INTRODUCTION

The control of *Culex tarsalis* Coquillett and *Anopheles freeborni* Aitken mosquito larvae in rice fields has been a concern of many mosquito control districts in the rice growing regions of California. Numerous methods and materials have been utilized in an attempt to maintain mosquito levels below a threshold that is tolerable to the general public. Studies dealing with the efficacy of bacterial insecticides (Garcia et al. 1981, Federici 1995) in relation to their rate of ingestion (Aly et al. 1988, Mahmood 1998); stability (Tousignant et al. 1992, Thiery and Hamon 1998); formulations (Wilmot et al. 1993, Su and Mulla 1999); and the influence of environmental factors (Mulla et al. 1990, Becker et al. 1992, Nayar et al. 1999) while dealing with different other species occurring in rice fields, would still apply in this situation.

Icy Pearl is a recently developed application method in which an aqueous suspension of *Bacillus thuringiensis var. israelensis* (B.t.i.)(serotype H-14) is applied in a frozen state. The development of the Icy Pearl method originated in Germany by Peter Mercatoris, IcyBac Corporation, and Dr. Norbert Becker, Scientific Director, German Mosquito Control of Mosquitoes (KABS/GFS). It is manufactured by introducing small drops of a B.t.i. suspension into a liquid nitrogen bath, which quickly freezes the liquid medium into very small pellets.

The rationale behind the process is that the method will improve delivery of B.t.i. to the target site. The density of the ice gives more weight to the individual unit containing the active ingredient, thus increasing a higher percentage of penetration to the water surface through dense and/or taller vegetation (in mid- to late season rice fields). In addition, since the ice floats, the active ingredient is released at the water surface. By increasing penetration of the material and with the release of the active ingredient at the water surface, a higher larval mortality should be noted, as less material would be lost to vegetation or substrate. The material is currently being used in Germany along the Rhine River to control *Aedes vexans* (Meigen) with satisfactory results (Peter Mercatoris, personal communication).

MATERIALS AND METHODS

Three rice fields in Sutter County, California were used for this trial. One field served as the untreated control (UTC) while the other two fields were treated with different concentrations of the Icy Pearl mixtures. A 3.33% B.t.i. suspension in ice was applied to the first field and a lower 2.2% mixture was applied to the second field as a comparison. The untreated control field (Sutter Yuba MVCD Field #799) was 61 ac., the field treated at the higher B.t.i. rate (Sutter Yuba MVCD Field #794) was 35 ac. and the field treated at the lower rate (Sutter Yuba MVCD Field #806) was 55 ac. in size as determined by GPS Model- Landstar MkIV, Type-90952-Sk8. Company: RACAL NCS. (America). Handheld recording device: Compaq Aero 2100 series. Software: Tripod Data Systems, Inc., Solofield for Windows CE. The field sites are located within a GPS location area reading of 38° 48' 39"N, 121° 30' 39"W for field #806, 38° 47' 47"N, 121° 29' 30"W for field #799, and 38° 47' 31"N, 121° 28' 59"W for field #794.

Sampling was accomplished using standard dipping technique (Reed and Husbands 1970) and was confined to the edges of the fields. The number of dips taken per field was determined by the field acreage. A total of 80 dips were taken for the smallest field of 35 ac. and 160 dips were taken for each of the two larger fields. All four sides of each field were sampled. An equal number of dips were taken for each field side. The sampling regimen consisted of a two-day pre- and post-treatment monitoring. The pre-treatment sampling was initiated on August 7th, 2001 and the final post-treatment samples were taken on August 10th and 11th.

Two WatchDog™ Data Loggers, Model 450 (Spectrum Technologies, Inc.), were placed in each field to monitor the air and water temperatures at 5-min intervals from the day of treatment to the final sampling day, 48 h later. The data loggers were placed in the fields to address the concern expressed by the rice growers that there might be a change in the water temperature with the application of Icy Pearl.

Four sentinel containers (Rubbermaid storage type containers) were used per field to monitor the Icy Pearl application. Each container had 6 styrofoam strips attached on its sides with Velcro to keep the container level and afloat and was filled with 3.9 gal of rice field water. Forty 4[th] instar *Culex tarsalis* larvae from the Sac-Yolo MVCD, lab-raised Bakersfield strain colony, were placed in each container. A total of 160 larvae were used as sentinels per field. The placement of the sentinel containers and the WatchDog™ Data Loggers was limited to areas of the fields devoid of rice.

A 3.33% VectoBac WDG (Valent BioSciences Corporation, Libertyville, IL) concentration was applied in field #794 with an application rate of 0.232 lbs product in 6.7 lbs ice per ac.; with a total of 235 lbs ice applied over 35 ac. For comparison to commercial products, this rate is equivalent to 3.45 lbs VectoBac G per ac. or 9 ounces of VectoBac 12AS per ac. In field #806, a 2.2% concentration of VectoBac WDG was applied at an application rate of 0.127 lbs product in 6.7 lbs ice/ac. A total of 369 lbs ice was applied over 55 ac. This rate is equivalent to 1.95 lbs VectoBac G/ac. or 5 ounces VectoBac 12AS/ac.

The Icy Pearls were stored in ice chests in a refrigerated trailer until the application time was set and loaded into a foam insulated sling hopper just prior to its application. A Bell Jet Ranger Helicopter towed the sling hopper. The sling attachment was 20-ft in length, and instructions were given to the pilot to fly at an altitude of 50-ft when dispersing the ice pellets, which would have maintained the hopper at a height of 20-ft above the fields. At the 20-ft delivery height of the sling hopper, the swath width was measured to be 60-ft. In addition the pilot was instructed to stop release of the material 100-ft prior to the edge of both fields. The pellets were applied by the pilot while he flew in an east and west direction on both fields. The application on field #794 began at 0912 h with the wind gusting from 6-13 mph from the SE during the entire treatment. Treatment of field #806 began at 0946 h with the wind from the SE gusting to a maximum of 6.8 mph.

RESULTS

Fields #794 and #806 displayed a decrease in the average collection of *Culex tarsalis* during the 48 h post-treatment sampling period of 90.2% and 35% respectively, while numbers in the untreated control field increased 109% (Table 1). Overall corrected *Culex tarsalis* mortality was 96.5% and 78% respectively for the two fields. (Table 1)

Data on *Anopheles freeborni* in field #794 showed 96.2% reduction during the 24 h post-treatment period (Table 2). However the 48 h sampling period the number of larvae collected increased with an uncorrected population reduction of 90.75%. In field #806 *An. freeborni* numbers decreased 100% over the sampling period. Populations in the untreated control increased 300% during the 48 h post-treatment sampling period. Overall corrected *Anopheles* mortality was 97.5% and 100% respectively for the two fields.

The untreated control field showed a slight reduction in larval numbers for the 24- h post-treatment sampling period for both *Cx. tarsalis* and *An. freeborni*. (Tables 1 and 2)

Larval mortality in sentinel containers was 100% in the two treated fields with the exception of container number 8 on the south side of field #794 where the mortality of only 17% was observed (Table 3). In the untreated field, overall sentinel mortality was 1.25%.

Table 1. Reduction in larval population *Culex tarsalis* in rice fields treated with the Icy Pearl formulation of *Bacillus thuringiensis israelensis* (VectoBac®).

Field Surveyed	2 Day Average Pre Treatment Sampling. Larvae/dip	24 Hour Post Treatment Sampling Reduction Rate %	48 Hour Post Treatment Sampling Reduction Rate %	Corrected Percent Control at 48 hours
#799, UTC	10.75	21%	-109%	NA
#794, 0912 h, 35 Ac. (3.33%)	10.25	75.6%	90.2%	96.5%
#806, 0946 h, 55 Ac. (2.2%)	10	20%	35%	78%

Table 2. Reduction in larval population of *Anopheles freeborni* in rice fields treated with the IcyPearl formulation of *Bacillus thuringiensis israelensis* (VectoBac®).

Field Surveyed	2 Day Average Pre Treatment Sampling. Larvae/dip	24 Hour Post Treatment Sampling Reduction Rate%	48 Hour Post Treatment Sampling Reduction Rate%	Corrected Percent Control at 48 hours
#799, UTC	0.625	20%	-300%	NA
#794, (3.33%)	13.5	96.2%	90.75%	97.5%
#806, (2.2%)	4.9	89.8%	100%	100%

Table 3. Mortality of *Culex tarsalis* sentinel larvae exposed to aerial application of Icy Pearl formulation of B.t.i. in rice fields.

Field Surveyed	Container Designation No.	Larvae # Start	24 Hour Post Treatment Sampling % Affected or Loss
#799, UTC	12	40	2.5%
	11	40	2.5%
	10	40	0
	9	40	0
#794, (3.3%)	8	40	17.5%
	7	40	100%
	6	40	100%
	5	40	100%
#806, (2.2%)	4	40	100%
	3	40	100%
	2	40	100%
	1	40	100%

A variety of factors were observed which might have hindered the application. Factors such as large trees and nearby homes in line with the flight path, and gusting winds, resulted in poor maneuverability of the aircraft, and thus had influence of inadequate coverage of the field. In addition, with unfamiliarity of proper usage of the sling hopper, a new piece of application equipment and technology, this too may have been a factor with respect to the success of application.

Specifications for application were originally gathered from Florida in which a different airship was used, and a lane separation of 60-ft was chosen for the rice field study based on the previous work. The airspeed was higher than the work in Florida, which may have narrowed the swath width and increased the coefficient of variation in the application rate, thus contributing to the inadequate coverage of the fields treated with Icy Pearl.

Monitoring of the rice field water temperature using the WatchDog™ Data logger showed no change in temperature after the Icy Pearl application. The size and amount of ice pellets applied to the water surface was miniscule compared to the volume of water contained in the rice paddies.

In conclusion, we had some unusual results. Overall, however, the Icy Pearl method provided good larval control. The method has proven successful in Germany, but further trials with the Icy Pearl method are necessary to improve success in controlling *Culex tarsalis* and *Anopheles freeborni* larvae in the rice fields of the Sacramento Valley.

Acknowledgements

The authors wish to thank Steve Abshier for finding and providing maps of the fields and also Ron McBride and Mike Kimball of Sutter-Yuba MVCD for their time and allowing us to use the fields within their district. We would also like to thank Stephanie Whitman of Valent Biosciences for her invaluable assistance, Peter Mercatoris of Phoenix Aviation/IcyBac Corporation for providing the know how and history, David Brown, James Clauson and Kara Kelley of Sacramento-Yolo MVCD for their assistance and Alpine Helicopter Service of Lodi, CA.

REFERENCES CITED

Aly, C., M.S. Mulla, B-Z. Xu, and W. Schnetter. 1988. Rate of ingestion by mosquito larvae (Diptera: *Culicidae*) as a factor in the effectiveness of a bacterial stomach toxin. *J. Med. Entomol.* 25: 191-6.

Becker, N., M. Zgomba, M. Ludwig, D. Petric and F. Rettich. 1992. Factors influencing the activity of *Bacillus thuringiensis var. israelensis* treatments. *J. Am. Mosq. Control Assoc.* 8: 285-9.

Federici, B.A. 1995. The future of microbial insecticides as vector control agents. *J. Am. Mosq. Control Assoc.* 11: 260-8.

Garcia, R., B. Desrochers and W. Tozer. 1981. Studies on *Bacillus thuringiensis var. israelensis* against mosquito larvae and other organisms. *Proceedings and Papers, Mosquito and Vector Control Association of California.* 49: 25-9.

Mahmood, F. 1998. Laboratory bioassay to compare susceptibilities of *Aedes aegypti* and *Anopheles albimanis* to *Bacillus thuringiensis var. israelensis* as affected by their feeding rates. *J. Am. Mosq. Control Assoc.* 14: 69-71.

Mercatoris, P. Personal communication.

Mulla, M.S., H.A. Darwazeh, and M. Zgomba. 1990. Effect of some environmental factors on the efficacy of *Bacillus sphaericus* 2362 and *Bacillus thuringiensis* (H-14) against mosquitoes. *Bull. Soc. Vector Ecol.* 15: 166-75.

Mulla, M.S., R.L. Norland, D.M. Fanara, H.A. Darwezah, and D.W. McKean. 1971. Control of chironomid midges in recreational lakes. *J. Econ. Entomol.* 64: 300-307.

Nayar, J.K., J.W. Knight, A. Ali, D.B. Carlson and P.D. O'Bryan. 1999. Laboratory evaluation of biotic and abiotic factors that may influence larvicidal activity of *Bacillus thuringiensis var. israelensis* against two Florida mosquito species. *J. Am. Mosq. Control Assoc.* 15: 32-42.

Reed, D.E., and R.C. Husbands. 1970. Integration of larval surveillance techniques in the operational program of the Fresno Westside mosquito abatement district. *Proc. Calif. Mosq. Control Assoc.*, 37: 98-101.

Su, T. and M.S. Mulla. 1999. Field evaluation of new water dispersible granular formulations of *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* against *Culex* mosquitoes in microcosms. *J. Am. Mosq. Control Assoc.* 15: 356-65.

Thiery, I. and S. Hamon. 1998. Bacterial control of mosquito larvae: Investigation of the stability of *Bacillus thuringiensis var. israelensis* and *Bacillus sphaericus* standard powders. *J. Am. Mosq. Control Assoc.* 14: 472-6.

Tousignant, M.E., J.L. Boisvert and A. Chalifour. 1992. Reduction of mortality rates of *Bacillus thuringiensis var. israelensis* aqueous suspensions due to freezing and thawing. *J. Am. Mosq. Control Assoc.* 8: 149-55.

Wilmot, T.R., D.W. Allen and B.A. Harkanson. 1993. Field trial of two *Bacillus thuringiensis var. israelensis* formulations for control of *Aedes* species mosquitoes in Michigan woodlands. *J. Am. Mosq. Control Assoc.* 9: 344-5.

Aedes albopictus Infestations in California

Kenneth J. Linthicum, Vicki Kramer, and The Surveillance Team[+]

Vector-Borne Disease Section, California Department of Health Services (CDHS), 2151 Convention Center Way, 601 North 7th Strreet, MS 486, PO Box 942732, Sacramento, CA 94234.

ABSTRACT: *Aedes (Stegomyia) albopictus* (Skuse) was discovered in June 2001 at the Port of Los Angeles in a cargo container from China containing a shipment of the commercial product known as "Lucky Bamboo" (*Dracaena* spp.) To keep the plants alive during the ocean transit, plants were shipped in 2-3 inches of water, providing an excellent habitat for *Ae. albopictus.* Mosquito infestations were subsequently detected at 15 nurseries/distributors in 6 counties in northern and southern California. Vector control operations were conducted and follow-up surveillance in and around infestation sites is ongoing. The Centers for Disease Control and Prevention issued an embargo on shipments of *Dracaena* spp. in standing water. Subsequent dry shipments have been received at several nurseries and no *Ae. albopictus* have been found during extensive inspections. Additionally, a thorough examination of individual *Dracaena* plants in one maritime container, embargoed because it had small amounts of standing water, failed to detect *Ae. albopictus.* As of December 2001 it is uncertain whether the distribution of *Ae. albopictus* is limited to those nurseries/distributors with documented infestations; however, infestations have persisted for more than 5 months near some of the nurseries, and eggs have been found up to 1000 meters from the original infestation sites.

Aedes (Stegomyia) albopictus (Skuse) was detected in June 2001 at the ports of Los Angeles and Long Beach and subsequently in at least fourteen wholesale/secondary distributor plant nurseries in California. This exotic mosquito was imported from China in shipments of *Dracaena* species sold as "Lucky Bamboo." Historically, this ornamental plant was imported to the United States from China and other Asian countries in dry containers via airfreight. However, due to increased demand for this product, shipments began to arrive in approximately January 2000 via cargo ships. To keep the plants green during this ocean-journey, the plants were shipped in 2-3 inches of water, thereby providing habitat for mosquito larvae. This was only the third time that *Ae. albopictus* has been found in California. It was previously found in tires in Oakland in 1971 and again in Oakland in the 1980s, but both introductions were very small in terms of numbers of mosquitoes and spatial extent, compared to the current infestation.

Aedes albopictus is a very aggressive biter and a known vector of dengue virus in Southeast Asia, Southern China, Japan, and the Seychelles, second in importance only to *Ae. aegypti.* As a maintenance vector and occasionally as an epidemic vector, it is responsible for many thousands of human cases of dengue, dengue hemorrhagic fever, and dengue shock syndrome in Asia. Dengue virus can infect *Ae. albopictus* oviducts and be transmitted to its eggs. This leads to inefficient but effective transovarial transmission of the virus. Dengue is hyperendemic in Southeast Asia and transmission is currently at an extremely elevated level. The mosquito is also potential vector of yellow fever, LaCrosse encephalitis, dog heartworm, and other diseases in this country.

Aedes albopictus was initially discovered in a cargo container by a USDA/APHIS Plant Protection and Quarantine Officer, Centers for Disease Control and Prevention (CDC) Quarantine Officers, and personnel from the Greater Los Angeles County Vector Control District. Subsequent investigations at wholesale nurseries by local mosquito and vector control agencies and county health departments documented additional infestations at 7 locations in Los Angeles County, 1 in Santa Clara County, 2 in San Bernardino County, 3 in Orange County, 1 in San Joaquin County and 1 in San Diego County (Table 1, Figure 1). Intensive vector control operations were conducted at all infestation sites. Mosquitoes were trapped and tested for the presence of viral pathogens, including dengue. On June 29, the CDC initiated an embargo of shipments of *Dracaena* in standing water, permitting shipments arriving before July 17 to be treated.

Mosquitoes from two sites were trapped and tested for the presence of viral pathogens. Seven and 14 *Ae. albopictus* submitted from Santa Clara County VCD and San Joaquin County MVCD, respectively, were tested for dengue, Saint Louis encephalitis, Murray Valley, and Japanese encephalitis; all were negative.

Local mosquito and vector control districts, county public health agencies, and VBDS expanded surveillance activities in and around all wholesale nurseries involved in *Dracaena* spp. distribution to determine whether other nurseries may be infested, and to determine if the infestation had spread outside of the nurseries. At two nurseries in Los Angeles County, adult mosquito activity was detected into November, more than five months

[+] Nabil Armanious, Ronald Hennessy, USDA/APHIS; Rey Fernandez, Michael Marty, CDC; Clifford Smith,USDA/APHIS; Minoo Madon, Michael Shaw, Susanne Kluh, Jack Hazelrigg, Greater LA Co. VCD; Mir Mulla, UCR; Kenn Fujioka, Warthuro Campugan, Angela Brisco, Gary Hawthorne, Melvin Cook, Kelly Middleton, Steve West, San Gabriel Valley MVCD; Noor Tietze, Mike Stephenson, Santa Clara Co.VCD; Jeff Beehler, Min-Lee Cheng West Valley MVCD; James D. Lang, Horace Ketcham, Michael Devine, Marilyn Corodemas, San Diego Co. DEH; Stacy Bearden, Ed Lucchesi, San Joaquin Conty MVCD; Robert Gay, Chindi Peavey, San Mateo Co. MAD; James Webb, Robert Cummings, Matt Robinson, Beth Drummond, Ralph Havickhorst, Bob Sjogren, Orange Co.VCD; Art Tilzer, Los Angeles Co..DHS; Todd Walker, Renjie Hu, Charles Myers, Marco Metzger, VBDS-Southern Region/CDHS; Ken Townzen, Mark Novak, VBDS-Northern Region/CHDS; Lucia Hui, Al Hom, Denise Steinlein, Stan Husted,VBDS-Coastal Region/CDHS; Steve Schutz, Contra Costa MVCD; Branka Lothrop, Coachella Valley MVCD; Wakoli Wakesa, San Bernardino Co. VCP; Zenaida Siruno-Savella, Lamar Rush, Long Beach DHHS; John Edman, Tom Scott, Robert Chiles, UC Davis 95616; Susan Dwyer, USPHS; Melvyn Seid, San Francisco DPH; Tom Zavortink, USF.

Table 1. Chronology of the discovery of *Aedes albopictus* in California in 2001.[1]

Date	Infestation Number	Location	Agencies Involved[2]	Findings/Actions[3]
June 7-15	First discovery in maritime container	Los Angeles Harbor	USDA/APHIS, CDC, GLACVCD, UCR	Adults collected, identified
June 16-20	Second discovery in maritime container	Long Beach L.A. County	GLACVCD, UCR, USDA/APHIS	Larvae collected, containers adulticided
June 22, 23	1	Rowland Heights L.A. County	GLACVCD, DHS	Adults feeding, larvae collected
June 27	2	Monterey Park L.A. County	San Gabriel Valley MVCD, DHS	Adults feeding
June 29, July 2	3	San Martin Santa Clara County	Santa Clara County VCD, DHS	Larvae and adults collected
July 3	4	Alhambra L.A. County	San Gabriel Valley MVCD	Adults collected
July 6	5	Chino San Bernardino County	West Valley MVCD	Larvae and adults collected
July 6	6	Chinatown L.A. County	GLACVCD	Larvae and pupae collected
July 9	7	Chino San Bernardino County	West Valley MVCD	Adults collected
July 12	8, 9, 10	Brea, Costa Mesa, Westminster Orange County	Orange County VCD	Adults collected
July 17	11	Vista San Diego County	San Diego VSP	Larvae collected, plant water drained. August 7 adults collected
July 19	12	South San Francisco San Mateo County	San Mateo County MAD, DHS	Pupa collected at USDA inspection site
July 20		Lodi San Joaquin County	San Joaquin County MVCD, DHS	Larvae, pupae and adults collected
July 23	13	City of Industry L.A. County	San Gabriel Valley MVCD	Larvae collected, plant water drained
August 13	14,15	El Monte L.A. County	San Gabriel Valley MVCD	Adults collected, plant water drained

[1] No *Aedes albopictus* infestations were detected by agencies conducting surveillance in the following counties: Alameda, Contra Costa, Fresno, Lake, Riverside, Sacramento, San Francisco, Santa Cruz, Solano, and Yolo.

[2] APHIS, Animal & Plant Health Inspection Service; CDC, Centers for Disease Control and Prevention; GLACVCD, Greater Los Angeles County Vector Control District; DHS, California Department of Health Services; L.A. County; Los Angeles County; MVCD, Mosquito and Vector Control District; UCR, University of California, Riverside; USDA, United States Department of Agriculture; VCD, Vector Control District; VSP, Vector Services Program; MAD, Mosquito Abatement District.

[3] Extensive larviciding and/or adulticiding was performed at all sites.

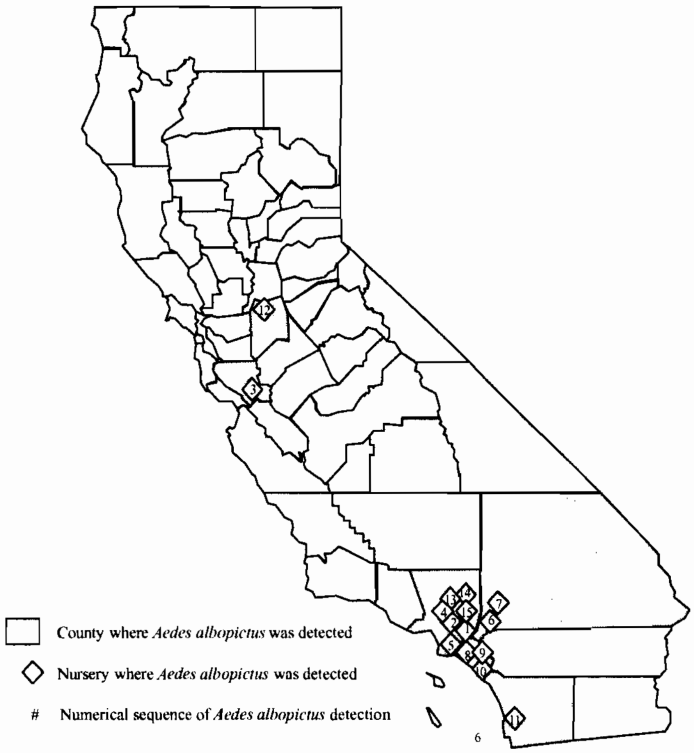


Figure 1. Location, by county, where *Aedes albopictus* infestations were discovered in California in 2001. Counties shown in blue had one or more documented infestations.

subsequent to initial detection. Surveillance will be conducted at all infestation sites in 2002 to determine whether *Ae. albopictus* persisted through the winter months.

VBDS recommended that the following activities be conducted to contain and/or eradicate *Ae. albopictus*: (1) apply adulticides to plant holding areas, and sustained release methoprene to water in plant holding containers where *Ae. albopictus* is detected; (2) continue to monitor adult populations after insecticide treatments to ensure that the population of *Ae. albopictus* is eradicated at the nursery; (3) monitor *Ae. albopictus* populations around the periphery of infested sites and in the vicinity of the site, utilizing oviposition traps or other appropriate surveillance methods to determine if populations have been established outside the borders of the nursery; (4) continue spot monitoring of infestation sites as future shipments are received; and (5) train warehouse staff on methods to reduce infestation risk (e.g. maintaining water levels to prevent repeated drying and reflooding of *Ae. albopictus* eggs should they be present).

Although there is an embargo on *Dracaena* shipments in standing water, the moist stalks of the plants may serve as a substrate for oviposition. Therefore, in collaboration with the CDC, VBDS developed recommendations for the processing and shipment of *Dracaena* species by exporters in Asia. Recommendations were designed to reduce the risk of further introductions of *Ae. albopictus* in dry shipments sent to California. Recommendations included ensuring that all steps in the processing of *Dracaena* shipments from the time plant stalks are first placed in water until the time the plants are placed in maritime containers to the port should be conducted in mosquito-free facilities. These facilities should have screens and doors that close automatically. Mosquito adulticides should be applied regularly as space sprays in the facility to kill any mosquitoes that may enter. Additionally we have recommended that a residual insecticide treatment be administered to each individual box containing *Dracaena* just before being packed into the maritime shipment container and sealed. This treatment is to control any adult mosquitoes that may be present in the maritime container or in the *Dracaena*. VBDS encouraged vector control districts and local health departments to share these recommendations with wholesale importers of *Dracaena* within their respective jurisdictions and that these guidelines be provided to exporters in Asia.

Acknowledgements

The authors acknowledge the significant contributions of the following individuals: Chester Moore, Roger Nasci, Harry Savage and Duane Gubler, Division of Vector-Borne Infectious Disease, CDC, Ft. Collins, CO; and Tony Perez and David Kim USPHS/CDC/PPQ/DGMQ, Atlanta, GA in providing expert advice and assistance in recognizing, controlling and preventing further infestations of *Ae. albopictus* in California. The authors also acknowledge all the participating staff of the various organizations involved in *Aedes albopictus* surveillance and control not previously mentioned. Special thanks to Curtis Fritz, VBDS-Headquarters/CDHS, for preparing the distribution map of infestation sites.

Development and Implementation of Greater Los Angeles County Vector Control District's Non-Medical Emergency Response Africanized Honeybee Program[1]

Jack Hazelrigg[2]

Greater Los Angeles County Vector Control District, 12545 Florence Avenue, Santa Fe Springs, CA 90670

ABSTRACT: Due to the expected entrance of Africanized honeybees (Apis mellifera scutellata) into California, the Los Angeles County Agricultural Commissioner formed an Africanized honeybee (AHB) task force in Los Angeles County late in 1993, an alliance of regional and local public health, education, and police and fire agencies. The purpose of the task force was to develop a response plan to the AHB entering Los Angeles County. A two part response plan was developed: 1) a medical emergency response plan, involving county and city fire departments responding to multiple stinging incidents; and 2) a non-medical emergency response plan, involving the 5 established county mosquito and vector control districts responding to service requests to remove swarms and hives. Early in 1994, prior to the arrival of AHB into Los Angeles County, the Greater Los Angeles County Vector Control District (GLACVCD) formed its non-medical emergency response program, consisting of specialized field equipment, additional trained staff, specific operational protocols, and published educational and informational materials. In additional, within one year, an outside centralized communications service agency was mutually employed by the 5 participating districts to facilitate and expedite the routing of county-wide AHB service request calls. Upon arrival of AHB into Angeles County in late 1998, GLACVCD's response program developed in 1993 proved inadequate and insufficient; the District needed to decide either to abandon or to augment the program. A decision was made to augment the program the following fiscal year and commit to a high level of service for a minimum of three years. Additional revenue was raised to increase personnel, refine elements of the program's administrative and operational procedures, and provide additional equipment. Since the decision to improve and supplement the AHB program was made, GLACVCD operates a satisfactory non-medical emergency response plan throughout its 1330 square mile service area.

INTRODUCTION

In October 1990, abejas asesinas (Spanish for the Africanized honey bee or "killer bee") entered Texas from Mexico. In California, the northward migration of the Africanized honey bee (AHB), *Apis scutellata*, into the United States eventually prompted the state of California's Department of Health Services (CDHS) to form a "Statewide AHB Steering Committee." The Committee of CDHS, university, and state and county agricultural officials, organized primarily to coordinate information regarding research, movement, and location of AHB. However, it also encouraged and urged officials of local health and agricultural agencies to consider developing emergency AHB public health response and education plans. Today, although the Steering Committee has no enforcement authority, its activities of reviewing AHB events, collecting, centralizing and disseminating AHB information, assisting local agencies in developing AHB training, response and educational plans, and reviewing and making recommendations on regulatory ordinances regarding the responses to AHB remain important functions.

Late in 1993, because of the westward migration and entrance of AHB from Texas into New Mexico and Arizona, officials from the Los Angeles County Agricultural Commissioner spearheaded the formation of a county-wide, local "Africanized Honey Bee Task Force," functioning independently of the state's Steering Committee. The task force, which remains active today, is led by the county's Agricultural Commissioner and is an alliance principally of representatives from the Agricultural Commissioner's office, the county and municipal health departments, the county and municipal fire departments, the local beekeeper's association, the pest control industry, and the five independent mosquito and vector control districts.

At its inception, the Task Force's primary consideration was to assemble an AHB action plan. One eventually emerged consisting of 4 major components: 1) AHB monitoring, a system of surveillance traps developed and implemented by the Agricultural Commissioner to detect the movement of AHB into Los Angeles County; 2) AHB education and public information, aimed at assisting and providing information at both the school and community level; 3) AHB training, preparing and certifying individuals from pest control and public health agencies in bee removal, and 4) an AHB response plan to protect the public from serious injury or fatality by AHB (Figure 1). In establishing the latter, the task force conceived two separate modes of action to the plan:

[1] M/S was submitted for publication in August 2001. It was inadvertently not included in the 2001 Proceedings. The Editor's apologies.

[2] General Manager, Greater Los Angeles County Vector Control District, 12545 Florence Avenue, Santa Fe Springs, CA 906070; jhazelrigg@glacvcd.org

- a medical emergency response mode – activates during a AHB stinging incident; uses 911 to alert trained emergency response services (fire, police, and emergency medical team (EMT) personnel) to respond to a multiple stinging incident;

- a non-medical emergency response (NoMER) plan – involves implementing operational programs that use trained and certified personnel to remove reported bee hives and swarms that threaten public health and safety.

Due to insufficient resources, the Los Angeles County Agricultural Commissioner could not perform the necessary non-medical emergency services, and, as a result, the 5 mosquito and vector control districts (Greater Los Angeles County Vector Control District, Los Angeles County West Vector Control District, San Gabriel Valley Mosquito and Vector Control District, Antelope Valley Mosquito and Vector Control District, and Compton Creek Mosquito Abatement District) agreed to seek approval from their governing boards to develop NoMER programs. Today, the Task Force meets occasionally to exchange information among the representative members. In November 1993, the events leading to the Greater Los Angeles County Vector Control District (herein, District or GLACVCD) eventually implementing a board approved, mandated AHB response plan program began.

GLACVCD NON-MEDICAL EMERGENCY RESPONSE PROGRAM (NOMER): PRE-ENTRANCE OF AHB INTO LOS ANGELES COUNTY

In November 1993, in preparation for arrival of AHB into California and eventually Los Angeles County, staff of GLACVCD sought and received authorization from its governing board of trustees to augment staff and acquire equipment for developing an AHB NoMER program—at a cost not to exceed $200,000. However, it did not, at that time, adopt a formal resolution, mandating or committing resources specifically to an AHB response program. Working cooperatively within the task force, the mosquito and vector control districts within the county agreed to implement the NoMER portion of the emergency response plan protocol as shown in Figure 1. The choice remained with each district to design its own NoMER program, differing, if necessary, in application of operational practices and procedures.

By mid 1994, using the NoMER protocol, GLACVCD added two additional full-time positions and acquired a 1-ton vehicle and especially equipped it with a motorized high-pressure orchard spray unit and the necessary support materials. All operational field staff, Vector Control Specialists (VCS), were trained and certified by University of California extension personnel in AHB biology, bee removal, and bee prevention methods. To gain bee and hive removal experience, the VCS responded to occasional feral European hive and swarm complaints. However, the official policy of the district remained not to respond to bee service request calls until the county Agricultural Commissioner confirmed entrance of AHB within Los Angeles County. In addition, the District's Public Information Officer worked in conjunction with the other county mosquito and vector control districts in developing educational pamphlets and brochures for informing and safeguarding the public against AHB.

By the end of July 1994, the District operated a two-person vehicle or AHB response unit, especially equipped with bee suits, application equipment, and wireless communications (cell phone and two-way radio) to respond to resident AHB service requests. The initial response practices and procedures were developed without actual experience responding to bee service requests. These included:

- clerical staff to receive and screen complaint calls, create a service request with essential data[1], and, if necessary forward the request to the AHB unit in the field. According to the NoMER protocol, screening calls serves to advise callers with structural bee infestations to contact a licensed structural pest control operator certified in bee removal[2]. Moreover, screening is important in minimizing unnecessary service responses associated with bee-mimics or bee-like insects.

- the AHB unit to respond within 24 hours of receiving a service request call; high-use public areas (e.g., parks and other outdoor recreational areas) and schools affected by bees to receive highest response priority.

- Once on site of a bee infestation, if the hive or swarm is accessible, the VCS to destroy[3] bees and remove the hive; if the hive is inaccessible, the VCS to apply Aero-cide®[4], seal the entrances with aerosol foam[5] insulation, and advise the resident or owner of his responsibility to remove the hive.

Some elements of these procedures changed and evolved after the District gained experience in actually responding to AHB service requests.

In May-April of 1993, University of California and California State Department of Agriculture officials estimated the arrival of AHB into California by 1995. By the end of 1993 and beginning of 1994, AHB had successfully migrated into New Mexico and Arizona. In Arizona they established along the lower Colorado River in Yuma, Arizona near the California border. By mid-summer of 1994, based on a recommendation from GLACVCD, all five districts collectively agreed to establish, advertise, and share the expense of a countywide 800-telephone number which residents could use specifically to make an AHB service request call. With this number, calls would be received by a contract firm and routed to the appropriate district quickly and efficiently,

[1] Complainant's name, phone number, location, and a brief description of the infestation (i.e., size, site location, when first observed).

[2] The amount of work associated with structural infestations exceeds the District's capabilities and resources; performing such work imposes too high a liability risk and may conflict with legal protections afforded licensed private structural pest control operators.

[3] An insecticidal soap with a Special Local Needs label was used; in October 1997, the same material gained EPA registration as M-Pede®.

[4] A 1% pyrethrin aerosol product from Whitmire Micro-Gen.

[5] A method later replaced by using a less costly resin-treated, polyester-fiber batting material that the bees could not "chew" through to escape.

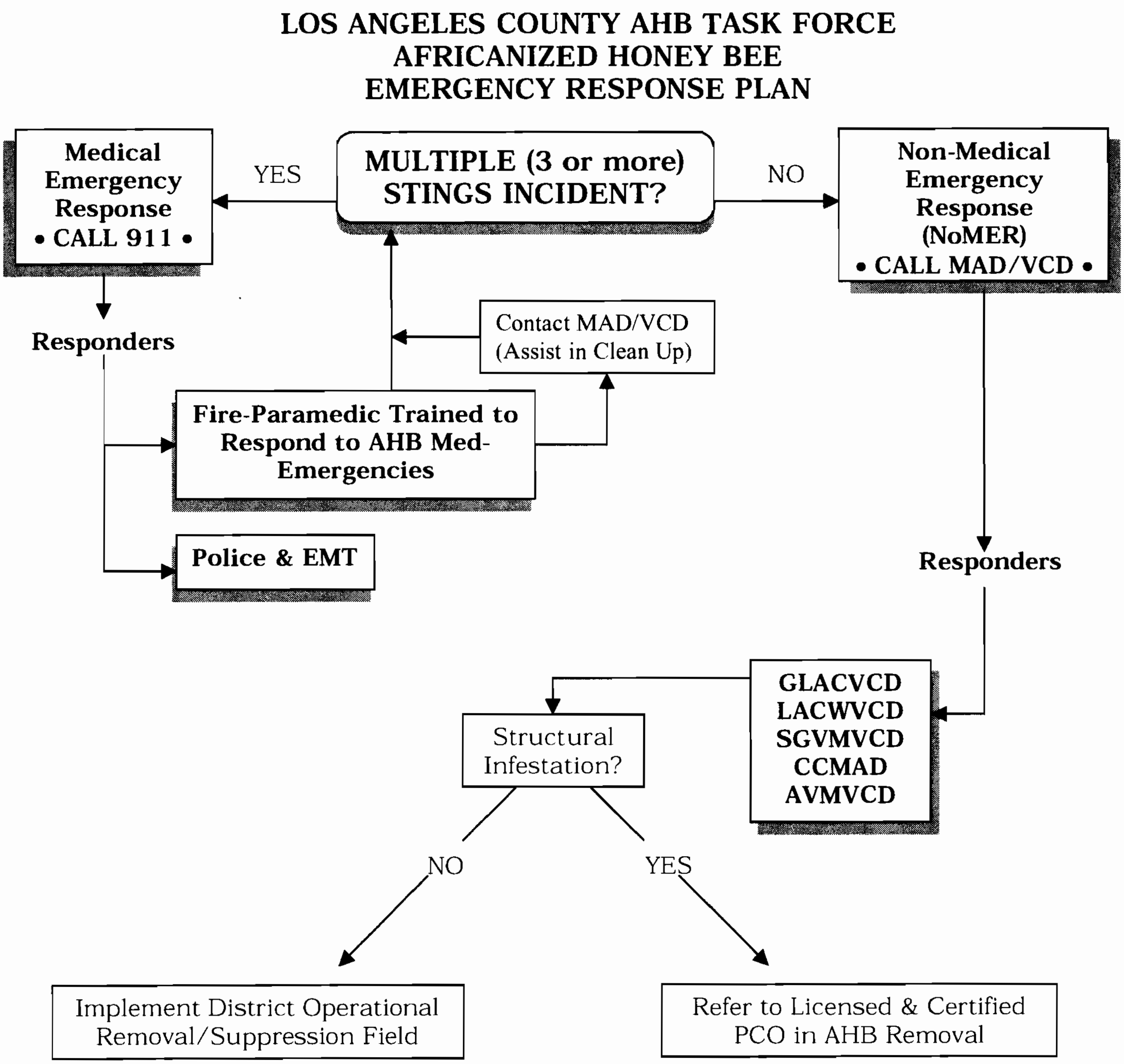


Figure 1. Emergency response plan developed by the Los Angeles County Africanized honey bee Task Force.

eliminating the caller's need to know the exact respondent agency. The districts' management felt this would facilitate communication, reducing residents' possible confusion and anxiety of the appropriate agency to call when troubled by bees. The location of a caller would be identified by zip code, site information of the bee problem recorded, and the data routed to the appropriate district. An outside professional telephone exchange provided the service. Initially, the service used an automated voice message to inform the public that AHB response services would not begin until the County Agricultural Commissioner officially announced arrival of AHB into the county. During 1994, the Agricultural Commissioner also established an 800-telephone number as a "hot line" (1-800-BEE-WARY) aimed at providing county residents only with AHB information.

On October 24, 1994, AHB was first detected in California in Riverside County near the town of Blythe just west of the Colorado River separating California and Arizona. By mid-1995, 7 AHB colonies were confirmed in Riverside and Imperial counties, the nearest colony approximately 140 miles northwest of Los Angeles County. During the next three years, AHB colonized approximately 25,000 square miles of area within Riverside, Imperial, San Diego, and San Bernardino counties, remaining localized within these counties. During this time, the only changes made to the district's response plan program were minor and consisted of medically screening the VCS for bee venom sensitivity and equipping the AHB unit with an Epipen®. In July 1998, just 3 months prior to detecting the first established colony of AHB in Los Angeles county, officials reported a colony of AHB in San Bernardino County 19 miles east of Los Angeles County[6].

On November 23, 1999 the Los Angeles County Agriculture Commissioner confirmed establishment of AHB in southwestern Los Angeles County in the City of Lawndale. Following the announcement, the districts' 800 response number was activated, and GLACVCD began actively responding to callers with bee complaints. By April 6, 1999 the Los Angeles County Agricultural Commissioner declared the entire 4,083 miles of the county colonized by AHB, with 27 confirmed established colonies, 4 within GLACVCD's boundaries.

GLACVCD NON-MEDICAL EMERGENCY RESPONSE PROGRAM: POST-ENTRANCE AND ESTABLISHMENT OF AHB INTO LOS ANGELES COUNTY

After April 6, 1999, the County Agricultural Commissioner halted its ongoing FABIS[7] tests on feral bee collections within the county, assuming most collections after April would likely be AHB. However, by this time the District received a monthly average of 236 bee service request calls and realized, based on that volume of need, that the staff and resources initially developed for conducting a satisfactory AHB response plan program were unprepared, inadequate, and insufficient.

From January through April 1999, the District logged a total 1,254 bee service requests. The increasing number of calls and public demand for service created significant problems. The office staff were overwhelmed with service requests, too many of which were poorly screened, resulting in excessive field responses involving wasps and bee-like insects. Likewise, supervisory and non-supervisory operational staff became overwhelmed. The response time to a service request well-exceeded 24 hours, as established "reasonable and expected" in the original operational response protocol; usually, a lapse time of several days occurred between receiving a service request and making a site response. Moreover, confusion and misunderstanding existed among emergency responders as to the District's role in providing AHB services, adding to inefficient use of response time and resources. It became apparent that elements of the District's NoMER program required refinement and improvement.

Several months prior to the District's 2000 fiscal year (July 1, 1999), the District's board of trustees approved a staff recommendation to increase the operating budget by 1.2 million dollars to allow for expansion and improvement of the existing response plan program into a mandated 3-year limited-term program. On July 1, the AHB program was scaled up dramatically to become a completely separate program within the District's overall vector control operations. Ten new employees were hired for 3 year-limited term contracts. They included 8 additional VCS and 2 newly created staff positions, a supervising Bee Coordinator and an Assistant to the Bee Coordinator, the latter responsible for thoroughly screening all incoming AHB calls and faxed data and computer processing all AHB service request and operational data. Also, three additional vehicles were acquired and outfitted as AHB units.

In addition to increasing staff and designating specialized positions and tasks, other improvements were made in the program, both administratively and operationally. Administratively, a special database was created for exclusively receiving service requests and tracking operational data. Also, the procedure of receiving service requests from the county-wide answering service was modified, enabling information to be "hot faxed" automatically and simultaneously downloaded into the computer, improving the speed and efficiency of processing service requests. Operationally, the procedures associated with screening service request calls were changed and refined to reduce unnecessary field responses (Figure 2), and the standard operating procedures used in the field were changed or modified. Instead of responding

[6] Prior to its occurrence and colonization in Los Angeles County, 103 confirmed colonies of AHB established in California, causing 18 multiple stinging incidents in humans (no deaths) and 6 in animals (3 dog deaths).

[7] **F**ast **A**fricanized **B**ee **I**dentification **S**ystem; from the beginning of the District's AHB response plan program, staff did not perform FABIS testing on collected bees primarily to save time and resources.

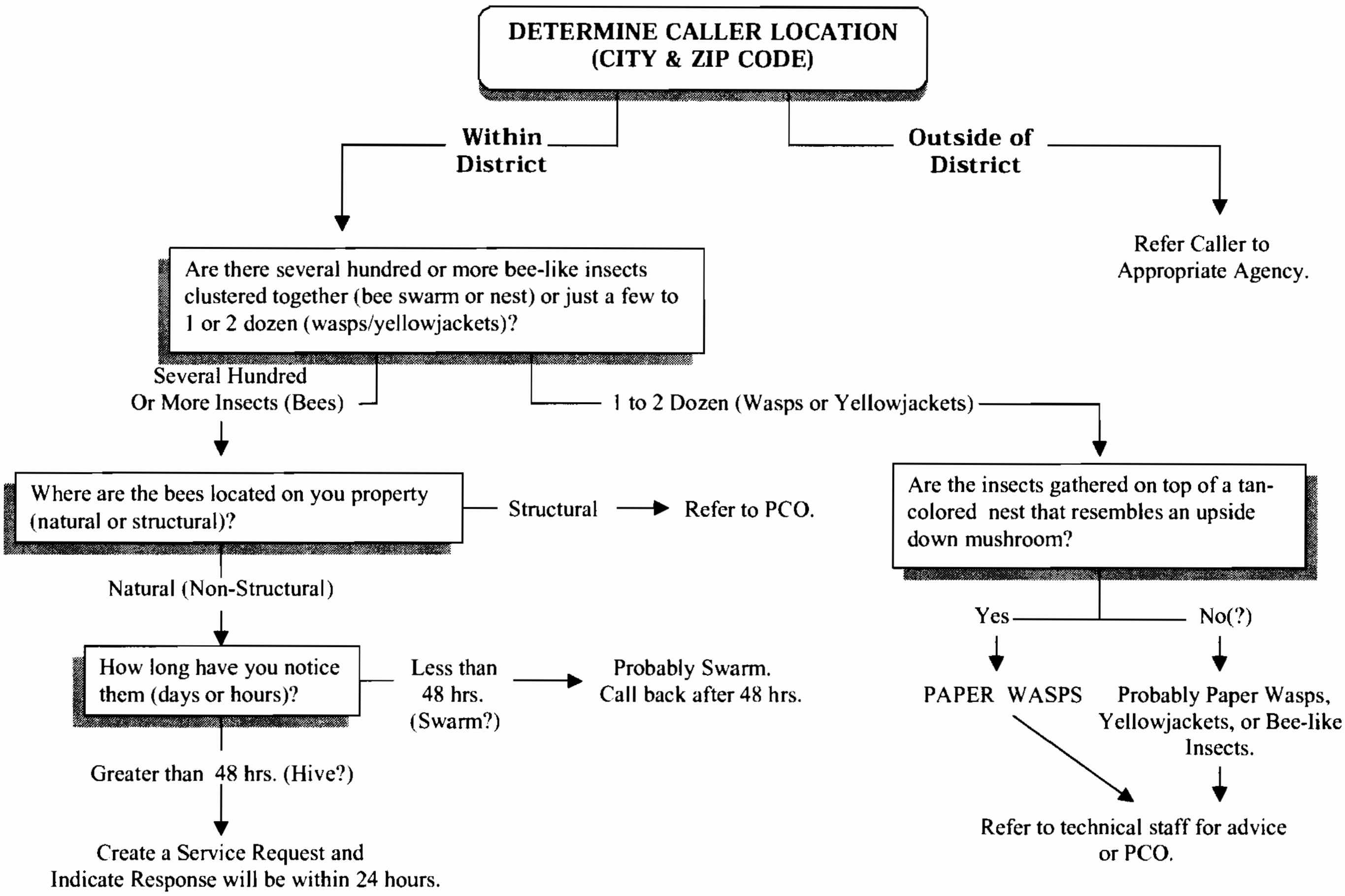


Figure 2. Protocol used in screening telephone calls associated with Africanized honey bee complaints.

immediately to settled and uncontrolled bee swarms, complainants were asked to observe swarm activity and call back if it persisted beyond 48 hours[8]. Operating hours of the VCS were expanded from dawn to dusk for the entire week, necessitating the creation of two work shifts and rotation of the VCS personnel assigned to mosquito control on a routine and periodic basis to work during weekends in the AHB response program. Upon completing hive removals, the VCS began leaving remnant traps baited with NGP (Nasinov Gland Pheromone) to capture straggler bees and collecting them within 24 hours to reduce the need for call-backs.

SUMMARY OF THE EXISTING AHB NOMER RESPONSE PROGRAM

Since the July 1999 expansion and improvements in the AHB program, the District now provides AHB response and removal services seven days a week from dusk to dawn. Although the highest volume of calls has been over 1000 for a single month, response to most service requests occurs within 24 hours or sooner.

Response service is performed on a priority based on degree of public risk or likelihood of serious injury and the priority schedule remains unchanged since the program's inception. Multiple stinging incidents receive highest response priority and involve staff providing immediate service assisting emergency services in any "mop up" of bees after a 911 incident. High-use public areas or schools threatened by a nearby hive or private residents having a hive and with older adults or children at high risk of being stung receive a high priority and response service immediately or within several hours, if possible. All other incidents receive a low or "to-do-as-soon-as possible" priority and are scheduled for following day response assignments by the AHB units.

The *in situ* infestation of a bee colony or swarm dictates the techniques and practices used by the VCS in destroying bees and removing hives. These differ slightly depending on the infestation, but, in general, the insecticidal soap, M-PEDE® is applied to kill

[8] Newly settled bee swarms usually are not threatening and often move several times before establishing a colony; typically the AHB unit would arrive to remove a swarm and find it gone, only to have it reappear nearby and cause another service request. Requesting residents to call back after 48 hours helps prevent the VCS from swarm "chasing," wasting time and effort.

the exposed bees and the hive or swarm bagged and removed. Protected or inaccessible hives are treated with Aero-cide® then sealed with resin-treated, polyester-fiber batting; removing the hive becomes an option of the owner, but his responsibility. A remnant trap to capture straggler bees is placed at all hive removal sites and collected after 24-48 hours. In all responses, hives or nests must be unattached to a habitable structure in order for the District to perform a removal, otherwise the resident is advised to call a private licensed pest control operator certified in bee removal

Since the nearly two years of AHB colonization in Los Angeles County, with the exception of a 78 year-old man living in the City of Long Beach fatally stung by AHB in early September 1999, no residents within the county have been reported stung by AHB. The Long Beach victim was a backyard bee hobbyist who neglected his hives. One hive became Africanized, and while mowing, he was attacked and stung over 300 times, dying after one week as a result of the attack.

During the 12-month fiscal period of July 1999 through June 2000, and implementation of the current response plan program, a total of 4,721 bee-related calls were received and screened. The months of April through October during this time marked the period of highest number of bee calls and averaged 525 calls per month. Of the 4,721 calls received, 33% were screened as "requiring-no-response." 52% were screened as "bees", and 15% as "others or bee-like". Of the total calls received, 2,917 were serviced or "ran" during this period. Of the "ran" calls, 86% were bees and 14% "bee-like" or "bee-related." Among the "bee-like" and "bee-related," approximately 10% were yellowjackets, mostly *Vespula germanica*, an introduced species from Europe. Until the recent implementation of the NoMER program, *V. germanica* was believed uncommon and to have a limited distribution in the Los Angeles basin. The many nest encounters with this insect presumably indicates otherwise. *V. germanica* is typically an arboreal nester, and in the basin it particularly showed an attraction or preference for Italian Cypress where many of the its colonies were encountered.

The District continues to maintain a highly active community information and education program on AHB, emphasizing the public's need to learn to live or coexist safely with AHB and to anticipate the District ending or significantly reducing its program on June 30, 2002. Beginning fiscal year 2003 (July 1, 2002), the District intends to scale down the AHB response program and provide service when receiving reports of hives/swarms in high-use public areas and schools only. Extending the NoMER program to private residents will cease. Despite the one fatality in Los Angeles county, AHB has yet appeared to constitute a significant public health risk. Inevitably, the feral bees in Los Angeles will become completely Africanized, replacing the European bee[9]. Based on that inevitability, it is not the intent of the District to sustain the program that was primarily intended to provide some initial comfort and relief from the fear and anxiety associated with AHB entering and colonizing Los Angeles County. To help minimize risk associated with AHB in the future, the Los Angeles County Agricultural Commissioner is seeking emendation of an existing county ordinance that would make it unlawful for residents or unregistered beekeepers to harbor bees. Several cities within Los Angeles County, independent of the county's action to seek regulatory changes, have adopted such anti-AHB ordinances. Residents in Los Angeles County can learn to dramatically reduce attack and injury and safely coexist with AHB as they've done with other venomous and undesirable arthropods.

[9] As of September 25, 2000, approximately 85% of the bees now collected and identified by the District appear to be Africanized.

Surveillance for *Aedes albopictus* to Determine Local Extent of Infestation

Susanne Kluh, Jack E. Hazelrigg, Michael W. Shaw & Minoo B. Madon

Greater Los Angeles County Vector Control District, 12545 Florence Avenue, Santa Fe Springs, CA 90670

ABSTRACT: Following the discovery of *Aedes albopictus* introduction into southern California in shipments of "Lucky Bamboo" from South China, Greater Los Angeles Vector Control District (GLACVCD) initiated an intense surveillance program for the Asian tiger mosquito around the focal points of infestation. Surveillance efforts within GLACVCD boundaries were concentrated in the Chinatown section of downtown Los Angeles as well as around a wholesale nursery in Rowland Heights. Initial trapping was performed with modified EVS/CO_2-light traps enhanced with shrimp-rinse water as an attractant, followed by ovitrapping from the focal points of infestation, expanding outward. *Ae. albopictus* adults were trapped in the vicinity of the wholesale nursery and *Ae. albopictus* eggs were detected multiple times in various ovitraps as far as 400 m from the nursery.

INTRODUCTION

Aedes albopictus, the Asian tiger mosquito, is common throughout Asia in urban, suburban, rural and forested areas (Hawley 1988). Several introductions into continental North America were recorded (Pratt et al. 1946, Eads 1972, Reiter and Darsie 1984). By 1999 infestations of *Ae. albopictus* were reported from 26 states east of the Mississippi River (Moore 1999).

Isolated incidences of *Ae. albopictus* introductions into California were recorded in 1971 (Eads 1972) and 1987 (CDC unpublished data). In June 2001, significant numbers of *Ae. albopictus* were introduced into California in containerized oceanic shipments of "lucky bamboo" (*Dracaena* spp.) from mainland south China (Madon et al. 2002 in press). Greater Los Angeles Vector Control District (GLACVCD) immediately implemented measures to monitor and suppress existing *Ae. albopictus* populations at the focal points of infestation in Los Angeles County. The Centers for Disease Control and Prevention (CDC) enacted an embargo to prohibit future shipments of *Dracaena* in standing water.

MATERIAL AND METHODS

Larval surveillance: All nurseries and wholesale distributors of "lucky bamboo" within GLACVCD boundaries were located and investigated for mosquito breeding. All water-holding containers on the premises were inspected for mosquito breeding. If present, larvae and pupae were collected and shipped to the CDC (and at a later date, to Tom Zavortink at USF) for species confirmation. Larval surveillance was also conducted at various cemeteries in the vicinity of infested nurseries. Larvae collected in these surveys were held in emergence jars and adults were identified at GLACVCD.

Adult surveillance: Adult trapping was conducted using modified EVS/CO_2 traps (enhanced with an oviposition attractant) in direct vicinity of the wholesale nursery/distributor and thereafter radiating in approximately ½ mi increments. The modification of the EVS/CO_2 traps was attained by the attachment of two small bird-feed containers to both sides of the fan housing unit (Fig. 1) containing either shrimp-rinse water (preferable), bamboo water or hay infusion as attractants. Strips of Balsawood were partially submerged in the attractant to provide a moist surface for oviposition. Aboveground adult trapping included daytime as well as nighttime surveillance. All underground storm drain systems (USDS) in the area around the wholesale nurseries (one sq mi and beyond) were also monitored with the modified EVS/CO_2 traps.

Since *Ae. albopictus* adults are not readily attracted to CO_2-baited EVS traps, human-baited "bite-counts" were also conducted at focal points of infestation (wholesale nursery). Biting mosquitoes were aspirated and sent for species determination.

Ovitrapping: Oviposition traps (Fig.2) were placed in the direct vicinity of the wholesale nursery/distributor, radiating away from the location in approximately 200 m increments in N, S, W & E directions. The USDS and catch basins were also surveyed locally using oviposition traps. These traps will be used for long-term surveillance. The oviposition medium (seed germination paper) was removed, inspected for mosquito eggs and replaced on a weekly basis.

RESULTS

Modified EVS/CO_2 sampling and trapping activities: On June 25, 2001, adult and larval sampling as well as CO_2–light trapping (modified EVS traps) was conducted at a local wholesale nursery in Rowland Heights, California. A number of adult and larval samples were sent to the CDC for identification. The specimens were confirmed to be *Ae. albopictus* as well as *Culex* spp., it, however, remained undecided whether the latter originated from China or were indigenous to southern California.

Accessible USDS in neighborhoods around the nursery were surveyed with the modified EVS/CO_2-light traps on June 26, 2001 (Table 1, Fig. 3).

On June 28, 2001, more larval samples were collected and bamboo cases were larvicided at the nursery. On July 2, modified EVS/CO_2–trapping was conducted in residential areas within

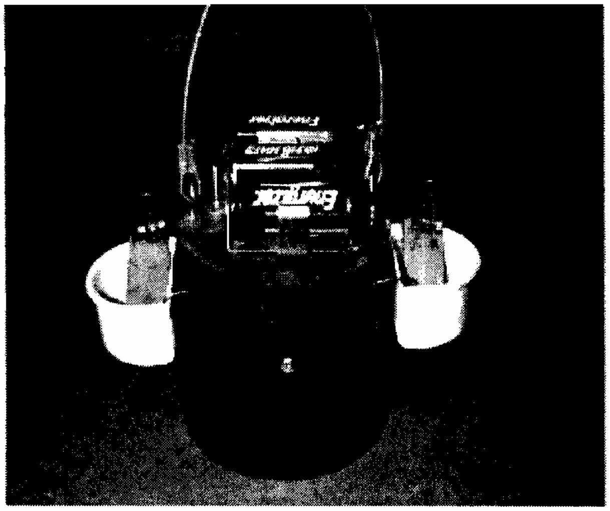


Figure 1. Modified EVS/CO_2 trap

Figure 2. Ovitrap

approximately 500 m around the nursery (Fig. 4). Trap counts are listed in Table 2. One *Ae. albopictus* was trapped at 18908 La Guardia and one *Aedes spp.* caught at 2305 Almeza Ave, was later confirmed to be *Ochlerotatus sierrensis*.

On July 5, twenty modified EVS/CO_2 traps were set overnight at various nurseries on Desire Ave (Fig. 4). All the mosquitoes trapped, were shipped to CDC for species determination and were identified as *Culex quinquefasciatus* and *Culiseta incidens*. Again it remains uncertain, however, whether all *Culex quinquefasciatus* were indigenous to southern California or whether some of them were perhaps imported from southern. China in "lucky bamboo" shipments.

Continuous day and nighttime trapping with modified EVS/CO_2 traps was conducted on July 12, and July 13, 2001, in residential areas approximately 1 km away from the nursery (Fig. 4). Traps were also placed at 2305 Almeza Ave in an attempt to collect additional specimens of the (at that time still unidentified) *Aedes spp.* (Table 3).

On July 18, 2001, additional larval and adult collections were conducted inside the hothouse at the nursery. Adult *Ae. albopictus* were collected with aspirators as well as with modified EVS/CO_2 traps (Fig. 4).

Modified EVS/CO_2–trapping in USDS in Chinatown (Fig. 5) was conducted on Wednesday, July 25 into Thursday 26, 2001. A trap placed in a catch basin at the end of Spring Street was removed by the Los Angeles Police Department as they were suspicious that it might be a "bomb" and unaware that it was a mosquito trap! No adult *Ae. albopictus* were trapped that night (Table 4). Upon inspection of air-freighted dry shipments of "lucky bamboo" in Chinatown, several *Ae. albopictus* larvae were detected in crates that now held the *Dracaena* in standing water. This proved that eggs of *Ae. albopictus* were imported on the "lucky bamboo" stems via the "dry" packaged air-freighted shipments.

Table 1. Adult mosquitoes trapped in USDS in Rowland Heights

Site	*Culex quinquefasciatus*
237 Batson Ave.	♀7
Pathfinder Rd. (opposite catch-basin)	-
Aquiro St./Gallio Ave.	♂2
Batson Ave./Gallineta	♀3
Killian St. (below Otterbein)	♀9

Figure 3. Modified EVS/CO_2 trap locations in USDS in Rowland Heights

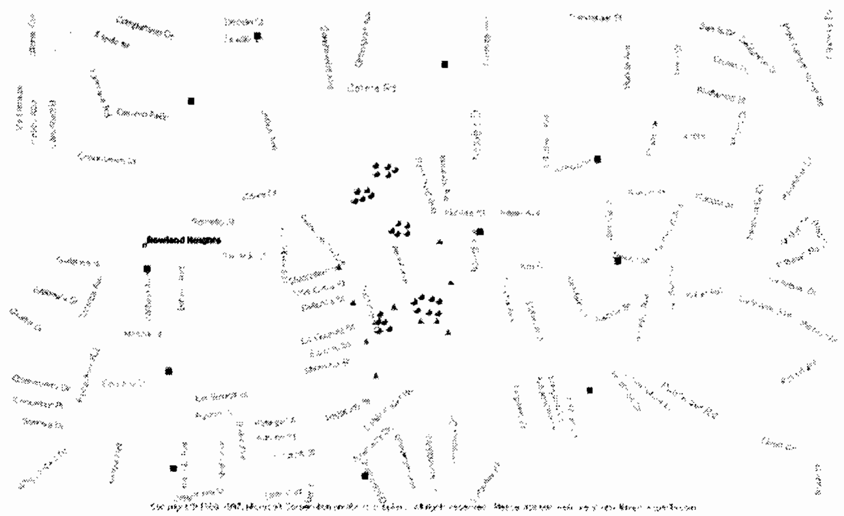


Figure 4. Modified EVS/CO_2 trapping sites in Rowland Heights
Green triangles: July 2 - 3, 2001 residential areas within approximately 500 m;
Red dots: July 5 - 6, 2001, various nurseries on Desire Ave.
Purple squares: July 12 - 13, 2001,residential areas within approximately 1km.

Table 2. Modified EVS/CO^2 light trap results in Rowland Heights within 500 m from nursery

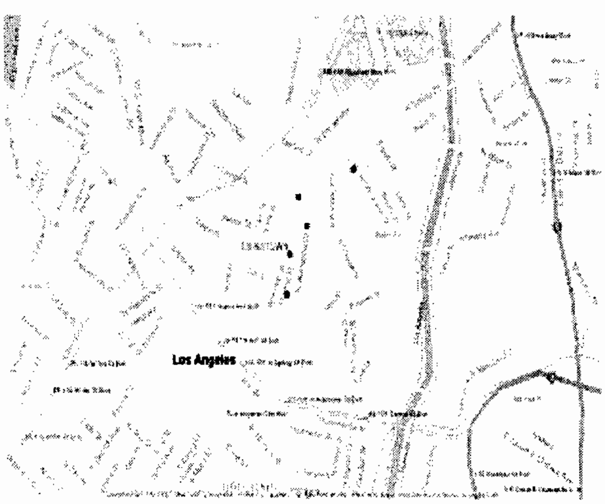
Site	*Culex quinque-fasciatus*	*Culex tarsalis*	*Culex stigmatosoma*	*Culiseta incidens*	*Aedes albopictus*
2302 Sierra Leone	♀4	-	♀1	♀2	-
2320 Sierra Leone	-	-	-	♀14	-
2442 Sierra Leone	♀3	♀1	-	♀5	-
2524 Sierra Leone	♀1	-	-	-	-
2307 Desire Ave	♀2	♀2	-	♀6	-
2024 Paso Real	♀1	♀1	-	-	-
2237 Paso Real	♀18 ♂1	♀13	-	-	-
18908 LaGuardia	♀9	♀13	-	♀6	♀1*
18941 LaGuardia	-	♀14	♀3	-	-
2305 Almeza	♀1	-	-	♀1	♀1**

* confirmed to be Ae. albopictus by CDC
** unidentified Aedes spp. according to CDC, later confirmed to be *Ochlerotatus sierrensis*

Table 3. Modified EVS/CO_2 light trap results in Rowland Heights within 1km from nursery

Site	*Culex quinquefasciatus*	*Culex tarsalis*	*Culiseta incidens*	*Aedes albopictus*
2305 Almeza(4traps)	♀37	♀11	♀2	-
1816 Nowell	♀12	♀1	-	-
1867 Valencia	♀10	-	♀1	-
19390 Dairen	♀4	-	-	-
19306 Oakview	♀2	-	-	-
2833 Blandford	♀2	-	-	-
2711 Batson	-	-	♀18	-
18321 Subido	♀3	♀1	♀2	-
18220 Galatina	♀1	-	-	-
18365 Camino Bello	-	-	-	-
18447 Seadler	♀6	-	-	-
262 Cypress	♀282	♀1	♀1	-

Figure 5. Modified EVS/CO2 trap locations in USDS in Chinatown.



Modified EVS/CO_2 trapping aboveground in Chinatown began on July 31, 2001, when 5 modified EVS/CO_2 traps were set overnight in the direct vicinity of the "lucky bamboo" importers (Fig. 6, Table 5). On August 6, eight modified EVS/CO_2 traps were set overnight in the Los Angeles downtown area within ~ 500 m in the vicinity of the "lucky bamboo" importers on N Spring St. (Fig. 6 and Table 6).

A final set of 8 modified EVS/CO_2 traps were placed overnight in the Los Angeles downtown area within approximately 1 km around the "lucky bamboo" vendors on N Spring St (Fig. 6, Table 7) on August 8, 2001. No *Ae. albopictus* were collected in any of the Chinatown trapping locations.

Monitoring with ovitraps: Ovitraps made out of 8 oz. drinking water bottles spray-painted black (Fig. 2), were chosen for the long-term surveillance. Shrimp-rinse water was used as an attractant and the traps were placed in direct vicinity of the wholesale nursery/distributor, radiating away from the location in approximately 200 m increments in N, S, W & E directions. Local USDS and catch basins were also surveyed using oviposition traps. The oviposition medium (seed germination paper) was removed, inspected for mosquito eggs and replaced on a weekly basis.

The ovitraps in Rowland Heights were set-up on July 30, 2001 (Figs. 7 & 8).

Table 4. Adult mosquitoes trapped in USDS in Chinatown (downtown L.A.)

Site	*Culex quinquefasciatus*	
N. Spring St./Ord St.	♀7	♂1
N. Spring Street/W. College St.	-	-
950 N. Broadway	♀4	♂1
W. Ann St./Wyse Ave.	♀19	♂3
N. Main St./Sotello Ave.	♀3	♂0

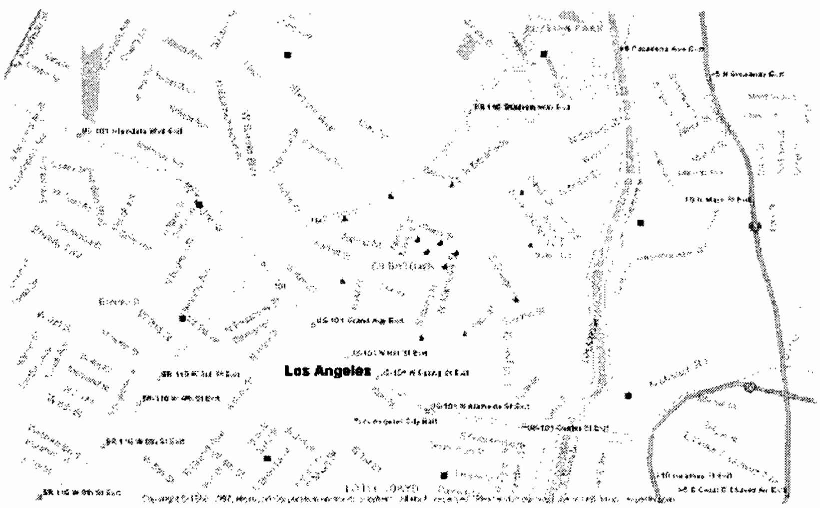


Figure 6. Modified EVS/CO_2 trapping sites in Chinatown.
Dots: July 31 - August 1, 2001, direct vicinity of the bamboo vendors;
Triangles: August 6 - 7, 2001, Los Angeles Downtown area within approximately 500 m;
Squares: August 8 - 9, 2001, Los Angeles Downtown area within approximately 1 km.

Table 5. Modified EVS/CO_2 light trap results in Chinatown (direct vicinity of the bamboo vendors).

Site	*Culex quinquefasciatus*	
821 N. Spring St. (inside bamboo store)	-	-
SE corner N. Spring/College	♂5	♀28
Bank of America (College/Broadway)	♂3	♀7
831 Broadway	♂1	♀8
Best Western (N. Hill St.)	♂2	♀7

Table 6. Modified EVS/CO^2 light trap results in Chinatown within 500 m of the bamboo vendors.

Site	*Culex quinquefasciatus*	
637 N. Spring St.	-	-
601 N. Grand	-	-
832 New Depot St.	-	-
Adobe St. (parking lot)	♂0	♀1
415 Bernard St.	♂0	♀1
204 Ann St.	♂7	♀15
N. College St.	♂3	♀7
N. Main St. (Post Office)	-	-

Table 7. Modified EVS/CO^2 light trap results in Chinatown within 1 km of the bamboo vendors.

Site	*Culex quinque-fasciatus*	*Culex tarsalis*	*Culex stigmatosoma*	*Culiseta incidens*
555 Mission Blvd.	♀23 ♂1	♀8	♀1	-
DWP Temple Ave	♀1	-	-	-
Caltrans yard (Spring St)	♀1	-	-	
1130 2nd St.	♀7	-	-	-
1201 Temple St.	-	-	-	
Stadium Way (Hospital)	♀1	-	-	♀7
1349 Broadway	♀3	-	-	-
Monlont Ave (The Brewery)	-	-	-	-

* confirmed to be Ae. albopictus by CDC
** unidentified Aedes spp. according to CDC, later confirmed to be *Ochlerotatus sierrensis*

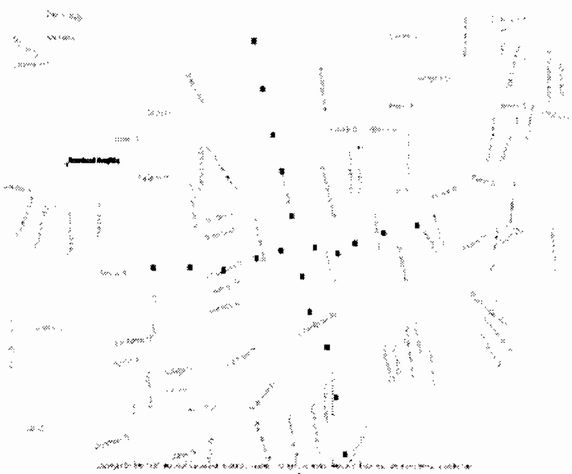


Figure 7. Distribution of ovitraps in the Rowland Heights: above ground.

Figure 8. Distribution of ovitraps in the Rowland Heights: inside catch basins.

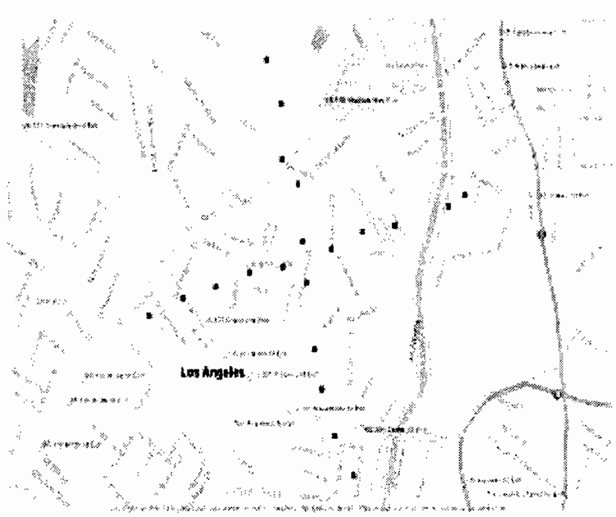


Figure 9. Distribution of ovitraps in the Chinatown section of downtown Los Angeles: above ground.

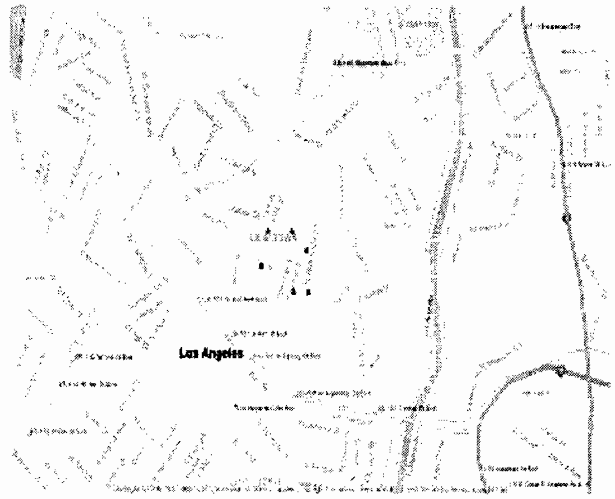


Figure 10. Distribution of ovitraps in the Chinatown section of downtown Los Angeles: inside catch basins.

Table 8. Positive ovitrap sites in Rowland Heights.

Date	Site #	# of eggs
8/7/2001	15	3
8/7/2001	16	40
8/14/2001	16	25
10/1/2001	4	24
10/10/2001	4	8
10/15/2001	16	18
11/19/2001	5	9

Ae. albopictus eggs were detected in Rowland Heights only. Sites # 15, 16 and 5 are located approximately 200 m from the wholesale nursery in southern, eastern and westerly direction. Site 4 is located approximately 400 m south of the nursery.

Ovitraps in the Chinatown area of downtown Los Angeles were set on August 16, 2001 (Figs. 9 & 10). To date, oviposition by *Ae. albopictus* has not yet been detected in Chinatown.

CONCLUSION

Intensive adulticiding and larviciding efforts to date have not successfully controlled the *Ae. albopictus* population in the Rowland Heights area. Container-breeding mosquitoes are extremely difficult to control once introduced – especially if they get established in a neighborhood, in catch basins or in the underground storm drain system. Extensive above and below ground surveillance will have to be conducted in spring and through late summer of 2002, to determine whether these mosquitoes are indeed established in the Rowland Heights area.

REFERENCES

Eads, R.B. 1972. Recovery of *Aedes albopictus* from used tires shipped to United States ports. Mosq. News 33: 113-114.

Hawley, W.A. 1988. The Biology of *Aedes albopictus*. J. Amer. Mosq. Control Assoc. Supplement. # 1: 1-40.

Madon, M.B., M.S. Mulla, M.W. Shaw, S. Kluh and J.E. Hazelrigg 2002. J. Vector Ecol. (in press).

Moore, C.G. 1999. *Aedes albopictus* in the United States: Current status and prospects for further spread. J. Am. Mosq. Control Assoc. 15(2): 221-227.

Pratt, J.J., R.H. Heterick, J.B. Harrison and L. Huber. 1946. Tires as a factor in the transportation of mosquitoes by ships. Military Surgeon 99: 785-788.

Reiter, P. and R.F. Darsie, Jr. 1984. *Aedes albopictus* in Memphis, Tennessee (USA): An achievement of modern transportation? Mosq. News 44: 396-399.

Evaluation of Mosquito and Arbovirus and Arbovirus Activity in Orange County During 2001

Robert F. Cummings, Stephen G. Bennett, Carrie L. Fogarty, Ralph Havickhorst, Greg Williams, Amber Mills, Shana Lowe and James P. Webb, Jr.

Orange County Vector Control District, P.O. Box 87, Santa Ana, CA 92702, 714-971-2421; email Rcummings@OCVCD.org

ABSTRACT: The Orange County Vector Control District continued its surveillance of mosquito and arbovirus activity throughout 2001 by collecting blood samples from wild birds and sentinel chickens, as well as collecting adult mosquitoes with CDC/CO2, gravid and stable traps. There were no positive mosquito pools, sentinel chicken seroconversions, or human cases in Orange County during 2001. Overall, 4 (0.15%) of the 2,711 sampled house finches and none of the 1,201 sampled birds of other species tested positive for SLE antibodies. *Culex quinquefasciatus* was the most commonly trapped mosquito, except for a freshwater wetland area of Irvine, where *Cx. tarsalis* was predominant.

INTRODUCTION

The Orange County Vector Control District (OCVCD) encompasses about 780 square miles (all of Orange County) and has almost 3 million residents. Most of the District consists of urban/suburban habitats with a variety of residential mosquito-breeding sources: improperly maintained swimming pools and ponds, debris-choked drainage channels, and other man-made habitats. Interspersed within this development are several natural, mosquito-producing fresh and salt-water wetlands. Three important encephalitis vectors, *Culex tarsalis* Coquillett, *Culex quinquefasciatus* Say, and *Culex stigmatosoma* Dyar (Reeves and Milby 1990) are found in the county. In 2001, the District continued its mosquito and encephalitis virus surveillance program by collecting blood samples from wild birds, sentinel chickens, and adult mosquitoes from a variety of trapping sites.

MOSQUITO SURVEILLANCE

Mosquitoes were collected weekly at 14 sites in the county (Figure 1), using 31 CDC/CO2 traps (Sudia and Chamberlain 1962), and six gravid female ovipositional traps (Cummings 1992). Engorged female mosquitoes were aspirated from an Australian crow trap (McClure 1984) modified to capture wild birds and the mosquitoes that feed on them. Mosquito numbers for all species were much higher in 2001 than in the previous year in urban/suburban habitats (Figure 2).

Culex quinquefasciatus was the mosquito species collected most frequently, but varied in abundance by season and habitat. Populations of this species were sampled most effectively with gravid traps in suburbanized areas of the county. Counts peaked in June and August for the season, and then declined through the winter at all locations (Figure 3).

Culex tarsalis was collected in substantial numbers at only two, relatively small, undeveloped areas of the District (Bolsa Chica and San Joaquin freshwater marshes). At the San Joaquin wetlands, numbers of host seeking *Cx. tarsalis* were highest in May (78 per trap night) and decreased gradually through the summer months, disappearing by the end of October for the remainder of the year (Figure 4). In contrast, during 2000, *Cx. tarsalis* collections in this habitat were highest in June, averaging 165 per trap night. Numbers of mosquitoes at this site (*Culex erythrothorax* Dyar being the most abundant) peaked between 173 - 350 per trap night in May through July 2001 and were less numerous than in 2000 (Figure 5).

During 2001, 3,295 post-blood fed (gravid or blood engorged) mosquitoes were selected from routine collections (nulliparous adults were not included), and were sent to the University of California-Davis, Center for Vector-borne Disease Research (UCD-CVDR) for testing (Table 1). The submissions included 108 pools of *Cx. quinquefasciatus*, 15 pools of *Cx. tarsalis* and one pool of *Cx. stigmatosoma*. None of these pools tested positive for either SLE or western equine encephalomyelitis (WEE).

SENTINEL CHICKENS

The District maintained one sentinel chicken flock of 10 chickens near a *Cx. tarsalis* - producing freshwater marsh at the San Joaquin Wildlife Sanctuary in Irvine. Blood samples from the chickens were tested biweekly for SLE and WEE antibodies by the California Department of Health Services' Viral and Rickettsial Diseases Laboratory (CDHS/VRDL) from April - November and at the District laboratory for the entire year. None of the chickens tested positive for SLE or WEE antibodies.

ENCEPHALITIS ANTIBODY SEROPREVALENCE IN WILD BIRDS

The wild bird encephalitis antibody seroprevalence program focused primarily on two abundant peridomestic passerines, House Sparrows (*Passer domesticus* L.) and House Finches (*Carpodacus mexicanus* Say). Birds were trapped in 11 modified Australian

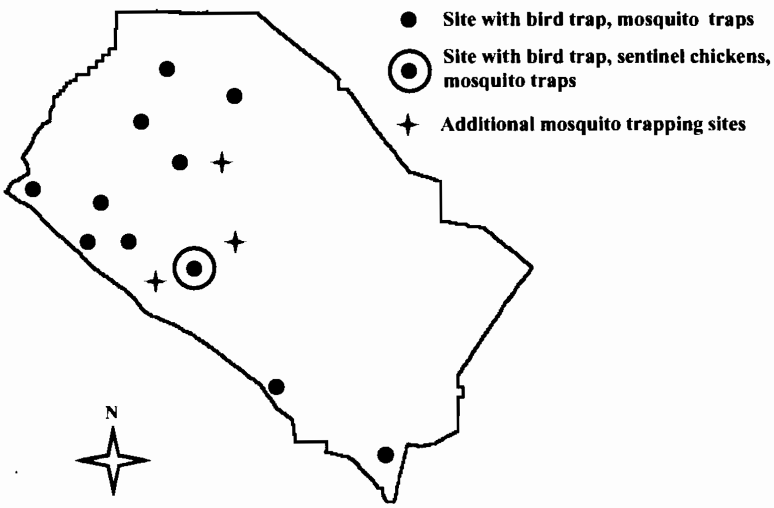


Figure 1. Arbovirus surveillance sites in Orange County, 2001.

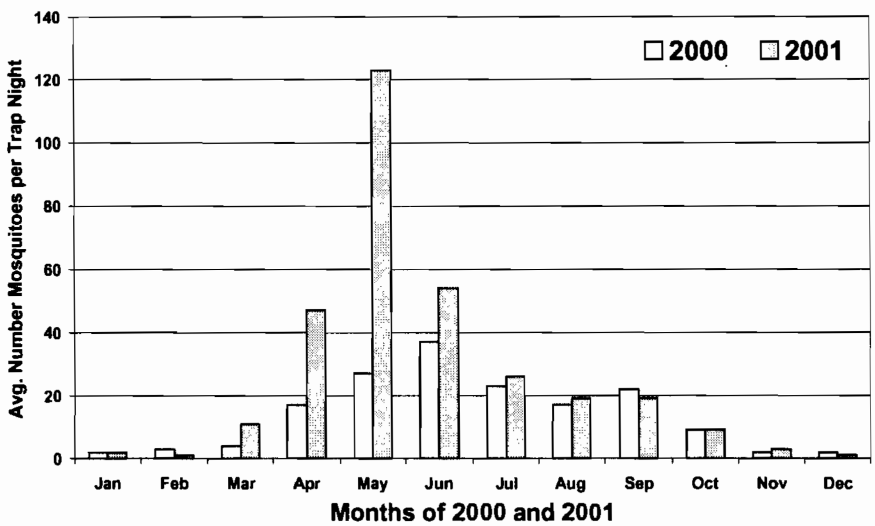


Figure 2. Host-seeking mosquito activity (all species, primarily *Cx. quinquefasciatus* and *Cs. incidens*) at 10 suburban mosquito collecting sites, Orange County, Calif., for 2000 and 2001.

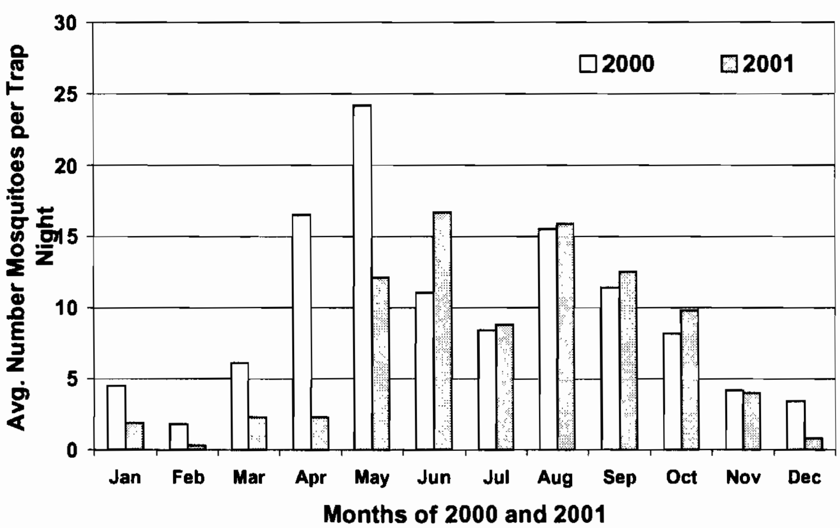


Figure 3. Numbers of gravid *Culex quinquefasciatus* collected from ovipositional traps at six sites in Orange County, Calif., for 2000 and 2001.

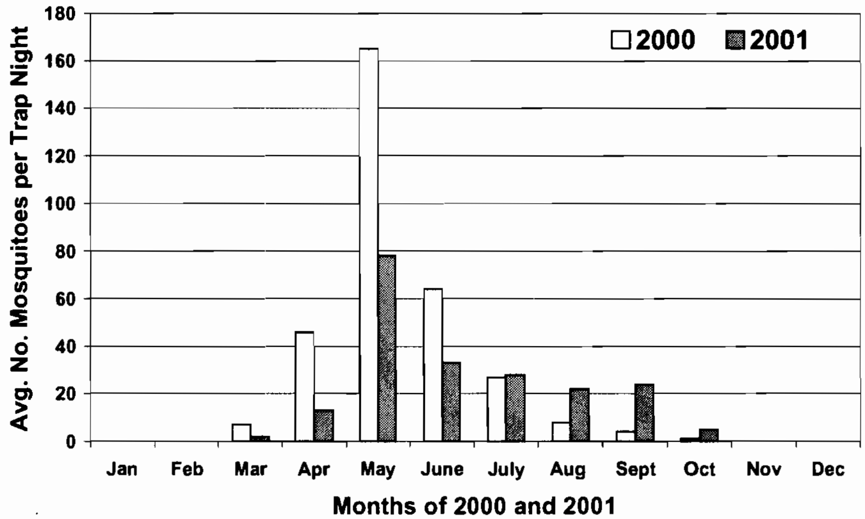


Figure 4. Host-seeking *Culex tarsalis* activity at the San Joaquin Marsh, Irvine, Calif., during 2000 and 2001.

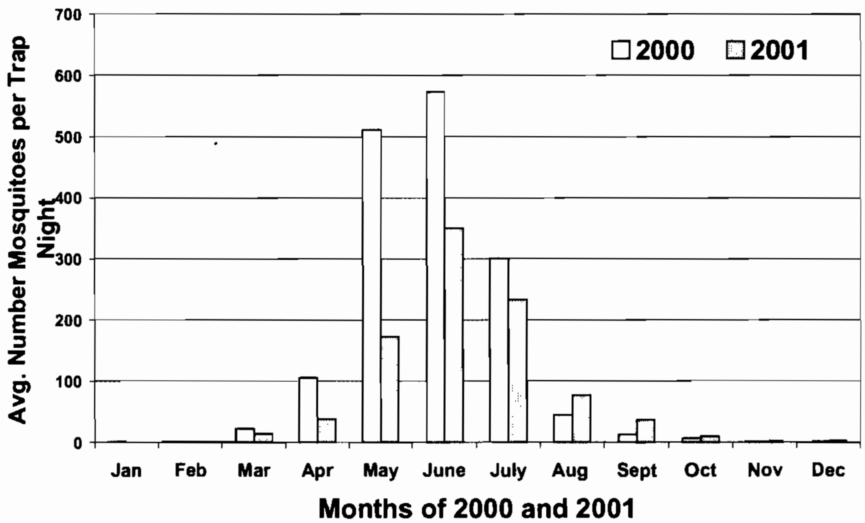


Figure 5. Host-seeking mosquito activity (all species, primarily *Cx. erythrothorax* and *Cx.tarsalis*) at the San Joaquin Marsh, Irvine, Calif., during 2000 and 2001.

Table 1. Number of mosquitoes and mosquito pools submitted for SLE and WEE virus testing by species and trap type from Orange County during 2001.

Species	No. of Mosquitoes	Gravid Trap Pools	Stable Trap Pools	CO_2 Trap Pools	Total Pools
Culex quinquefasciatus	2,717	88	20	0	108
Culex tarsalis	567	0	15	0	15
Culex stigmatosoma	11	0	0	0	1
Totals	**3,295**	**88**	**35**	**0**	**124**

Crow traps at sites also used to sample the adult mosquito population. Six trap sites were located in riparian corridors surrounded by suburban development, where House Finches were predominant. House Sparrows were collected almost exclusively at two sites located in urbanized communities with few open areas. Birds were sampled at each site on alternate weeks (5-6 sites/ week). Newly captured birds were banded, recorded, bled and released. Blood samples (0.2-ml) were taken from the jugular vein with a 1.0-ml syringe and a 28-gauge needle, dispensed into a 1.8-ml field diluent solution, kept cool and processed at the District laboratory using a hemagglutination inhibition (HAI) assay (Gruwell et al. 1988)

Of the 2,711 House Finches sampled in 2001, four birds (0.15%) tested positive for SLE antibodies. None of the 1,006 House Sparrows and 195 birds of other species were positive during the year (Table 2). Antibody-positive birds were detected in April, May and June only (Figure 6)

.

LONG TERM TRENDS

In the past five years, 1997 - 2001, the wild bird arbovirus surveillance program has detected a period of relatively low SLE and WEE antibody prevalence, in contrast to three active years from 1990 to 1992 (Figure 7). Approximately 5% (110 birds) of the 2,246 House Sparrows sampled in 1991 were positive for SLE antibodies (Gruwell et al. 2000). Numerous chicken seroconversions and two confirmed human case were also detected in 1991 and 1992 in Los Angeles and Orange counties (Bennett et al. 1992, 1993). Although seroconversion rates in all avian sentinel systems in the Los Angeles Basin have been low recently, data from OCVCD's ongoing wild bird program suggest that enzootic transmission of SLE still persists at a very low rate.

Table 2. Small bird seroconversions for SLE and WEE antibodies in Orange County during 2001.

Species	No. Samples	SLE Positive	WEE Positive	% SLE	% WEE
House Finch	2,711	4	0	0.15	0
House Sparrow	1,006	0	0	0	0
Song Sparrow	92	0	0	0	0
White-crowned Sparrow	85	0	0	0	0
Other Species	68	0	0	0	0
Totals	**3,912**	**4**	**0**	**0.10**	**0**

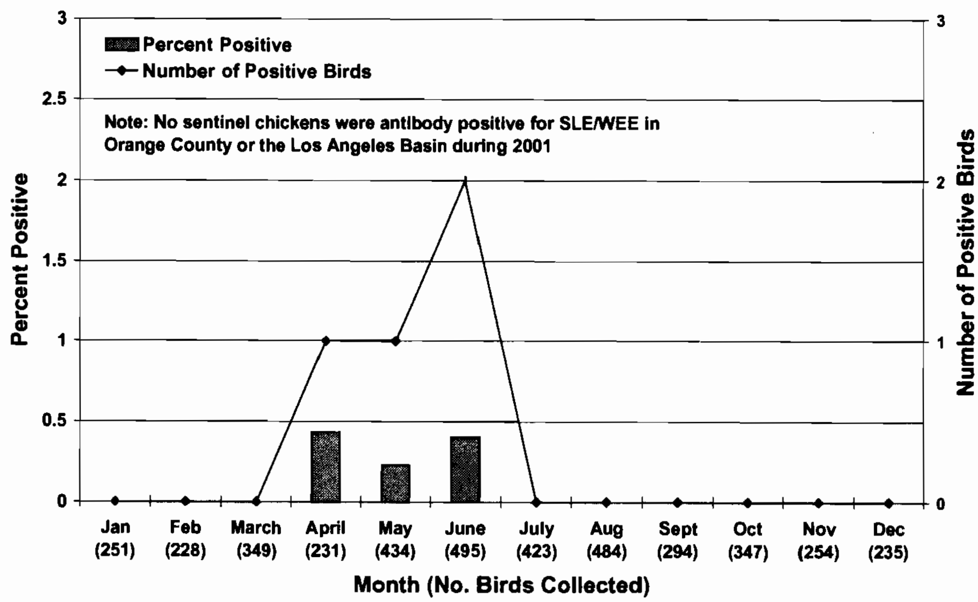


Figure 6. Seroprevalence of SLE antibodies in wild birds (House Finches and House Sparrows) from Orange County, Calif., during 2001.

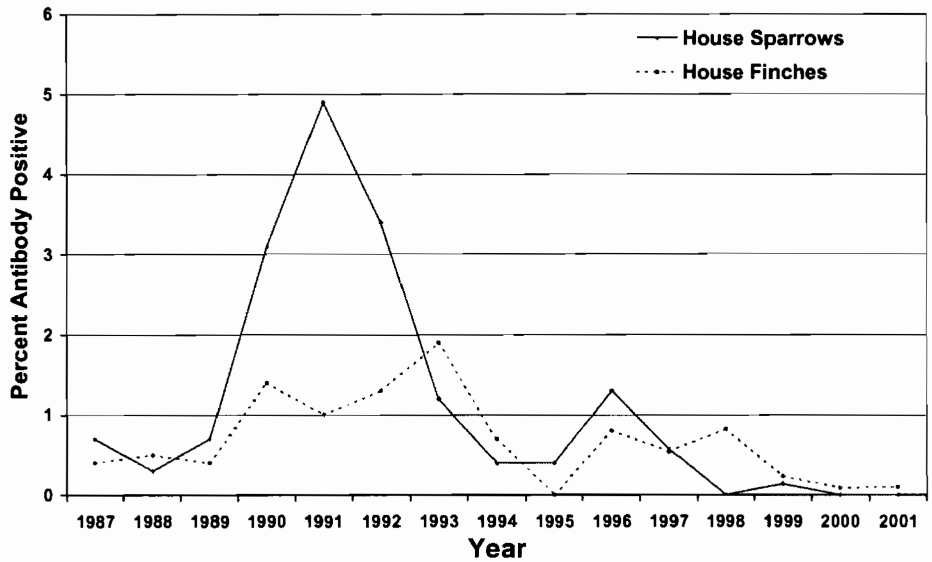


Figure 7. Long-term trends in SLE antibody seroprevalence in wild birds (House Finches and House Sparrows) in Orange County, Calif., from 1987 – 2001.

REFERENCES CITED

Bennett, S.G., J.P. Webb, A. Reining, T.J. Smith, J.S. Dean and L.H. Shaw. 1992. Evaluation of mosquito and arbovirus activity in Orange County, California, 1991. Proc. Calif. Mosq. Vector Control Assoc. 60:44-51.

Bennett, S.G., J.A. Gruwell, C.L. Fogarty, T.J. Smith, J.S. Dean, A. Reinig, V. Saylor and J.P. Webb. 1993. Evaluation of mosquito and arbovirus activity in Orange County, California, 1992. Proc. Calif. Mosq. Vector Control Assoc. 61:16-23.

Cummings, R.F. 1992. The design and use of a modified Reiter gravid mosquito trap for mosquito-borne encephalitis surveillance in Los Angeles County, California, 1987. Proc. Calif. Mosq. Vector Control Assoc. 56:58-68.

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. 60:170-176.

Gruwell, J.A., C.L. Fogarty, S.G. Bennett, G.L. Challet, K.S. Vanderpool, M. Jozan and J.P. Webb, Jr. 2000. Role of peridomestic birds in the transmission of St. Louis encephalitis virus in Southern California. Journal of Wildlife Diseases. 36(1): 13-34.

McClure, E. Bird Banding. 1984. Boxwood Press, Pacific Grove, CA., 341 pp.

Reeves, W.C. and M.M. Milby. 1990. Natural infections in arthropod vectors. *In*: W. C. Reeves (ed.), Epidemiology and Control of Mosquito-borne Arboviruses in California, 1943 – 1987. California Mosquito and Vector Control Association, Sacramento, CA. pp. 128 – 144.

Sudia, W.D. and R.W. Chamberlain. 1962. Battery-powered light trap, an improved model. Mosq. News 22:126-129.

Operational Applications of GIS Technology in the Detection and Eradication of Red Imported Fire Ants (*Solenopsis invicta*) in Orange County California

Michael G. Hearst[1], Shana Lowe[1], Maria Diuk-Wasser[2], and Richard P. Meyer[2]

[1] *Orange County Fire Ant Authority, P. O. Box 59, Santa Ana, CA 92702*
[2] *Orange County Vector Control District, P. O. Box 87, Santa Ana, CA 92702*

ABSTRACT: The Red Imported Fire Ant or RIFA (*Solenopsis invicta*) has infested Orange County, and has been found in twelve other counties in California. Recognizing the importance of eradicating this invasive pest, the California Department of Food and Agriculture (CDFA) contracted with county agencies and special districts to implement an eradication program (Bowen, 2001). Detection of the last remaining colonies will be the greatest challenge facing all agencies involved in this effort. The Orange County Fire Ant Authority (OCFAA), a division of Orange County Vector Control District has developed a strategy of utilizing Trimble GeoExplorer 3® GPS units to more precisely locate colony positions plus providing a digital field log for simultaneous entry of pesticide application and treatment data. Points of introduction, the preferred habitat, and likely dispersal distance have all been characterized by the application of GIS technology. Data currently being gathered also will allow staff to include wind direction, and soil disturbance in future RIFA analysis. This paper describes the ways the District has been able to apply GIS technology to aid in the RIFA eradication effort in Orange County, California.

INTRODUCTION

The economic and public health impact of the Red Imported Fire Ant or RIFA (*Solenopsis invicta*) has been so great that this species has become one of the most studied, and best understood of North American invasive species. Effective treatment protocols have been developed and are well documented (Barr et al. 1999; Barr et al. 2000). Considering recent successes in the application of GIS technologies, the District has dedicated considerable resources to applying GIS to selectively eliminate fire ant colonies with minimum of impact on non-target species. The ability to map eradication activities has also been a tremendous logistical asset in keeping state and local officials appraised of ongoing District activities. Initially, GIS technology was applied to mapping to graphically display the spatial attributes of colony distribution and associated numerical status of RIFA throughout Orange County. Following the initial "mapping phase", subsequent data were obtained and applied to treatment/detection activities in a manner to assist with economizing the resources required to find and destroy RIFA colonies.

From the beginning, the District was fully aware that the greatest obstacle to eradicating fire ants was locating *all* colonies. Operationally, a key component of the eradication effort has been the recruitment of the residents of Orange County into a "citizen-based" fire ant detection team. Through participation in numerous community outreach events, a cable TV campaign, and a very supportive press, the tactic has yielded thousands of "Hotline" calls reporting fire ant colonies that have subsequently been included in the GIS database. In addition to the countywide projected eradication of fire ants, the District has the added task of providing a fire ant free buffer around each of the 158 production nurseries located throughout Orange County. Since the size of the buffer has become the subject of some debate, it is apparent that fire ant dispersal be quantified over time and space. Therefore, GIS is being applied to delineate and assess the effectiveness of buffer management actions.

MATERIALS AND METHODS

Locality, activity, and treatment data has been collected at each of the 21,459 fire ant treatment sites. Each site record contains thirty-seven separate fields, including coordinates, address and ownership information, survey results, number of mounds, treatment history, and method of discovery. Each site was located using the Trimble GeoExplorer® 3 (TGE3) mapping system. This unit also allows gathering and entering all the required data, which is downloaded into the OCFAA database via Pathfinder® Office software. TGE3 data is downloaded daily, converted to a .dbf database file, imported into ArcView®, and converted into an ArcView® shapefile. Shapefiles contain locations, shapes, and attributes of all sites. Survey efforts were augmented by CDFA systematic grid surveys to the extent that the entire County has been surveyed twice. This information was integrated with various other datasets to reveal spatial relationships. With the inclusion of a countywide vegetation map, street map, parcel map, as well as zip code, and city boundary map, an operational "base map" was created along with a County aerial photograph with a pixel resolution of 1 meter. Additional inputs included wind direction information and soil grading augmentation to locate recent soil disturbances and local soil movement.

Relative to fire ant dispersal and spread, a GIS-based proximity analysis was applied using the locality data obtained from treatment records. It has been reported that newly mated queens may not disperse more than 5 km (Voght et al., 2000) and that newly mated polygyne queens may fly even shorter distances (Goodisman et al., 2000). Thus, using ArcView® *Point Proximity Analysis* (Bauer, 2001), a distance of 1000 meters was iterated (in lieu of direct flight measurements) and applied to determine what percentage of sites were within this distance of adjoining site(s).

Another GIS based objective was to determine the rate and extent to which the County RIFA infestation has spread and what various environmental factors may have influenced dispersal. Using locality data obtained for sites discovered by CDFA ArcView® script, *Animal Movement* (Spatial Tools. 1998) was applied to generate Minimum Convex Polygons (MCP) enclosing clusters of known fire ant sites as of January 2000. MCPs were overlaid against the current known sites to illustrate the extent of expansion that had occurred over the last two years.

All of the known fire ant sites have been found in association with disturbed soil with the exception of two wetlands reconstruction sites. The two wetlands sites represent the only natural vegetation type that has supported fire ants. It is a simple task to generate the polygon via ArcView® to facilitate the production of site maps needed to conduct detection surveys at both wetland locations.

RESULTS

Fire ant colony proximity analysis determined that 95.67% of the active sites were within 1,000 meters of another site and that 98.29% of all sites were within 2,000 meters of another site (Fig. 1). Considering that several colony locations are likely the result of human assisted and not natural dispersal (i.e., landscape material), this phenomenon suggests that fire ants are dispersing at shorter versus longer distances. This is consistent with longer duration "natural" flight distances of newly mated queens determined by onsite ambient measurement, (Markin et al., 1971) and flight energetics studies (Voght et. al., 2000).

The MCPs described by the clusters of known sites (778) in January of 2000, contained 88% (691) of all known sites. There were several sites attributed to human assisted dispersal prior to CDFA involvement. When a map of the current sites, 3,115, exactly four times the number from two years earlier, was superimposed over the polygon described by the sites known from two years ago, it was found that 93% (2,904) of these sites fell within the polygon described by the earlier cluster (Fig. 2).

When known fire ant sites were compared to vegetation types in an attempt to determine habitat preference, several sites showed up in areas of natural vegetation, other than wetlands. A quick look at the aerial photograph revealed the problems of dealing with three-year-old vegetation maps in a county that is growing as fast as Orange County. All of the sites shown to occur in natural vegetation were found in the photograph to actually be in developments that didn't exist when the vegetation map was produced. Fortunately, due to their environmental value, wetlands are not undergoing the same rate of removal, and the map of 2000 remains applicable to accurately locate this potential RIFA habitat.

DISCUSSION

Based on the percentage of fire ant colonies found within 1000 meters of another colony (95%), indications are that RIFA queens usually do not disperse more than 1000 meters from the colony of

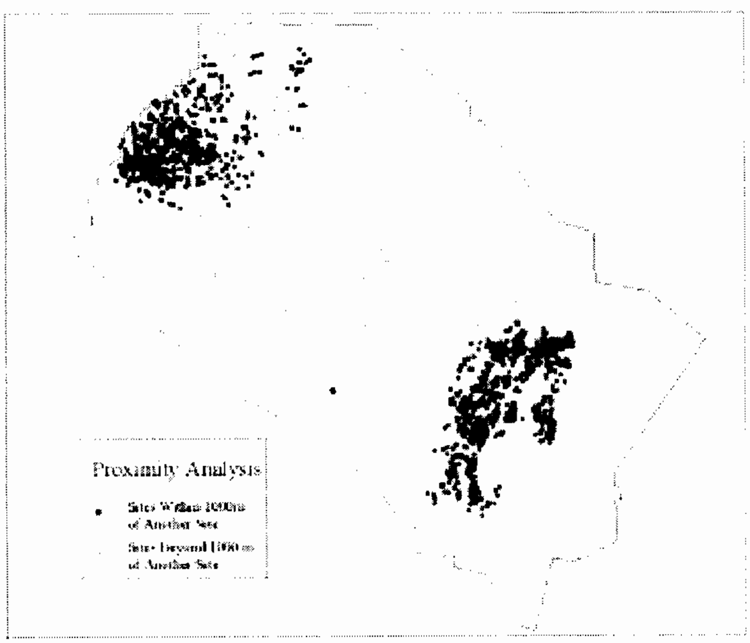


Figure 1. Map of Orange County showing the locations of sites that are within 1000 meters of another, and those outside the 1,000-meter perimeter

Figure 2. Map of Orange County showing the known sites in 2000, the extent of that cluster, and the currently known sites.

their origin. Indeed, direct measurement would provide a more accurate measurement of how far fire ants disperse; however our application of GIS demonstrates that fire ants are "behaving" differently (i.e., reduced dispersiveness) in coastal Southern California. This information may prove useful in developing a mechanism or protocol to relax the quarantine on Orange County nurseries, especially those nurseries located within several kilometers of known RIFA colonies.

The analysis of infestation cluster boundaries revealed an apparent lack of notable expansion in either of the two infested areas (north and south central county); this is good news attributing to the success of the program. Concurrent systematic bait surveys by CDFA also demonstrate that countywide infestations appear to be limited to the clusters circa north and south Central County. The spatial agreement between our data and that of CDFA suggests that fire ant dispersal is progressively being "checked" by existing control efforts. Existing data indicates the size of the infested area is shrinking, and even though there are still new finds, they are limited to areas already known to be infested. Survey and treatment data shows a reduction rate exceeding 95% following just one treatment cycle (Bowen, 2001). Success of the program will ultimately be judged by bait surveys that repeatedly fail to reveal the presence of ants.

In addition to our early applications of mapping and reporting, GIS technology has allowed us to determine the limits of the RIFA infestation, concentrate most of our resources on containment and control, and at the same time characterize high risk areas prone to new RIFA infestations.

REFERENCES

Barr, Charles L., Bill Summerlin, and Bastiaan M. Drees. 1999. A cost/efficacy comparison of individual mound treatments (IMT) and broadcast baits. Proceedings of the 1999 Imported Fire Ant Conference, Charleston, South Carolina. Pp.31-36.

Barr, Charles L., Rody Best, and Bastiaan M. Drees. 2000. Field comparison of five broadcast baits as full rate hopper blend and skip swath applications. Proceedings of the 2000 Imported Fire Ant Conference, Chattanooga Tennessee. Pp.84-90.

Bauer, Nick. 2001. Point proximity analysis. http://gis.esri.com/arcscripts.

Bowen, Richard A. 2001. Eradication of RIFA in Orange County; program design. Proceedings of the 2001 Imported Fire Ant Conference, San Antonio, Texas.

Goodisman, Michael A.D., Christopher J. DeHeer, and Kenneth G. Ross. 2000. Unusual behavior of polygyne fire ant queens on nuptial flights. *Journal of Insect Behavior*. 13: 455-468.

Markin, G. P., J. H. Diller, S. O. Hill, M. S. Blum, and H. R. Herman. 1971. Nuptial flight and flight ranges of the imported fire ant, *Solenopsis saevissima richteri* (Hymenoptera: Formicidae). Journal of the Georgia Entomological Society. 6: 137-144.

Spatial Tools. 1998. Animal movement. http://gis.esri.com/arcscripts.

Voght, James T., Arthur G. Appel, and Mark S. West. 2000. Flight energetics and dispersal capability of the fire ant, *Solenopsis invicta* Buren. Journal of Insect Physiology. 46: 697-707.

Vertical Distribution of Mosquitoes in the Prado Basin, Riverside County, CA

Lal S. Mian

Department of Health Science and Human Ecology, California State University, San Bernardino, CA 92407-2397

ABSTRACT: Of the 4845 mosquitoes collected in CO_2-baited traps over five nights, *Culex quinquefasciatus* accounted for 63.9%, followed by *Anopheles hermsi* (17.8%), *Culex erythrothorax* (7.3%), *Culex stigmatosoma* (6.0%), *Culex tarsalis* (4.7%), and *Culiseta inornata* and *Culiseta particeps* (0.3%). Of the three trap heights (2-, 6- and 18-ft), *An. hermsi* and *Cx. erythrothorax* were found significantly at the lower levels, whereas Cx. *quinquefasciatus*, *Cx. stigmatosoma* and *Cx. tarsalis* preferred higher tree canopy levels.The findings necessitate further research and to include the use of sentinel bird hosts to determine if encephalitis virus transmission would occur.

INTRODUCTION

Mosquito ecology plays an important role in our understanding of the epidemiology of mosquito-borne encephalitis and other diseases. Mosquito age and flight pattern are key determinants of host-seeking or blood-feeding behavior and subsequent pathogen transmission to animal or human hosts. Literature on vertical host-seeking or feeding zone (s) and parity is scanty or inconclusive. Parous *Culex thalassius* Theobald were found as high as 30 ft, whereas nulliparous individuals stayed at the lower level (3 ft) in Gambia, South Africa (Snow and Wilke 1977). The occurrence of nulliparous mosquitoes was attributed to incomplete development of wings and flight muscles. Pfuntner et al. (1988) found no significant differences in the numbers of *Culex* mosquitoes caught at near ground level and as high as 30 ft under dairy conditions in the Chino area. In another study conducted near a dairy and the Prado Basin wetland, *Culex quiquefasciatus* Say and *Culex tarsalis* Coquillett were reported to exhibit a bimodal distribution with peaks at 2- and 18-ft levels (Mian et al. 1990).

The Prado Basin wetland located close to dairies and human habitation, provides ideal breeding habitats to a variety of mosquito species. It also harbors a diverse group of wildlife including mammals and especially birds that play a major role in the transmission of encephalitis viruses. The present study was undertaken to determine the vertical distribution—host-seeking or feeding zones of adult mosquitoes in the Prado Basin wetland.

MATERIALS AND METHODS

In the study area situated in the northeastern part of the Prado Basin wetland, three willow trees were selected along the north bank of northeastern diversion channel of the Santa Ana River. The trees were halfway surrounded by a mixture of dense vegetation, predominantly willows and reed. A nylon rope (50 ft long, 7/8th in. thick) was made into a loop around a strong branch (~20 ft high) on each tree. Each tree had three carbon dioxide (CO_2)-baited CDC traps hung at 2-, 6- and 18-ft level from the rope. The traps were set in the evening and picked up the following morning. The tests were run over five nights—August 24, 31, October 2, 16 and November 6, 2001. In the laboratory, mosquitoes were anesthetized with triethylamine and then identified to sex and species, using identification keys by Loomis (1959). Statistical analysis was done using PSI (1993).

RESULTS AND DISCUSSION

A total of 4845 mosquitoes were collected during the five-night trapping. The most abundant species was *Cx. quinquefasciatus* (63.9%), followed by *Anopheles hermsi* (17.8%), *Culex erythrothorax* Dyar (7.3%), *Culex stigmatosoma* (6.0%), *Cx. tarsalis* (4.7%), *Culiseta inornata* and *Culiseta particeps* (0.3%). Data on mosquito distribution showed that *An. hermsi* and *Cx. erythrothorax* were found in significantly higher numbers at the lower levels, whereas *Cx. quinquefasciatus*, *Cx. stigmatosoma* and *Cx. tarsali,* all tended to be at higher tree canopy levels (Table 1). Percent distribution profiles of the five species and their host-seeking/feeding zones clearly illustrate the vertical distribution of each species (Fig. 1). For example, *An. hermsi* and *Cx. erythrothorax* being present in greater numbers near ground, might be feeding on smaller hosts such as rodents and lagomorphs. Higher numbers at the lower levels might also include younger females with wings and flight musculature not developed enough to fly to higher levels. Species host-feeding at higher levels may result in proportionally greater feeding on avian hosts, and thus the likelihood of encephalitis virus transmission. The findings in this study suggest a need for further research to include sentinel bird hosts instead of traps at higher levels in order to determine if any seroconversion takes place.

Acknowledgements

The author would like to thank Dr. Major S. Dhillon, District Manager, Northwest Mosquito and Vector Control District (NWMVCD), for logistic support during the course of this study. The author also thanks Cecilia Reed, Tanya Sahagun, Heather Atamian Jared Dever and Brian Baharie of NWMVCD, for their field assistance.

Table 1. Mosquito distribution by height at the Prado basin wetland*.

Species	2 ft.	6 ft.	18 ft.	P-value
Anopheles hermsi	36	19	1	0.002
Culex erythrothorax	5	10	9	0.312
Culex quinquefasciatus	11	27	168	0.008
Culex stigmatosoma	1	4	15	0.029
Culex tarsalis	3	3	9	0.005
*Culista spp.***	<1	<1	0	0.411

*mean of 3 trees and 5 trap-nights (15).
***Cs. inornata* and *Cs. particeps*.

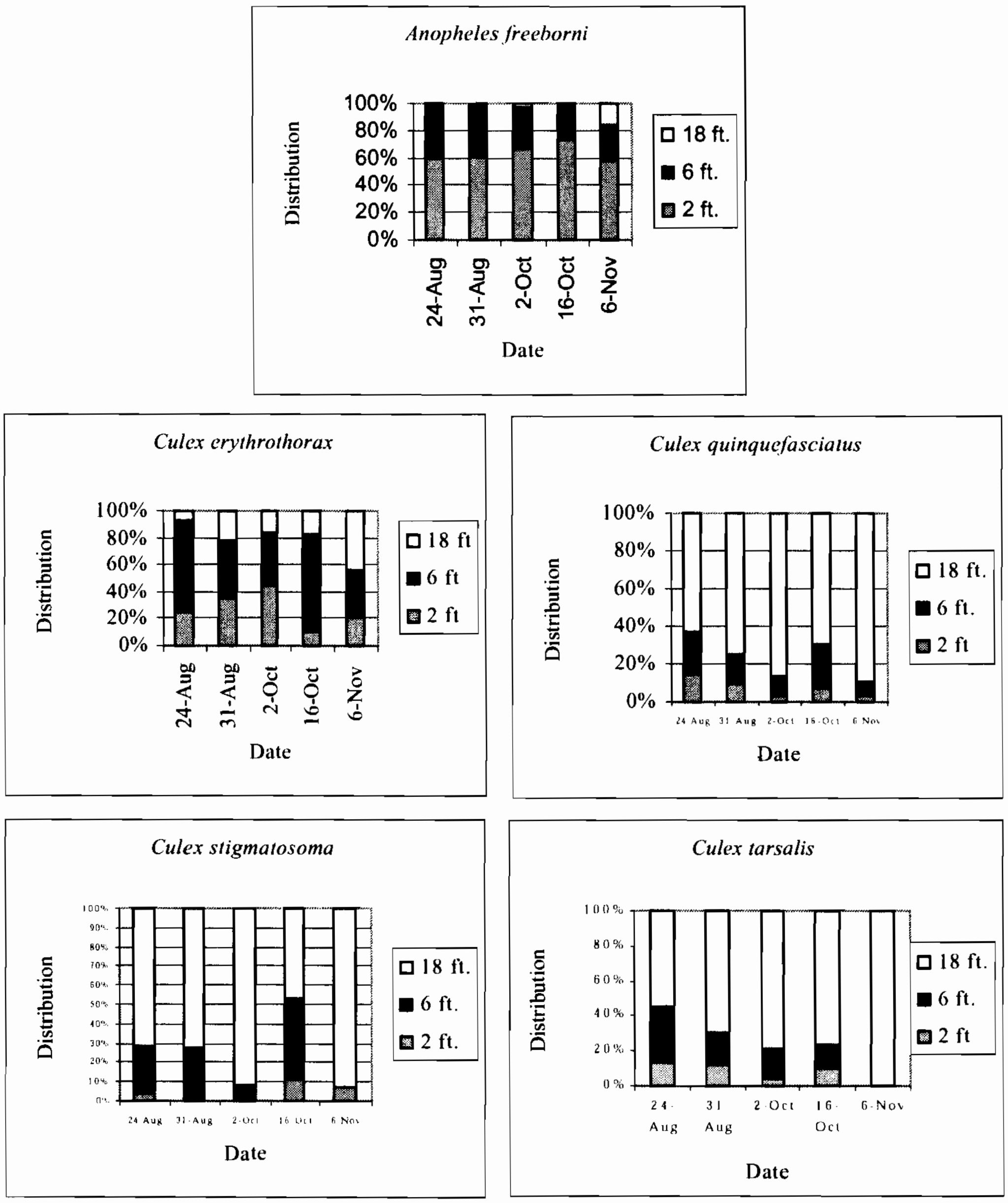


Figure 1. Percent vertical distribution of adult mosquitoes in the Prado Basin wetland.

REFERENCES

Loomis, E.C. (ed.). 1959. A Field Guide to Common Mosquitoes of California. Entomology Committee, California Mosquito Control Association. Sacramento. 26 pp.

Mian, L.S., M.S. Mulla, H. Axelrod, J.D. Chaney and M.S. Dhillon. 1990. Studies on the bioecological aspects of adult mosquitoes in the Prado Basin of southern California. J. Am. Mosq. Control Assoc. 6: 64-71.

Pfuntner, A.R., W.K. Reisen and M.S. Dhillon. 1988. Vertical distribution and response of *Culex* mosquitoes to differing concentrations of carbon dioxide. Proc. & Papers, Calif. Mosq. and Vector Control Assoc. 56: 69-74.

Poly Software International (PSI). 1993. PSI-PLOT: Technical Plotting and Data Processing, 4th Edition. Salt Lake City. 248 pp.

Snow, W.T. and T.J. Wilke. 1977. Age composition and vertical distribution of mosquito populations in the Gambia, West Africa. J. Med. Entomol. 13: 507-513.

Comparison of the Efficacy of Three Pesticides for Control of Ground Nesting Yellowjackets (*Vespula vulgaris* L.)

James P. O'Brien and Chindi A. Peavey

San Mateo County Mosquito Abatement District, Burlingame, CA 94010

ABSTRACT: Three pesticides were evaluated for their ability to destroy ground nests of yellowjackets (*Vespula vulgaris*). Drione®, an insecticidal dust containing silica gel and pyrethrins was applied to 22 nests; RealKill®, an aerosol spray containing chlorpyrifos and pyrethroids was applied to 20 nests. Twenty additional nests were treated with Victor® Poison-Free Wasp and Hornet Killer, an aerosol product containing sodium lauryl sulfate and mint oil. Nests were excavated 2 to 4 days after treatment, noting nest size, depth and entrance tunnel structure. Drione® eliminated 100% of treated nests. Aerosol products were less effective. Forty-three percent of nests treated with RealKill® required further treatment, as did 70% of those treated with Victor® Wasp and Hornet Killer. Control of nests treated with RealKill® and Victor® appeared to be related to entrance tunnel structure.

INTRODUCTION

During 2001 the San Mateo County Mosquito Abatement District (SMCMAD) responded to 159 requests from the public to destroy the nests of yellowjackets, *Dolichovespula* and *Vespula*. The majority of these calls involved subterranean nests of *V. vulgaris* L. Operations were limited to destruction of nests and did not involve baiting or atmospheric dispersion of pesticides.

With increasing frequency, the public requesting yellowjacket control at residences or places of business has shown concern as to the relative toxicity of pesticide applications. It is not unusual for the SMCMAD to receive requests for pesticide-free destruction and or removal of yellowjacket nests. The SMCMAD's policy is to conduct operations with a minimal impact on the environment while achieving a high degree of control. In the interest of accommodating members of the public who wish to have poison-free destruction of nests while still maintaining a high rate of treatment success, the SMCMAD decided to evaluate the efficacy of a pesticide labeled "poison-free" for use in treatment of yellowjackets.

Our current program for treatment of yellowjacket nests includes the use of Drione®, manufactured by Roussel Uclaf Corporation, and RealKill®, manufactured by United Industries Corporation. During normal treatment of yellowjacket ground nests by SMCMAD, choice of pesticide is made on an individual basis. An assumption being made that different formulations may contribute to a higher degree of control based on nest characteristics such as orientation of nest entrance, comb size, and length and configuration of entrance tunnel. Nests that appear to have a relatively short and straight entrance tunnel are often treated with an aerosol pesticide. Those nests that are presumed to have long and circuitous entrance tunnels (such as those in rocky ground or in the root ball of trees) are most often treated with an insecticidal dust.

We decided to test the operational efficacy of Victor Poison-Free Wasp and Hornet Killer® manufactured by Woodstream Corporation, along with Drione® and RealKill®. Efficacy of the pesticides was evaluated relative to practical considerations and treatment of non-uniform nest structure.

MATERIALS AND METHODS

For uniformity, the study was limited to treatments of underground nests of *V. vulgaris*. This is the most commonly encountered species of ground nesting yellowjackets on the San Francisco peninsula. Yellowjackets from each nest were identified from specimens taken before application of pesticide and after nest excavation. Identification to species was per key by Akre et al. (1980). Nests containing other species were excluded from the data analysis.

Field evaluations of efficacy were conducted from June through September of 2001. All nests were located in residential neighborhoods within San Mateo County, California. For the purposes of this study, choice of pesticide was random. Nest characteristics were not used as criteria in whether to use any of the pesticides being evaluated.

Three pesticides were tested: Drione® (DR) 1% pyrethrins, 10% piperonyl butoxide, technical, 40% amorphous silica gel, 49% inerts; RealKill® (RK) 0.25% chlorpyrifos, 0.05% d-trans allethrin, 99.70% inerts; Victor Poison-Free Wasp and Hornet Killer® (VIC) 8% Mint Oil, 1% sodium lauryl sulfate, 91% inerts.

RK and VIC were applied directly from manufacturer's aerosol cans. DR was applied with a Centrobulb D® bulb duster manufactured by Central Rubber Products Co. For both RK and VIC, the entire aerosol can was applied to a nest. The weight of total ingredients for RK was 481 g. and for VIC 469 g. Approximately 15 g of DR was applied to each nest. Formulations were applied directly into the surface opening of ground nests. The nozzle of the applicator was placed into or as close to the opening as was possible.

DR was applied to 22 nests, RK to 21 nests, and VIC to 20 nests. Time of day, and atmospheric conditions were noted at time of treatment. Forty-eight to 120 hr after treatment, nests were excavated and the following data recorded: exposure of nest tunnel opening (in shade, on flat ground, etc.), the number of combs in the nest, straight distance across largest nest comb, shortest distance from the surface opening to the nest cavity, and configuration of nest tunnel. Success or failure of the treatment was determined by the number of *V. vulgaris* adults found alive on nest combs at the time of excavation. A nest was considered successfully controlled if <10 live adults were found in the excavated cavity.

STATISTICAL ANALYSIS

The proportion of nests killed after treatment with different materials was analyzed by the Chi square test. This test was also used to compare treatment success among nests with different tunnel configurations. Comparisons of treatment success between pairs of groups were analyzed with Fisher's exact test. Analysis of variance was used to analyze differences in the diameter of the largest comb, number of combs in a nest and distance of the nest cavity from the surface entrance, among nests treated with different materials. Comparison of these parameters between nests that survived treatment and those that did not was done with an unpaired t-test. Statistical analyses were performed using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego California USA, (www.graphpad.com).

RESULTS

Nest Measurements

The dimensions of excavated nests are presented in Table 1. Nests ranged from 2 - 9 in. diameter (average 5.3 in.) and contained an average of 3.6 combs each (range 2 – 7 combs). Distance of the nest cavity from the surface entrance ranged from 1 to 24 in. (average 7.7 in.). Nest size, as indicated by diameter of the largest comb and number of combs, did not differ significantly among nests treated with different products. Nests treated with DR were significantly closer to the surface than those treated with RK or VIC ($P = 0.0028$) (Table 1). There was no significant difference between the depth of nests treated with RK or VIC.

Configuration of the entrance tunnel fell into 5 categories: vertical, horizontal, sloping, right angle, and re-curved (Fig. 1). Over half (60%) of the nests excavated fell into 2 categories: right

Table 1. Description of nests treated with different materials.

Material		N	Diameter of largest comb (inches)	No. of combs	Distance from surface entrance to nest (inches)
Drione	Killed nests	22	4.8 +1.2[1]	3.4 + 0.8	5.4 + 2.5
	Treatment failures	0	-	-	-
	Total	**22**	**4.8 +1.21**	**3.4 + 0.8**	**5.4 + 2.5**
RealKill	Killed nests	12	5.1 + 1.0	3.4 + 0.5	8.1 + 3.7
	Treatment failures	9	6.2 + 1.3	5.0 + 1.3	13.2 + 6.1
	Total	**21**	**5.8 + 1.3**	**4.1 + 1.2**	**10.3 + 5.4**
Victor	Killed nests	6	4.5 + 1.2	3.0 + 1.1	3.3 + 2.4
	Treatment failures	14	5.9 + 1.3	3.9 + 1.1	9.3 + 4.8
	Total	**20**	**5.5 +1.4**	**3.6 + 1.1**	**7.5 + 5.0**
Overall	Killed nests	40	4.9 + 1.4	3.3 + 0.8	5.9 + 3.2
	Treatment failures	23	6.2 + 1.3	4.3 + 1.3	10.8 + 5.6
	Total	**63**	**5.3 + 1.3**	**3.7 + 1.1**	**7.7 + 4.8**

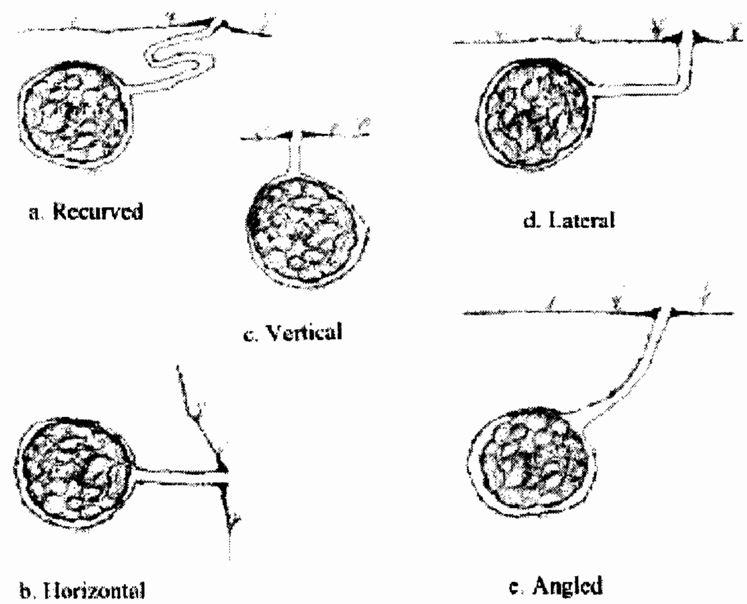


Figure 1. Yellowjacket ground nest entrance tunnel configurations.

angle (33%) or sloping (27%). The remaining nests fell into 3 categories: vertical (17%), recurved (14%), and horizontal (8%) (Table 2).

Efficacy of Different Products

The 3 products tested differed significantly in their efficacy (P < 0.0001) (Table 2). DR provided 100% control for all nests treated with it. Treatment success was significantly lower for aerosol products (P < 0.0001 for either product when compared with DR). Although RK controlled a higher proportion (57%) of nests than VIC (30%), this difference was not statistically significant (P = 0.1180).

Treatment Success Relative to Nest Characteristics

Nest Size

Among nests treated with aerosol products, those that survived treatment were significantly larger in diameter (P = 0.0014) and had more combs (P = 0.0007) than those that did not (Table 1). The distance between the surface opening and the nest cavity was also significantly greater for nests in which treatment failed (P < 0.0001) (Table 1).

Entrnce Tunnel Configuration

Drione® successfully controlled 100% of nests, regardless of tunnel configuration. Both RK and VIC controlled 100% of nests with vertical tunnels (Table 2). Among nests treated with aerosol products, the proportion of nests killed was significantly higher among nests with sloping tunnels than those with right angle tunnels (P = 0.0472). Treatment success for nests with right angle tunnels was lower among nests treated with VIC (12%) or RK (60%) than for those treated with DR (100%) (Table 2). This difference was statistically significant for VIC (P = 0.0014) but not for RK (probably due to small sample size). Neither RK nor VIC successfully killed any nest having a horizontal or recurved tunnel (Table 2). However, the number of nests in these categories was too small to analyze these results statistically.

Environmental Conditions

Environmental conditions at the time of treatment or excavation did not appear to affect treatment success. There was no statistically significant difference in control among nests treated at different times of the day or under different temperature or weather conditions (overcast or clear) (data not shown). Similarly, treatment success did not differ among nests with various degrees of shading or exposure of the nest entrance. Treatment success was evident 24 hr. after application of any of the pesticides used, and did not differ among nests excavated at different intervals after treatment.

DISCUSSION

Drione® was significantly more effective in controlling ground nests of *V. vulgaris* than either of the aerosol products tested. This product effectively eliminated 100% of nests treated, regardless of their size, distance from the surface entrance, or configuration of the entrance tunnel. This level of control can be attributed to the fact that it is carried into the nest cavity on the bodies of the yellowjackets themselves. In contrast, the aerosol products tested (RK and VIC) contain liquids whose movement into the nest cavity may be hindered by the configuration of the entrance tunnel. The effectiveness of RK and VIC was reduced in nests that had entrance tunnels with sharp changes in direction or approached the nest horizontally from the side of an embankment.

The efficacy of aerosol products was also affected by length of the entrance tunnel. This has been noted previously by others.

Table 2. Proportion of nests with different entrance tunnel types that were killed by treatment with either Drione®, RealKill®, or Victor Poison-Free Wasp and Hornet Killer®.

Tunnel Configuration	Number of Nests Killed/Number Treated (%) Drione	RealKill	Victor	Total
Vertical	5/5 (100%)	2/2 (100%)	4/4 (100%)	11/11 (100%)
Sloping	4/4 (100%)	7/10 (70%)	1/3 (33%)	12/17 (71%)
90^{o} angle	8/8 (100%)	3/5 (60%)	1/8 (12%)	12/21 (57%)
Recurved	3/3 (100%)	0/3 (0%)	0/3 (0%)	3/9 (33%)
Horizontal	2/2 (100%)	0/1 (0%)	0/2 (0%)	2/5 (40%)
Total	22/22 (100%)	12/21 (57%)	6/20 (30%)	40/63 (63%)

MacDonald (1980) observed that the effectiveness of aerosols was reduced in nests with longer entrance tunnels. Likewise, Bell and Wagner (1981) noted that household aerosol sprays labeled for yellowjacket control were only effective in killing individual insects and are inadequate for actual nest destruction. The effectiveness of aerosol products can be dramatically enhanced by excavation of the nest during treatment, and application directly to the comb. However, excavation of active nests is hazardous and should not be attempted without protective clothing.

Additionally and anecdotally, SMCMAD has had treatment failures when using DR to treat subterranean yellowjacket nests beyond the sample groups of this study. Irrigation and rainwater tend to cake or disperse DR and prevent its carriage into the nests by individual yellowjackets. Rainfall and irrigation were not present during any treatments in the course of this study and therefore their effects could not be evaluated. Rainfall is, as a rule, infrequent in San Mateo County during the summer season (the time at which SMCMAD receives its greatest number of requests for yellowjacket treatment). Therefore, it is not likely to be a significant factor in selection of materials for control of yellowjackets in this area. Failures have also been noted among nests that had multiple cavities or additional undetected entrance tunnels. All of the nests in the present study were contained within a single cavity.

Environmental and human health factors often displace efficacy in choosing a material to control vectors. While these issues are of tremendous importance, the efficacy of products used by public health agencies should be carefully evaluated. VIC is not an effective alternative to pesticides currently used by the SMCMAD for destruction of undergound yellowjacket nests. This material may be of value to members of the public who wish to pursue treatment of nests themselves and are adamantly opposed to the use of more traditional pesticides. However, anyone treating yellowjacket nests should be advised to exercise extreme caution, including the use of protective gear when using materials that require excavation of the nest cavity.

Acknowledgments

We thank the personnel of the SMCMAD for their assistance. In particular James Counts' comments on operational technique, are appreciated. Angela Rory participated in data collection. Alexandra Porshnikoff kindly lent her artistic skill to the production of Figure 1.

REFERENCES

Akre, R.D., A. Greene, J.F. Macdonald, P.J. Landolt and H.G. Davis. 1980.Yellowjackets of America north of Mexico. U.S. Department of Agriculture, Agriculture Handbook No. 442, 102 p.

Bell, M.R. and R.E. Wagner 1981. Control of yellowjackets and similar wasps. Division of Agricultural Sciences, University of California, Leaflet 2527. 2 p.

MacDonald, J.F. 1980. Biology, recognition, medical importance and control of Indiana social wasps. Purdue Univ. Coop. Ext. Serv. Publ. E-91. 24 p.

William C. Reeves
New Investigator Award

The William C. Reeves New Investigator Award is given annually by the Mosquito and Vector Control Association of California 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.

There were no competitors for this award at the 2002 Seventieth Annual Conference.

Previous William C. Reeves New Investigator Award Winners:

2001 - Christopher Barker

2000 - Jason Rasgon

1999 - Parker D. Workman

1998 - Yvonne Ann Offill

1997 - John Gimnig

1996 - None

1995 - Margaret C. Wirth

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

Geospatial and Statistical Modeling of Mosquito Distribution in an Emerging Focus of La Crosse Virus*

Christopher M. Barker[1,2], Carlyle C. Brewster[1], and Sally L. Paulson[1]

[1]Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0319
[2]Current address: Arbovirus Field Station, 4705 Allen Road, Bakersfield, CA 93312

ABSTRACT: Several human cases of encephalitis in southwestern Virginia have been attributed to La Crosse virus (LAC) in recent years. The emergence of LAC in the region has generated interest in studying the distribution and phenology of the principal vector, *Ochlerotatus triseriatus*, and a potential accessory vector, *Aedes albopictus*. A Bayesian statistical model was used to incorporate ovitrap survey data and a landcover map derived from satellite imagery of Wise County, Virginia to create surface maps showing the probability of high mosquito abundance for the region. The model accurately predicted that *Oc. triseriatus* would be most abundant in forested areas and that *Ae. albopictus* is most likely to be found in urban and residential areas. The model was also used to predict the occurrence of these mosquito species for different time periods during the season.

INTRODUCTION

La Crosse virus (LAC) causes encephalitis that affects mainly children, causing febrile illness, often with swelling of the central nervous system, sometimes followed by seizures which can last for years after the initial viral infection. Although death from this disease is rare, infections and their sequelae can cause seizures and reduced learning potential in some children, not to mention the financial strain caused by extended hospital stays (Grimstad 1988).

From 1975 through 1993, only one case of La Crosse encephalitis was reported from the entire state of Virginia. However, between 1994 and 1997, 5 cases were reported from Tazewell County, and in 1997, 5 cases were reported from Wise County (Virginia Mosquito Control Association 1998). In 1998, 1 case was reported from each of the following counties: Wise, Dickenson, and Buchanan. Due to these recent cases, southwest Virginia is emerging as a significant focus of LAC activity (Fig. 1).

The principal vector of LAC is *Ochlerotatus triseriatus* (Say), the eastern treehole mosquito (Berry et al. 1974, Pantuwatana et al. 1974, Watts et al. 1974, Balfour et al. 1975, Beaty and Thompson 1975). As its common name indicates, this mosquito develops in treeholes, which are very common in eastern deciduous forests. As a result, this mosquito also is encountered commonly throughout the forested areas of the eastern and midwestern United States.

In the natural cycle of LAC, the virus is amplified in a mammalian reservoir host such as the eastern chipmunk, *Tamias striatus* (Gauld et al. 1975), or the grey squirrel, *Sciuris carolinensis* (Moulton and Thompson 1971, Ksiazek and Yuill 1977). Infected female *Oc. triseriatus* are also capable of transmitting LAC transovarially to their progeny (Miller et al. 1977). LAC overwinters in transovarially infected eggs, and infected larvae hatch the following spring (Lisitza et al. 1977). Miller et al. (1977) showed that this virus can persist for at least 4 years in the absence of horizontal amplification in vertebrate reservoir hosts. Therefore, transovarial transmission and the overwintering of LAC-infected eggs are critical for virus maintenance in nature (Miller et al. 1979).

Humans are dead-end hosts for LAC, being susceptible to infection, but incapable of developing a sufficient viremia to permit passage of the virus from a human to a susceptible mosquito. As humans have populated forested areas, contact with *Oc. triseriatus* has increased. Another factor that increases *Oc. triseriatus*-human contact is that *Oc. triseriatus* also will breed in artificial containers near homes, particularly if the containers are located in shaded areas (Nasci 1988).

Another mosquito that has spread throughout the eastern United States is *Aedes albopictus* (Skuse). Like *Oc. triseriatus*, this mosquito breeds in containers and commonly inhabits areas near human dwellings. Breeding populations of *Ae. albopictus* were first found in the United States in 1985 near Houston, Texas (Sprenger and Wuithiranyagool 1986), and since then this species has spread at an impressive rate so that its range now includes most of the U.S. east of the Mississippi River. Many of these same areas in the eastern United States are also endemic for LAC, and *Ae. albopictus* is a competent experimental vector, with infection and oral transmission rates equal to or higher than those for *Oc. triseriatus* (Grimstad et al. 1989, Streit and Grimstad 1990). The rates for transovarial transmission of LAC in *Ae. albopictus* are lower than those for *Oc. triseriatus* (Tesh and Gubler 1975), but recent isolations from wild-caught vertically infected *Ae. albopictus* indicate that this species may become an important bridge vector (Gerhardt et al. 2001).

Because both *Oc. triseriatus* and *Ae. albopictus* preferentially lay eggs in certain habitats, geographic information systems and remote sensing techniques can be used to identify habitats in

* presented at the 69[th] Annual Conference of the MVCAC, January 21-24, 2001.

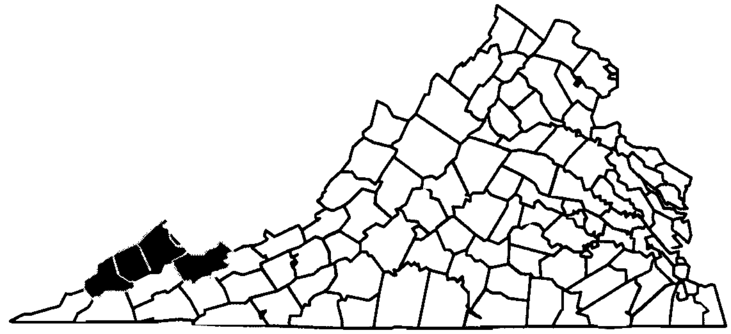


Figure 1. Virginia counties reporting cases of La Crosse encephalitis (1994-1998).

which these species breed. Such techniques have proven useful in several recent studies showing relationships between landcover characteristics and vector abundance (Beck et al. 1994, Wood et al. 1992, Roberts et al. 1996). The abundance of malaria vectors near villages in Mexico (Beck et al. 1994) and at study sites Belize (Roberts et al. 1996) was successfully predicted as either high or low based on satellite-derived landcover composition surrounding the study areas. Because environmental factors such as rainfall, elevation, and temperature influence both vegetation type and vector abundance, it seems logical that a relationship would exist between vector population levels and landcover type.

Bayesian modeling procedures provide a way to produce distribution maps by linking survey data for a given species with satellite imagery. Although Bayesian procedures have been used primarily to model vertebrate distributions (Aspinall 1991, Aspinall and Veitch 1993, Hepinstall and Sader 1997, Tucker et al. 1997), this approach also has been used to predict insect abundance levels (Wood et al. 1992). Bayesian procedures use survey data to calculate prior probabilities of finding the species at any point within the landscape, regardless of other variables. The formula also requires the assignment of conditional probabilities to pertinent variables, such as habitat type, given the presence or absence of the species. The combination of prior and conditional probabilities using the Bayesian formula results in a map showing the distribution of the species as a probability of high abundance, *i.e.*, abundance exceeding a predefined threshold. In a study by Wood et al. (1992), Bayesian procedures were used to predict high and low mosquito-producing rice fields in California, indicating that Bayesian modeling could be useful for directing vector control efforts or public disease risk awareness campaigns.

MATERIALS AND METHODS

Mosquito Survey

Mosquitoes were collected by ovitrap during a 16-week period from May 29 through September 18, 2000. All ovitraps were collected one week after they were set out, and traps were distributed throughout the county each week, so that the entire county was represented during each sampling period. During the first 4 weeks of collection, 22 new ovitrap sites (21 during week 1) were set out each week, with a single ovitrap at each site. Following the initial 4-week collection period, each ovitrap site was repeated at 4-week intervals through the end of the 16-week period so that collections were made four times at each location. The majority of these traps were placed in habitat types where highest numbers of *Oc. triseriatus* and *Ae. albopictus* were expected (forested areas or urban/residential areas), and remaining ovitraps were placed in less prevalent habitat types that were expected to have lower numbers of mosquitoes, such as herbaceous rangeland, coniferous forest, shrub and brush rangeland, and barren land. Although the number of ovitraps in a habitat type was partially based on anticipated numbers of mosquitoes, individual sites were chosen that were representative of the habitat type, not because of their expected suitability as mosquito oviposition sites. In addition to these repeated sites, 6 new sites were sampled each week after the first 4-week period from the landcover types where lower numbers of mosquitoes were expected to obtain a more representative estimate of oviposition activity in these habitats. Each ovitrap location was recorded using a hand-held GPS III Plus receiver (Garmin International, Inc., Olathe, KS) so that their exact locations could be mapped. Eggs from each site were identified to species (Linley 1989a,1989b) and counted weekly. These numbers were used to determine prior and conditional probabilities for high and low mosquito abundance for input into the Bayesian model.

Image Classification

Landsat Enhanced Thematic Mapper (ETM+) imagery was used to create a landcover map of Wise County using landcover classes described by Anderson et al. (1976). These included: Forest, Urban/Residential, Rangeland (Shrub & Brush and Herbaceous), Barren Land, and Water. GPS coordinates were recorded for various landcover types and these, along with ovitrap site descriptions, were used in a feature mapping process to create a training data set for the supervised classification. These training data for each landcover class then were used in a maximum likelihood classification of the Landsat imagery to create the landcover map of Wise County. Only 6 of the Landsat ETM+ spectral bands were used in the classification: blue, green, red, near-infrared, and mid-infrared (2 bands). The thermal band was not used because of its coarser spatial resolution (60m) compared to that of the visible color and other infrared bands (28.5m).

Bayesian Classification

The Bayesian model consists of 4 main components: prior probabilities of high or low species abundance, and conditional probabilities of high or low species abundance. These probabilities are used to calculate a posterior probability of high species abundance for a given condition (landcover type, in this case). Bayes' theorem, as used in this study, is as follows:

$$P(M_{high}|Habitat) = P(M_{high}) * P(Habitat|M_{high}) / [P(M_{high}) * P(Habitat|M_{high}) + P(M_{low}) * P(Habitat|M_{low})]$$

where

$P(M_{high})$ = Prior probability of high mosquito abundance
$P(M_{low})$ = Prior probability of low mosquito abundance
$P(Habitat|M_{high})$ = Conditional probability of finding a specific habitat given high mosquito abundance
$P(Habitat|M_{low})$ = Conditional probability of finding a specific habitat given low mosquito abundance, and
$P(M_{high}|Habitat)$ = Posterior probability of high mosquito abundance

Prior probabilities of high and low mosquito abundance and conditional probabilities for each landcover type were calculated from the mosquito survey data. Prior and conditional probabilities were calculated for each pixel in the landcover map to produce a probability surface, which showed the posterior probabilities for high mosquito abundance for each pixel in the county based on landcover type. Surfaces were created for each of eight 2-week periods during the collection season.

Model Validation

Statistical comparisons based on error matrices (Fielding and Bell 1997) were used to test for agreement between model predictions and actual ovitrapping survey data. Buffer zones of 200 m, a radius that encompasses the flight range for most *Oc. triseriatus* and *Ae. albopictus* (Mather and DeFoliart 1984, Hawley 1988), were created around each ovitrap location, and posterior probabilities for high mosquito abundance were averaged within this zone for each site. These average probabilities then were compared with actual mosquito numbers collected at the respective sites. Average probabilities greater than 50% were assumed to predict high abundance, and actual mosquito numbers were defined for each species as either high or low based on whether they were above or below the mean number per trap-week for the entire collecting season.

RESULTS AND DISCUSSION

The ovitrapping data showed that there are significant differences in oviposition preference among the landcover classes for both *Oc. triseriatus* and *Ae. albopictus*. In particular, forested areas yielded significantly higher numbers of *Oc. triseriatus* than urban areas (P=0.0004), but the opposite was true for *Ae. albopictus*, with higher numbers of this species collected in urban areas (P=0.0001).

The landcover map of Wise County that was created from the satellite imagery had an overall accuracy of 98% based on a comparison of output classification and user-defined ground truth data. Accuracy levels for individual landcover classes were also high, indicating that minimal error was introduced in the classification process.

Temporal dynamics of *Oc. triseriatus* seemed to be somewhat bimodal, with peaks in late June (223 eggs/trap week) and early September (166 eggs/trap week). The model reflects these fluctuations, with accuracy levels [(ovitrap sites correctly predicted as having high or low *Oc. triseriatus* abundance)/(total number of sites)] between 55 and 79%. Accuracy levels decreased when the mosquito population was high. Predicted and actual numbers of *Oc. triseriatus* were highest in forested areas.

Low numbers of *Ae. albopictus* (<10 eggs/trap week) were present during June, and the population increased steadily during July and August, peaking in late August (55 eggs/trap week) and declining thereafter. *Ae. albopictus* abundance was generally predicted with better accuracy (70–94%) than *Oc. triseriatus*. Accuracy levels early in the season were very high (94%) because of the large number of sites where actual and predicted mosquito abundance levels were low. As mosquito populations increased later in the season, the accuracy levels declined. Unlike *Oc. triseriatus*, urban areas had the highest actual and predicted numbers of Ae. albopictus during most weeks.

Kappa values, which take into account correctly classified pixels in addition to false positives and negatives, varied from very low to moderate values (0.00-0.53 for Oc. triseriatus; 0.00-0.49 for Ae. albopictus) for both species.

The moderate levels of accuracy achieved with the current model indicated the need for inclusion of other ecologically relevant factors in the model. Rainfall and elevation data were obtained for the study period, but their relationship to mosquito abundance could not be shown for the time period in question. Rainfall was relatively frequent during the collection period, so it might be more closely correlated with mosquito abundance during years when it is more sporadic and serves to limit production of container-breeding LAC vectors. To improve the model, relevant climatic data should be included and multiple ovitraps should be used at each collection site to reduce variation resulting from trap placement selection. Also, sampling in each landcover type at an intensity representative of its actual prevalence within the county would provide more accurate predictions of mosquito abundance.

In conclusion, the present study demonstrates that ovitrapping survey data can be combined with statistical modeling and remote sensing methods to predict the abundance of Oc. triseriatus and Ae. albopictus. Predictions from this type of model may improve and focus control strategies or public awareness campaigns in areas where mosquito populations are high.

REFERENCES

Anderson, J. R., E. E. Hardy, J. T. Roach, and R. E. Witmer. 1976. *A Land Use and Land Cover Classification for Use with Remote Sensor Data*. U. S. Geological Survey Professional Paper 964. Washington, DC: U. S. Government Printing

Office, 28 pp.

Aspinall, R. J. 1991. Use of an inductive modeling procedure based on Bayes Theorem for analysis of pattern in spatial data. *Computer Modeling in the Environmental Sciences* (D.G. Farmer and M.J. Rycroft, editors), Institute of Mathematics and its Applications Conference Series No. 28, Oxford University Press, Oxford, pp. 325-339.

Aspinall, R. and N. Veitch. 1993. Habitat mapping from satellite imagery and wildlife survey data using a Bayesian modeling procedure in a GIS. Photogrammetric Engineering and Remote Sensing. 59(4): 537-543.

Balfour, H. H. Jr., C. K. Edelman, F. E. Cook, W. I. Barton, A. W. Buzicky, R. A. Siem, and H. Bauer. 1975. Isolates of California encephalitis (La Crosse) virus from field-collected eggs and larvae of *Aedes triseriatus*: Identification of the overwintering site of California encephalitis. J. Infect. Dis. 131(6): 712-716.

Beaty, B. J. and W. H. Thompson. 1975. Emergence of La Crosse virus from endemic foci. Fluorescent antibody studies of overwintered *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 24(4): 685-691.

Beck, L. R., M. H. Rodriguez, S. W. Dister, A. D. Rodriguez, E. Rejmankova, A. Ulloa, R. A. Meza, D. R. Roberts, J. F. Paris, M. A. Spanner, R. K. Washino, C. Hacker, and L. J. Legters. 1994. Remote sensing as a landscape epidemiologic tool to identify villages at high risk for malaria transmission. Am. J. Trop. Med. Hyg. 51(3): 271-280.

Berry, R. L., B. J. LaLonde, H. W. Stegmiller, M. A. Parsons, and G. T. Bear. 1974. Isolation of La Crosse virus (California encephalitis group) from field collected *Aedes triseriatus* (Say) larvae in Ohio (Diptera: Culicidae). Mosquito News. 34(4): 454-457.

Fielding, A. H. and J. F. Bell. 1997. A review of methods for the assessment of prediction errors in conservation presence/ absence models. Environmental Conservation. 24(1): 38-49.

Gauld, L. W., T. M. Yuill, R. P. Hanson, and S. K. Sinha. 1975. Isolation of La Crosse virus (California encephalitis group) from the chipmunk (*Tamias striatus*), an amplifier host. Am. J. Trop. Med. Hyg. 24(6): 999-1005.

Gerhardt, R.R., K.L. Gottfried, C.S. Apperson, B.S. Davis, P.C. Erwin, A.B. Smith, N.A. Panella, E.E. Powell, and R.S. Nasci. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. Emerging Infectious Diseases. 7(5): 807-811.

Grimstad, P. R. 1988. California Group Virus Disease. In: *The Arboviruses: Epidemiology and Ecology* vol. II (T. P. Monath, editor), CRC Press, Boca Raton, FL, pp. 99-136.

Grimstad, P. R., J. F. Kobayashi, M. Zhang, and G. B. Craig, Jr. 1989. Recently introduced *Aedes albopictus* in the United States: Potential vector of La Crosse virus (Bunyaviridae: California Serogroup). J. Am. Mosq. Control Assoc. 5(3): 422-427.

Hawley, W. A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 4(Suppl.1): 1-40.

Hepinstall, J. A. and S. A. Sader. 1997. Using Bayesian statistics, thematic mapper satellite imagery, and breeding bird survey data to model bird species probability of occurrence in Maine. Photogrammetric Engineering and Remote Sensing. 63(10): 1231-1237.

Ksiazek, T. G. and T. M. Yuill. 1977. Viremia and antibody response to La Crosse virus in sentinel gray squirrels (*Sciuris carolinensis*) and chipmunks (*Tamias striatus*). Am. J. Trop. Med. Hyg. 26(4): 815-821.

Linley, J. R. 1989a. Scanning Electron Microscopy of the Egg of *Aedes (Protomacleaya) triseriatus* (Diptera: Culicidae). J. Med. Entomol. 26(5): 474-478.

Linley, J. R. 1989b. Comparative Fine Structure of the Eggs of *Aedes albopictus*, *Ae. aegypti*, and *Ae. bahamensis* (Diptera: Culicidae). J. Med. Entomol. 26(6): 510-521.

Lisitza, M. A., G. R. DeFoliart, T. M. Yuill, and M. G. Karandinos. 1977. Prevalence rates of La Crosse virus (California encephalitis group) in larvae from overwintered eggs of *Aedes triseriatus*. Mosquito News. 37(4): 745-750.

Mather, T. N. and G. R. DeFoliart. 1984. Dispersion of gravid *Aedes triseriatus* (Diptera: Culicidae) from woodlands into open terrain. J. Med. Entomol. 21(4): 384-391.

Miller, B. R., G. R. DeFoliart, and T. M. Yuill. 1977. Vertical transmission of La Crosse virus (California encephalitis group): Transovarial and filial infection rates in *Aedes triseriatus* (Diptera: Culicidae). J. Med. Entomol. 14(4): 437-440.

Miller, B. R., G. R. DeFoliart, and T. M. Yuill. 1979. *Aedes triseriatus* and La Crosse virus: Lack of infection in eggs of the first ovarian cycle following oral infection of females. Am. J. Trop. Med. Hyg. 28(5): 897-901.

Moulton, D. W. and W. H. Thompson. 1971. California group virus infections in small, forest-dwelling mammals of Wisconsin: Some ecological considerations. Am. J. Trop. Med. Hyg. 20(3): 474-482.

Nasci, R. S. 1988. Biology of *Aedes triseriatus* (Diptera: Culicidae) developing in tires in Louisiana. J. Med. Entomol. 25(5): 402-405.

Pantuwatana, S., W. H. Thompson, D. M. Watts, T. M. Yuill, and R. P. Hanson. 1974. Isolation of La Crosse virus from field collected *Aedes triseriatus* larvae. Am. J. Trop. Med. Hyg. 23(2): 246-250.

Roberts, D. R., J. F. Paris, S. Manguin, R. E. Harbach, R. Woodruff, E. Rejmankova, J. Polanco, B. Wullschleger, and L. J. Legters. 1996. Predictions of malaria vector distribution in Belize based on multispectral satellite data. Am. J. Trop. Med. Hyg. 54(3): 304-308.

Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J. Am. Mosq. Control Assoc. 2(2): 217-219.

Streit, T. G. and P. R. Grimstad. 1990. Vector competence of *Aedes albopictus* for La Crosse encephalitis virus. Proc. Ohio Mosq. Cont. Assn. 19: 45-50.

Tesh, R. B. and D. J. Gubler. 1975. Laboratory studies of transovarial transmission of La Crosse and other arboviruses by *Aedes albopictus* and *Culex fatigans*. Am. J. Trop. Med. Hyg. 24(5): 876-880.

Tucker, K., S. P. Rushton, R. A. Sanderson, E. B. Martin, and J. Blaiklock. 1997. Modelling bird distributions – a combined GIS and Bayesian rule-based approach. Landscape Ecology. 12(2): 77-93.

Virginia Mosquito Control Association. 1998. Website: Reported Cases of Mosquito Transmitted Diseases in Virginia. http://www.mosquito-va.org/disease.html.

Watts, D. M., W. H. Thompson, T. M. Yuill, G. R. DeFoliart, and R. P. Hanson. 1974. Overwintering of La Crosse virus in *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 23(4): 694-700.

Wood, B. L., L. R. Beck, R. K. Washino, K. A. Hibbard, and J. S. Salute. 1992. Estimating high mosquito-producing rice fields using spectral and spatial data. Int. J. Remote Sensing. 13(15): 2813-2826.

Vectolex CG® Trial on *Ochlerotatus washinoi*, Shasta County, CA*

John Albright

Shasta Mosquito and Vector Control District, P.O. Box 990331, Redding, CA 96099-0331

ABSTRACT: Vectolex CG® was tried in March 2000 on a 2 acre, intermittently-flooded, wooded area along the Sacramento River infested with third instar *Ochlerotatus washinoi* at two larvae/dip. It took two weeks for the larvae to die. However, the source remained free of third instar through pupal stages until it dried up in Mid-May indicating an excellent residual effect.

BACKGROUND

Ochlerotatus washinoi is a hard-biting early spring mosquito species often found in the District boundary. Larvae frequently occur in areas that are intermittently flooded or subject to groundwater seepage in the late winter and early spring. Turtle Bay is a heavily wooded area adjoining the Sacramento River. It was once part of the gravel quarry used in the construction of Shasta Dam. Historical excavation has created numerous low spots where water accumulates creating ideal *Oc. washinoi* habitat. Depending on precipitation, river levels and groundwater conditions these sources may persist as late as June generating *Culiseta spp., Culex spp.* and *Anopheles spp.* as the weather warms up. If *Bacillus sphaericus* is successful in controlling *Oc. washinoi* at Turtle Bay, the residual effect could conceivably control the other mosquitoes following later in the year and greatly reduce the labor needed to treat this area annually.

MATERIALS AND METHODS

Dip counts were taken following the procedure provided to us by Peter Dechant and Stephanie Whitman of Valent Biosciences. Vectolex CG®, a granular commercial preparation of *Bacillus sphaericus* strain 2362, was applied at a rate of 5 lb./ac. to a 2 ac. source averaging 2 ft. in depth. Dip counts prior to the application averaged 2.0 third and fourth instar larvae/dip. No other larval instars were observed. Dip counts were taken on a weekly basis from just prior to the application on March 6th until May 16, 2000, at which time the source had dried up. Dip count data were put in a Microsoft Excel Spreadsheet for graphical analysis. Percent effectiveness was determined by comparing counts of 3rd and 4th instar larvae pre- and post-treatment. Residual effect was determined by absence of 3rd and 4th stage larvae in the dip counts despite the presence of 1st and 2nd instar larvae (Figure 1).

RESULTS

Control reached 90% after 2 weeks and fluctuated between 90 and 100% for the remainder of the trial (Figure 1).

First and 2nd instar larvae of *Anopheles spp.* and *Culiseta spp.* were first observed three weeks post treatment. No 3rd or 4th instar larvae of *Anopheles spp.* and *Culiseta spp.* developed at any point in the experiment indicating residual control from March 6 through May 9. The source dried up by May 16.

DISCUSSION

Cold weather had several important effects on the results obtained with this experiment. Initial dip-counts were probably "skewed downward" because the larvae were swimming on the bottom of the source and rarely surfacing for air. This also led to the appearance of an increase in the dip counts of 3rd or 4th instar larvae between four days and one week post-treatment (Figure 2). The cold water was probably responsible for the long time required for the *Oc. washinoi* larvae to die. This may be due to less larval feeding, slower larval metabolism, a decrease in the rate of activity of the Vectolex CG® or some combination of these factors. There seems to be a correlation between mean daily temperatures of 60° F, larval activity at the surface, and Vectolex CG® control (Figure 3).

It would be interesting to see if making an application of Vectolex CG® during a warm spell might lead to a quicker kill. It would also be interesting to see if this would alter the residual effects of the product. Other sources in this area were similarly treated and subjective observations seem to support the results of this experiment. However, there was still a significant population of *Oc. washinoi* adults noticed in this area. This may be the result of missed sources or immigration of adults from elsewhere rather than a failure of the product.

*Presented and manuscript submitted at the 69th annual conference of MVCAC, January 21-24, 2001. The Editor apologizes for the oversight in not having this paper published in the above dated proceedings. Since the original presentation was given the sub-genus *Ochlerotatus* was elevated to genus status, changing the name of *Aedes washinoi* to *Ochlerotatus washinoi*.

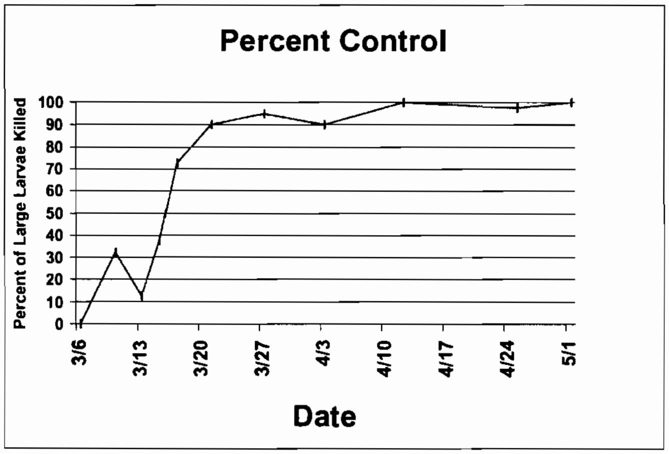


Figure 1 – Mortality of *Oc. Washinoi* 3rd and 4th Instar Larvae

Anopheles spp. and *Culiseta spp.* first and second instar larvae began to appear after three weeks but control continued until the source dried up in early May (Figure 2).

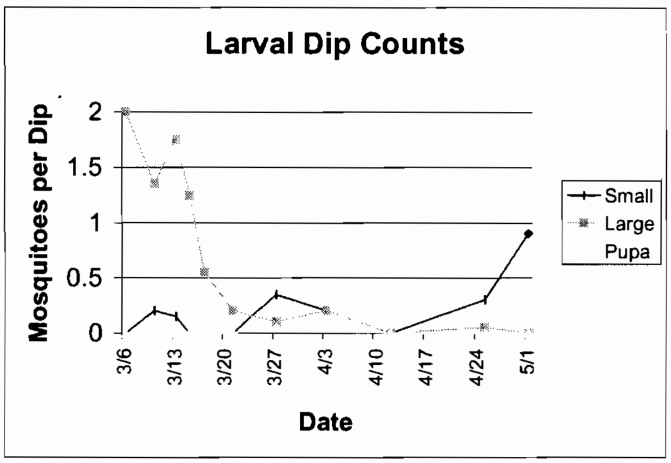


Figure 2 – Dip Count Comparisons of 1st and 2nd Instars vs. 3rd and 4th Instars vs. Pupae

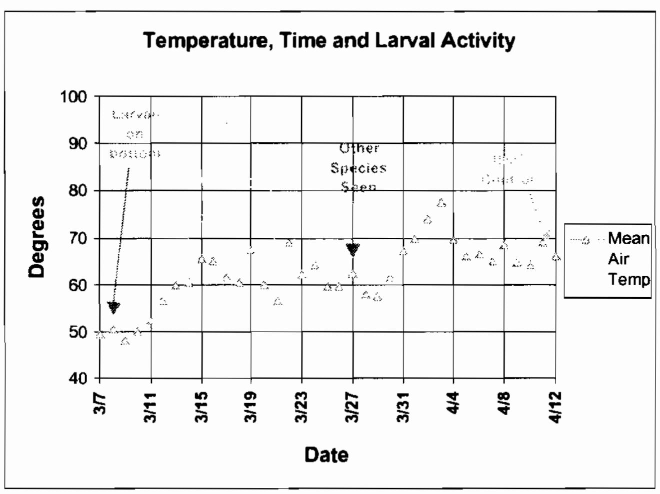


Figure 3 – Observations of Larval Activity

Early Season Factors Affecting *Vespula pensylvanica* Peak Populations, Shasta County, CA*

John Albright

Shasta Mosquito and Vector Control District, P.O Box 990331, Redding, CA 96099-0331

ABSTRACT: The importance of *Vespula pensylvanica* (yellowjackets) as a nuisance vector in the Shasta Mosquito and Vector Control District (SMVCD) is highly variable from one year to the next. The level influence of different factors affecting yellowjacket populations also varies greatly from year to year. Having a prudent, effective and proactive control program requires some effective means of predicting peak yellowjacket activity early in the year based on objective observable criteria. A 3-year long experimental control program monitoring *V. pensylvanica* populations has not found a good correlation between early season conditions and late season yellowjacket populations.

BACKGROUND

In 1997, it was noticed that a substantial amount of District time not devoted to mosquito control was spent answering public inquiries about yellowjacket control. In 1998 we began monitoring *Vespula pensylvanica* populations in several municipal parks to obtain base knowledge from which to determine the necessity and feasibility of controlling yellowjackets in public areas. Several control techniques were tried at different locations. It was apparent from the data obtained that the populations at the test locations varied considerably from year to year. Also the number of inquiries from the public regarding yellowjacket control varied from overwhelming to nonexistent depending on the year. It seemed that an analysis of data that might effect yellowjacket populations over time might enable us to make early predictions regarding the level of peak yellowjacket activity. We wished to objectively test the following assumptions:

High queen numbers mean high nest numbers, which means high yellowjacket numbers.

Favorable conditions will lead to early establishment of nests, which leads to larger nests and higher peak populations.

Early observations taken at a few known problem areas provide a representative picture of overall yellowjacket activity within the District boundaries.

MATERIALS AND METHODS

Vespula pensylvanica queens and workers were trapped using Rescue® Yellowjacket traps baited with heptyl butyrate and turkey ham over a 3-year period beginning in 1998. The traps were set at 4 public municipal parks within Redding city limits. Traps were set in April every year and maintained through October. Two sites were trapped for queens from early April until queens ceased to appear in the traps. All locations were then trapped for the remainder of the season to monitor worker populations. The traps were emptied weekly, re-baited and the trapped yellowjackets were counted. Two locations were not trapped for queens and were untreated controls. The worker numbers used for predicting population trends were taken from the control sites to avoid influences on the data that may have been due to such factors as early-season queen-trapping or late-season use of toxic bait. Yellowjacket population data was stored in a Microsoft Access® database. Weather information came from historical data and Weatherview® software associated with SMVCD weather stations and data from the Internet. Graphs were generated using Microsoft Excel®.

RESULTS

The year 2000 was the most active year for queens with 248 queens trapped, compared to 82 and 58 for the years 1998 and 1999 respectively (Figure 1). The year 1999 was the most active for workers with a total of 3,793 per trap compared to 726 and 2,735 for the years 1998 and 2000 respectively (Figure 2). The ratio of the total workers/trap to the number of queens caught was 8.85, 65.40 and 11.03 for the years 1998, 1999, and 2000 respectively (Figure 3). Earliest nest establishment occurred in 2000 with later establishment in 1999 and the latest establishment of nests in 1998. This can be shown by dramatic increases in the numbers of foraging workers observed (Figure 4). This occurred around April 19, 2000, May 24, 1999 and June 7, 1998. The year 2000 was the peak year for yellowjacket activity at sites where queens were trapped and workers were treated with toxic baits. The year 1999 was the peak year at the control sites (Figure 5).

DISCUSSION

The data do not support the idea that high queen numbers in the early spring are a good predictor of *V. pensylvanica* peak populations. The highest yellowjacket populations occurred in 1999 (Figure 2), which was the year of the lowest queen numbers

**Presented and manuscript submitted at the 69th annual conference of MVCAC, January 21-24, 2001. The Editor apologizes for the oversight in not having this paper published in the above dated proceedings.*

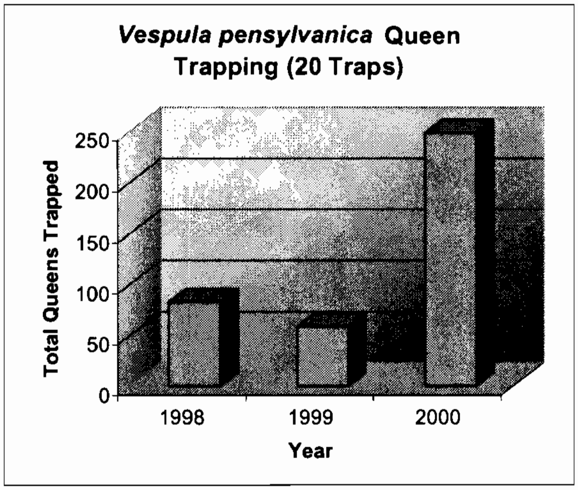


Figure 1.

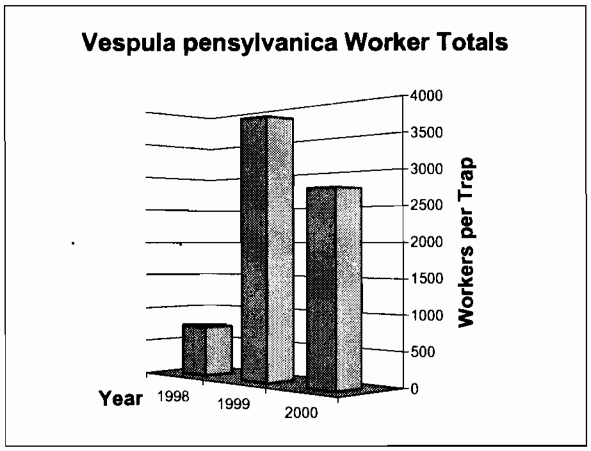


Figure 2.

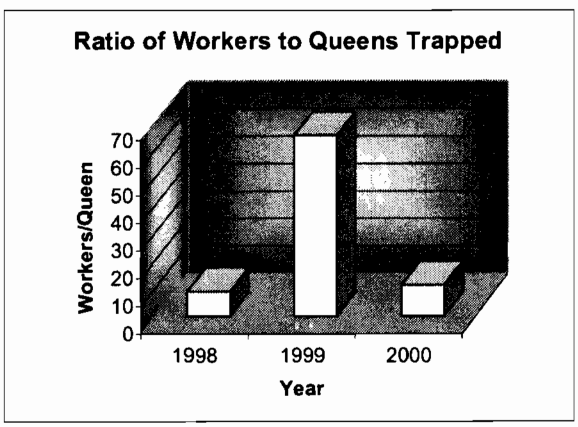


Figure 3.

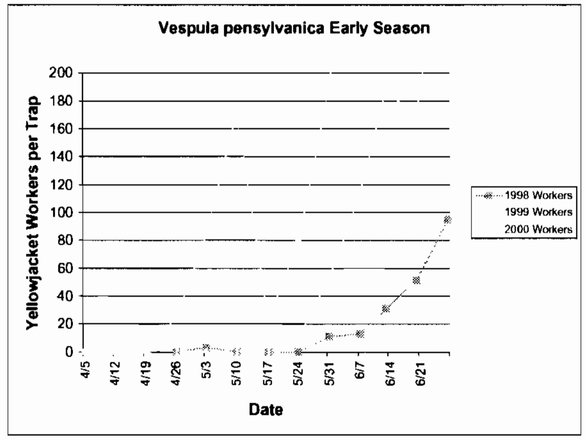


Figure 4.

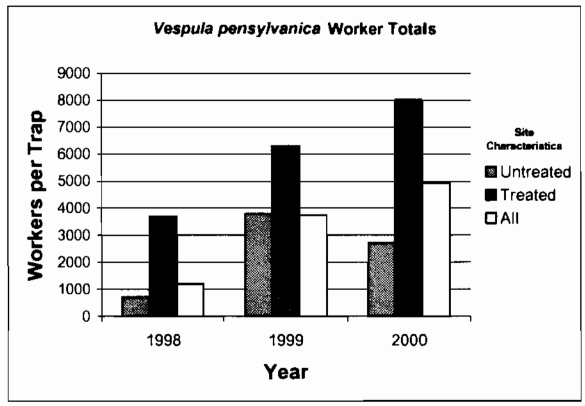


Figure 5.

(Figure 1). The number of workers, in fact correlates very poorly with the number of queens caught in the early season (Figure 3). There was a 5.9 to 7.4-fold increase in the ratio of workers to queens in 1999 compared to 1998 and 2000.

Mild conditions in April and May, which might encourage early development of nests, does not appear to be a good predictor of yellowjacket worker populations later in the season based upon our observations. Foraging workers were trapped nearly a month earlier in 2000 when compared to 1999, which was the peak yellowjacket year at the untreated sites (Figure 4). However, there is evidence that extremely poor early season conditions in fact lead to low late season yellowjacket numbers. El niño conditions in the spring of 1998 led to rainfall and cool temperatures that persisted until late June. All early season yellowjacket activity was therefore, significantly delayed compared to the other 2 years. This factor alone is an adequate explanation for the fact that 1998 had the lowest yellowjacket population of any of the years observed so far.

There also seems to be some problem with the assumption that observations at a few known problem areas can be used to evaluate the current or future state of the yellowjacket population throughout the entire District. Some of the discrepancies between late season observations and expected populations may be due to statistical artifacts resulting from experimental methods based upon this assumption. Since the trapping of queens and use of toxic bait may affect late season workers, early and late season observations were not made at the same locations. In our project we trap queens and use poisoned bait at the locations that have had the worst yellowjacket problems historically (treated sites). Our statistics change quite a bit when the worker populations at these sites are included. Worker populations at treated sites were higher in 2000 than in 1999, which was the peak year at the control sites (Figure 5). The fact that almost no yellowjacket complaints were reported to the District in 2000 (the year of peak activity at the treated sites) indicates that *V. pensylvanica* population information obtained at these problem areas does not adequately reflect the severity of the yellowjacket problem for the entire District.

CONCLUSION

This study has not yet come up with early season factors that can reliably predict the severity of the yellowjacket season ahead. The production of large numbers of well-populated, healthy *V. pensylvanica* nests that will create significant pest problems seems to be largely the result of conditions which occur after the nests have begun to be established. An increase in the number of sites being surveyed, more detailed observations in the months of June and July, and more years of study may be necessary before any reliable early predictive indices for *V. pensylvanica* populations can be developed from this research.

A Two-year Study of the Application of Altosid® (Methoprene) for Controlling Ground-nesting Yellowjackets (Hymenoptera: Vespidae)

Lucia T. Hui, William W. Pitcher, Ronald Keith[1], Robert Schoeppner[2], Charles H. Dill[1], and Charles P. Hansen[2]

California Department of Health Services, Vector-Borne Disease Section, 2151 Berkeley Way, Berkeley, CA 94704
[1] Marin-Sonoma Mosquito and Vector Control District, 595 Helman Lane, Cotati, CA 94931
[2] San Mateo County Mosquito Abatement District, 1351 Rollins Road, Burlingame, CA 94010

ABSTRACT: A two year study investigated the application of methoprene, in the form of Altosid Liquid Larvicide (ALLª), for the control of ground-nesting yellowjackets (Hymenoptera: Vespidae), *Vespula vulgaris* (L.) and *Vespula pensylvanica* (Saussure). Bait stations were provisioned with Altosid-treated canned mackerel and placed in picnic areas of two recreational parks in Marin and San Mateo Counties. The bait stations were monitored and rebaited bi weekly. The first-year study focused on monitoring the bait consumption and foraging activity of yellowjackets, evaluating the higher dosages of ALL® for repellency and evaluating the overall control effectiveness. In the second year, applications of higher dosages of methoprene, up to a concentration six times the recommended field formulation (5% methoprene), were made to shorten the time needed for control. Bait stations were placed adjacent to established nests to evaluate the material's effectiveness for short-term control. Results indicate that methoprene had a detrimental effect on yellowjackets in the small recreational area, but it took approximately two months to bring about a noticeable reduction of foraging workers. The study failed to demonstrate that the material could be relied upon to abate the yellowjacket populations as a short-term control method. However, the application of five percent methoprene to the established nests for two months had significant impact on the development of larvae, pupae and queen cells in *V. vulgaris* (L.).

INTRODUCTION

The common yellowjacket, *Vespula vulgaris* (L.) and the western yellowjacket, *Vespula pensylvanica* (Saussure) are two major nuisance and stinging pests in the northwestern United States. Both species are ground nesters and scavengers. Their ability to interact with humans and attain very high population densities in a given area has resulted in severe economic impact not only in recreational sites, but also in residential areas. During the months of August, September, and October, yellowjacket control is one of the most important activities conducted by the mosquito and vector control districts in California. The use of protein bait mixed with an insecticide is an old technique that has been used for 60 years in Washington and California (Akre et al. 1980). Many of the organochlorine insecticides (chlordane, mirex) previously used successfully for controlling yellowjackets are no longer registered in California (Grant et al. 1968; Keh et al. 1968; Rohe and Madon 1969; Wagner and Reierson 1971). Currently, most agencies participating in yellowjacket baiting programs rely on a microencapsulated form of Diazinon (Knoxout 2FM®) as the toxicant (Ennik 1973; Chang 1988). This material has produced good control over the years, but it is important to evaluate alternative materials in the event that Diazinon becomes unavailable, loses its registration, or becomes ineffective due to pesticide resistance.

The alternative material that was selected for this study was methoprene, an insect growth regulator (IGR).

The use of IGRs and chitin inhibitors as toxic baits are advantageous because of their specificity for the target species and their limited toxicity to vertebrates and plants (MacDonald et al. 1976). Studies of IGRs on social hymenoptera have demonstrated efficacy in destroying nests of the pharaoh's ant (Edwards 1975; Hrdy et al. 1977; Wilson and Booth 1981), the red imported fire ant (Vinson and Robeau 1974), and the eastern yellowjacket, *Vespula maculifrons* (Buysson) (Parish and Roberts 1983). The Marin/Sonoma Mosquito and Vector Control District, San Mateo County Mosquito Abatement District, and the California Department of Health Services, Vector Borne Disease Section collaborated on a two year study investigating the application of methoprene, in the form of Altosid Liquid Larvicide (ALLª), for the control of scavenging yellowjackets.

The first-year study goals were to 1) monitor bait consumption over a season and document the foraging activities within the test areas, 2) determine a point at which the yellowjacket nest might be in decline, and 3) evaluate the use of higher dosages of ALLª for repellency. For the second year, the goals were to 1) apply ALLª at the highest possible recommended dosage to the nest and determine the time necessary to reduce the colony to a point where it was no longer a nuisance, 2) evaluate the development of queen cells and the overall condition of the nest after exposure to ALL®, and 3) evaluate the overall effectiveness of short-term control on yellowjackets.

METHODS

Study sites were located at Samuel P. Taylor State Park (SPTSP) in Marin County and Huddart County Park (HCP) in San Mateo County. Both study sites had incurred numerous stinging incidents and annoyance complaints each year. San Mateo County Mosquito Abatement District conducted numerous controls studies at HCP (Grant et al. 1968; Jewell 1986; Rogers, 1972A, 1972B). The habitat at both study sites was redwood forest bisected by a riparian zone. The primary scavenging yellowjacket species present in both of the study sites were *V. vulgaris* and *V. pensylvanica.*

First-year study (1995): The bait mixture was prepared by folding the liquid ALLâ into canned Jack Mackerel© (Orleans Food Co., Inc., New Orleans, LA) at a ratio of one ounce of ALLâ to six ounces of mackerel (0.83% methoprene), the recommended formulation by the manufacturer (Zoecon Corporation). The bait was then measured onto aluminum ashtrays and placed into wire-mesh cages that had ½ inch wide access openings (Rogers and Lauret 1968). The individual bait station was tethered in a tree branch at a height of ten to twelve feet. Bait stations were placed at approximately 200 foot intervals to produce the most effective coverage (Rogers 1972A). Two other sites were also selected and provisioned with higher concentrations of ALLâ (2.5% and 5% methoprene). Bi-weekly, researchers would monitor yellowjacket foraging activity at the stations and record bait consumption. Records were kept by weighing the bait tray before placement and after removal from the bait station. In mid-July of 1995, bait stations with untreated canned mackerel were placed in the picnic areas at the study sites. This pre baiting was done to identify the yellowjacket species and document foraging activity within the test areas. The actual application of ALLâ began in late July of 1995 at both HCP and SPTSP.

Second-year study (1996): The repellency data indicated that 80% of the workers of *V. pensylvanica* foraged within 1,100 feet of the nest (Akre et al.1975). Therefore, the four bait stations were placed within twenty feet of the nest. It was very difficult to locate early stage nests because they were small. The researchers did not locate any nests until late July. The bait stations were pre baited with mackerel for about two weeks to establish a foraging pattern. The stations were provisioned for two months with a bait mixture containing ALLâ at methoprene concentrations of 0.83 percent, 2.5 percent and 5 percent. At the end of this period, the nests were excavated and placed into plastic bags with dry ice. The nests were stored inside a Bio Freezer to await processing in the laboratory. The nests were evaluated by confirming the yellowjacket species, locating the queens, performing a dissection of the abdomen of the queens, and evaluating the overall condition of queen cells, larvae, and pupae.

RESULTS

In a typical year, queens emerge from diapause in search of nesting sites and visit flowers for nectar in April and early May, and the first workers emerge in early June (Akre et al. 1976). The wet spring of the first year (1995) may have interfered with the normal yellowjacket developmental cycle. The researchers did not observe foraging workers until July at either SPTSP or HCP. A total of 40 bait stations (test-20; control-20) were established at each study site. Evidence indicated that the bait stations were being visited by small rodents, dermestid beetles, and foraging ants. The amount of bait taken by these animals could not be determined. Even though the bait stations were placed 10 12 feet high and into dense redwood growth, there was a considerable amount of vandalism by park visitors.

No yellowjacket nests were recovered from the test and control areas of either site in 1995. The effort to track foraging yellowjackets back to nest sites had limited results. Workers were captured and marked with colored pens and strings, which were attached to their legs, but with poor results similar to previous studies (Akre et al. 1976). Other attempts, such as walking up and down the study sites to locate the nests, were also unsuccessful in the fall. The life expectancy of a colony of a ground nesting yellowjacket starts in early June and ends in late October. The peak of nest activity is from August to early October (Edwards 1980). As such, failure to locate the active nests due to seasonal decline or the effect of methoprene in the fall could not be determined. The bait stations were retrieved during the last week of October, and the project terminated. The researchers were not able to detect evidence of either reduced bait consumption or the emergence of males and queens at the end of the study. However, there was a sharp reduction in worker foraging activity by the end of the study in both test areas in 1995 (Figures 1 & 2).

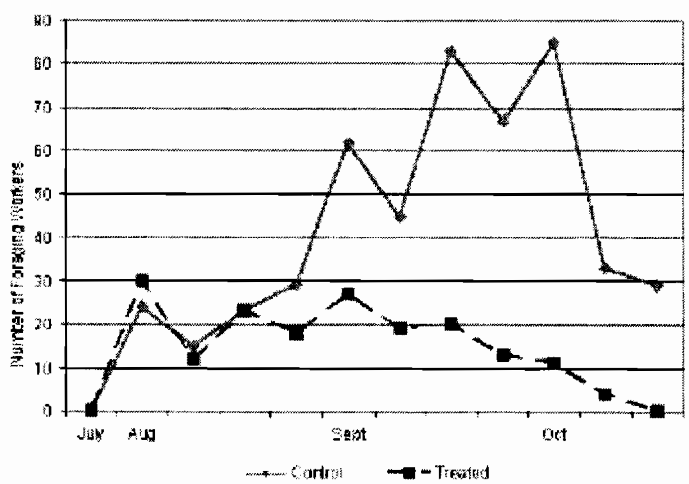


Figure 1. The total number of foraging workers observed at the bait stations/minute, Samuel P. Taylor State Park, Marin County, 1995.
Source: California Department of Health Services

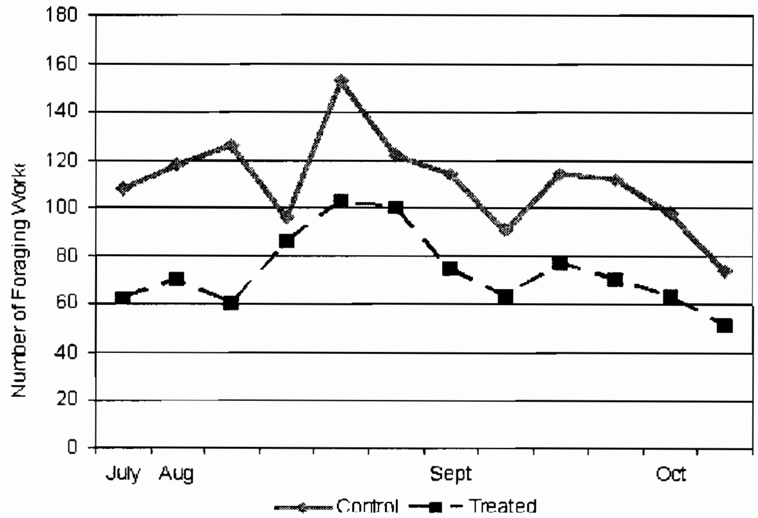


Figure 2. The total number of foraging workers observed at the bait stations/minute, Huddart County Park, San Mateo County, 1995.

Source: California Department of Health Services

In the spring of 1996, 20 sugar-baited traps (made of Hawaiian Punch) mixed with heptyl butyrate were placed within the test and control areas, this attempt to capture the over-wintered queens were not successful. Use of the attractant traps in the spring was ill-advised since queens of *V. vulgaris* rarely were collected and were not attracted to heptyl butyrate (Macdonald et al. 1976). In addition, many attempts were made to test the larvae and pupae for methoprene residual. This analysis was not practical because the fatty acids in the yellowjacket larvae were similar to methoprene breakdown products (*Culex* larvae- hydroxy ester, *Musca* larvae-hydroxy acid) (Questad et al. 1975). Conclusions were drawn from the bait consumption and counts of foraging workers at the bait stations. No adjustment for evaporation was made because the parameter was constant for all feeders (Parrish and Roberts 1983).

The counts of foraging workers at the SPTSP bait stations treated with 0.83 percent methoprene were characterized by a sharp rise in yellowjacket activity in mid-August. However, the number of foraging workers then gradually declined over the next month, resulting in significantly reduced activity by early October (Figure 1). These results differed markedly from the control area, which demonstrated a gradual but a steady increase in foraging activity until mid-October. Comparisons between the number of visiting workers from the test area versus the control area indicated that the treated area demonstrated a decline much sooner (early September) than the control area (mid-October). The reduction of the foraging workers may have been caused by the noticeable effect of methoprene on the development of the yellowjacket larvae. However, it took almost two months of baiting to demonstrate a significant reduction on the worker population. The weekly rate for bait consumption (Figure 3) paralleled the foraging results until mid-October, when there was a sharp increase in foraging activity before the baiting was terminated. The increased consumption of the bait resulted from a reduction in the number

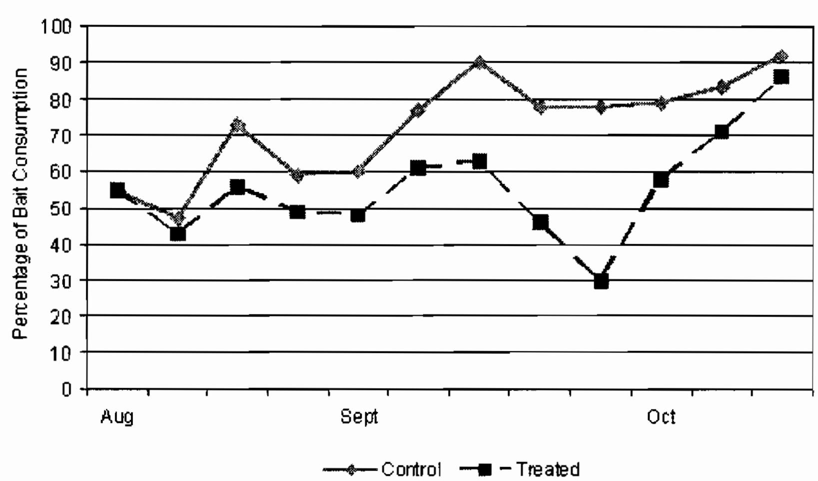


Figure 3. Bait consumption of Altosid Liquid Larvicide® (0.83% methoprene) in mackerel, Samuel P. Taylor State Park, Marin County, 1995.

Source: California Department of Health Services

of visiting workers causing an increased effect of evaporation (cake effect was observed in the unconsumed mackerel baits at the treated site).

The results in the HCP test area and control were similar to the Marin County site (Figure 2 & 4). In early September, a *V. vulgaris* nest was identified approximately midway between the test area and the control. The apparent number of foraging workers in and out of this nest and at the nearby bait stations began to decline in the fall. This was possibly due to the effect of the methoprene. No remnants of the nest could be found after the completion of baiting. A vertebrate predator may have been attracted to the decaying nest and may have destroyed it before it could be excavated. In addition, it was noted that *V. pensylvanica* was the prevalent species at the bait stations in mid-September.

The results of the preliminary repellency tests of 1995 from the Marin and San Mateo sites are depicted in Figure 5. Concentrations of ALL[a] were applied at three times (2.5% methoprene) and six times (5% methoprene) above the recommended rate (0.83% methoprene). At both sites, there was no evidence that the number of visiting workers declined. Therefore, no noticeable effect of repellency to yellowjackets was observed by applying methoprene at the higher dosages. This result differed from the data obtained from a study on the eastern yellowjacket, *V. maculifrons* (Parish and Roberts 1983). In 1996, the test formulation for baiting nests, based on the repellency data of 1995, was to apply ALL[a] at 0.83 percent, 2.5 percent and 5 percent methoprene. The bait consumption at the higher dosage failed to show any evidence of repellency (Table 1).

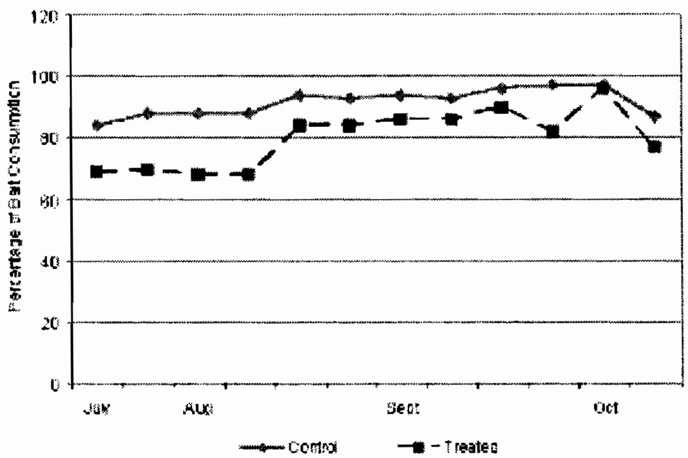


Figure 4. Bait consumption of Altosid Liquid Larvicide® (0.83% methoprene) in Mackerel, Huddart County Park, 1995

Source: California Department of Health Services

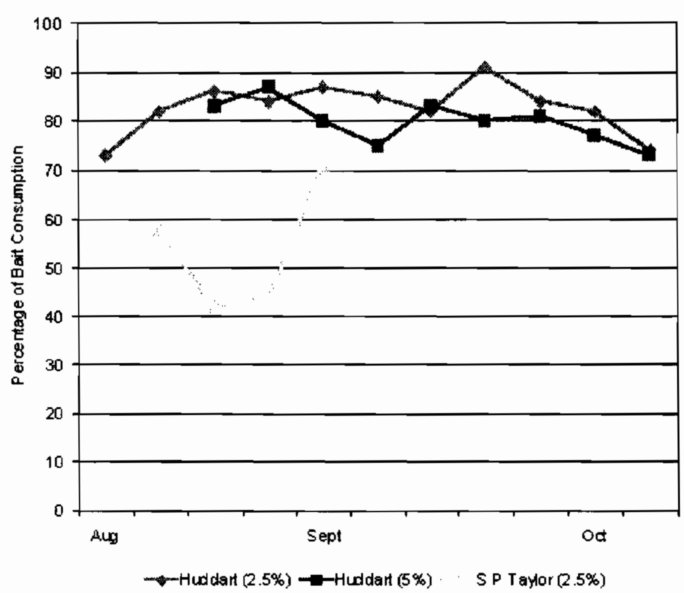


Figure 5. Methoprene repellency - bait consumption, 1995.

Source: California Department of Health Services

Nest Site	County	ALL® (% methoprene)	Species	Queen Cells	Queens Reproductive	Larvae & Pupae	Nest Status
SPTSP 1	Marin	5%	*V. vulgaris*	None	Discolored Atrophic	Normal	Decline
SPTSP 2	Marin	5%	*V. pensylvanica*	200	Normal	Normal	Active
Creek Side HCP	San Mateo	5%	*V. pensylvanica*	100	No queens were collected	Normal	Active
Redwood 1 HCP	San Mateo	0.83%	*V. pensylvanica*	300	No queens were collected	Normal	Active
Redwood 2 HCP	San Mateo	2.5%	*V. pensylvanica*	250	No queens were collected	Normal	Active
Redwood 3 HCP	San Mateo	5%	*V. vulgaris*	None	Discolored Atrophic	Discolored Disformed	Decline

Table 2. Nest observations: Altosid Liquid Larvicide® (% methoprene), 1996.

Source: California Department of Health Service

Date	Creek Side 5% methoprene	Redwood 1 0.83% methoprene	Redwood 2 2.5% methoprene	Redwood 3 5% methoprene	Control
8/22	50%	100%	100%	100%	100%
8/26	50%	100%	100%	100%	100%
8/28	50%	100%	100%	100%	100%
8/30	35%	100%	100%	100%	100%
9/3	34.5%	98.6%	100%	100%	100%
9/6	17.4%	99.3%	100%	100%	100%
9/11	21%	95%	100%	100%	100%
9/13	1.5%	48.75%	49.5%	95.6%	100%

Table 1. Repellency test: Percentage of bait consumption of different concentrations of methoprene at the treated and control nests, Huddart County Park, San Mateo County, 1996.

Source: California Department of Health Services

At the end of the two-month test period, the nests were excavated at the time when males, new queens, and healthy immatures would be expected to be developing in the larval or pupal stage. The immature yellowjackets were evaluated for methoprene effects based on morphological anomalies, and the queens were dissected to examine the reproductive structures. Even though all treated nests were still active at the completion of the test period, there appeared to be a species variation in the condition of the colonies.

The observation of applying different concentrations of methoprene to nests at both study sites is depicted in Table 2. The *V. vulgaris* nests from both test areas appeared to be in decline at the time of excavation. At SPTSP, the dissected queens from the study site treated with five percent methoprene revealed discoloration of abdominal contents, and the reproductive tree appeared atrophic. The yellow area of the external abdominal tergites were also noticeably darkened or discolored. In the nest, the mature larvae and pupae appeared normal but no early instar larvae were present, and no queen cells had been constructed. At the HCP site (5% methoprene), workers had been observed removing dead larvae from the nest and discarding the bodies a short distance away from the nest (Redwood 3). This "Nest Sanitation" of removing dead or diseased larvae could be implied as an early sign of a declining colony (Akre et al. 1976). All the larvae and pupae removed from this nest were deformed or discolored (Figure 6). No queen cells had been constructed and the nest appeared to be in decline as indicated by ants invading the nest.

The *V. pensylvanica* nests from both test sites appeared to be normal. The dissection of the queen from the SPTSP nest (5% methoprene) revealed white internal tissues and the reproductive tree was normal. No queens were recovered from the HCP nests (0.83%, 2.5%, and 5% methoprene). At both test sites, large numbers of mature worker larvae and pupae were present in a large percentage of the cells. In the SPTSP nest, at least 200 queen cells had been constructed and some had first instar and second instar larvae inside. In the three HCP nests, there were about 100 to 300 queen cells that had been constructed. Immature larvae were present in about ten of the queen cells in the SPTSP nest, and no larvae were present in the queen cells of the HCP nests (Table 2).

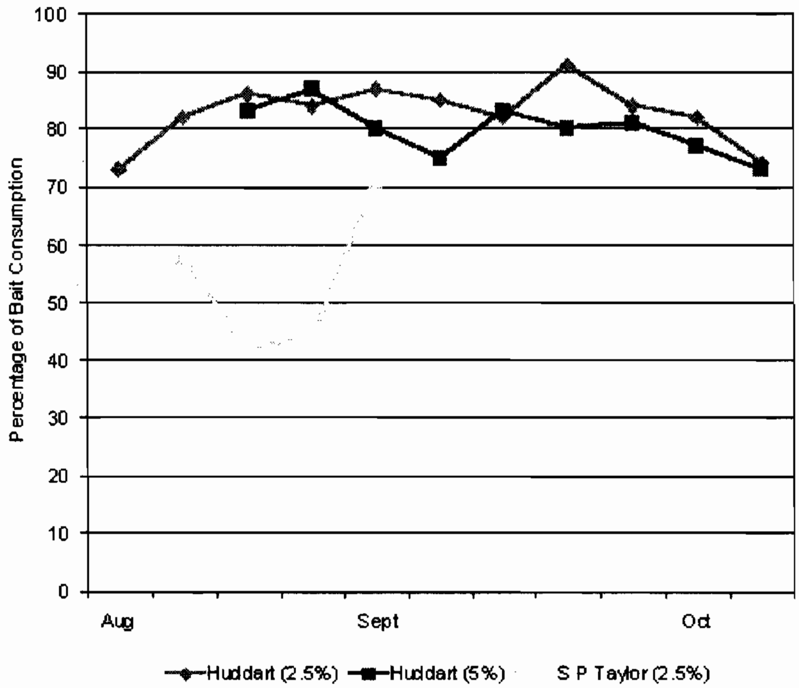


Figure 5. Methoprene repellency - bait consumption, 1995.
Source: California Department of Health Services

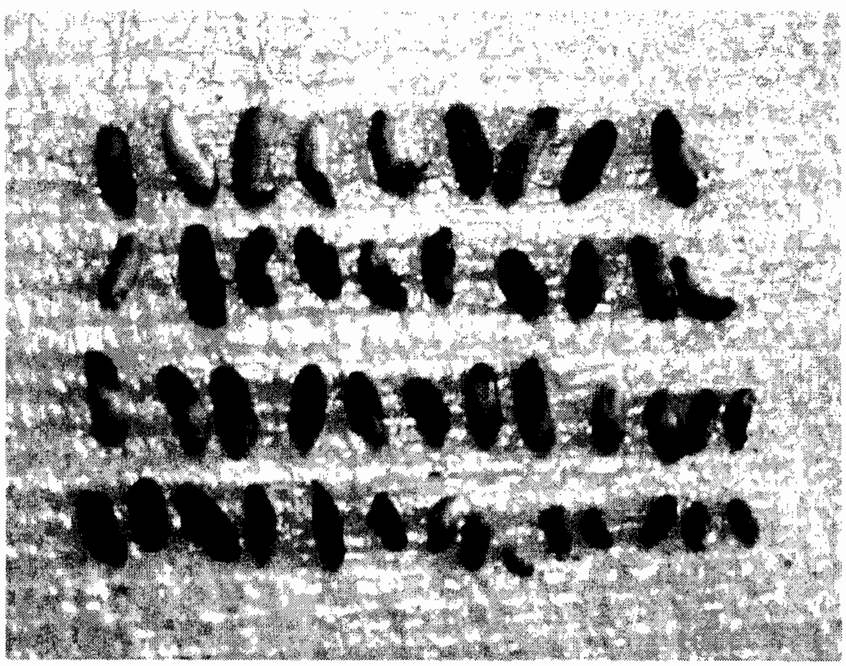


Figure 6. Larvae removed from the nest of *Vespula vulgaris,* Huddart County Park, San Mateo County, 1996 (treated with 5% Methoprene).
Source: California Department of Health Services

DISCUSSION

From this study, it can be concluded that the methoprene bait appeared to reduce the yellowjacket population. However, a more significant effect has been observed on *V. vulgaris* than on *V. pensylvanica*. This result was encouraging because *V. vulgaris* was difficult to abate by applying toxic baits in most recreational areas (MacDonald et al. 1976).

In the first year of the study, it was documented that both species of yellowjackets readily accepted the bait at concentrations up to six times the recommended ALL® formulation of five percent methoprene with no repellency. At the SPTSP study area the number of foraging workers dropped off markedly in mid-September when compared to the control area. This was not observed at the HCP study areas. Based on the second year's finding, methoprene had a significant impact on the colony of V. *vulgaris*. As such, the foraging results in the first year's study were suspect because of the late appearance of *V. pensylvanica* workers at the treated site. This species did not appear to be as susceptible to the methoprene as *V. vulgaris*. This may be the result of behavioral resistance and differential attraction of the *V. pensylvanica* workers to the meat bait. The colonies of *V. pensylvanica* in southern California appeared heavily committed to scavenging, whereas colonies of this species in Washington and Idaho, while still pestiferous, were not totally committed to meat bait for obtaining protein (Akre et al. 1980). The higher *V. vulgaris* susceptibility may account for the rapid decline of the yellowjacket population at the nest found midway between the test area and the control at HCP. There were no remnants of the nest found when it was excavated. It was assumed that a vertebrate predator may have destroyed the nest, but no evidence was left to support that statement.

At the conclusion of the second year's study, the results were more dramatic. The two *V. vulgaris* nests were in decline as cited above. At one of the HCP nests treated with five percent methoprene, workers were observed removing dead larvae from the nest and were fighting off an infestation of ants in early September. All the larvae and pupae removed from this nest were deformed or discolored. No queen cells had been constructed in either of the nests in the test areas. The nests of the *V. pensylvanica* appeared to have no visible affects, and many workers defended the nest during the excavation.

A successful baiting program typically demises the colony in two weeks (MacDonald et al. 1976; Ennik 1973). The purpose of this study was to seek an alternative material for controlling the scavenging yellowjackets. Unfortunately, the two-year investigation did not establish that methoprene was an effective short-term control method. Because it took two months of intensive bi-weekly baiting program with methoprene to reduce the population in the small recreational area, the control measure failed to have a major impact. Logistically, this baiting program was not only labor intense but also very costly. However, yellowjackets are beneficial insects and should be controlled only in areas where they were proven nuisances. The yellowjacket populations of *V. vulgaris* in a given area vary from year to year and peak at three to five year intervals. Worker densities reach highest numbers in late August to October in northern Idaho and Washington forests (MacDonald et al. 1976). A large nest can produce 3,500 to 15,000 foragers and many hundreds of fertilized females (Akre et al. 1980). Even if mortality of the queens was 99.9%, there would still be nine queens out of 10,000 surviving from the over-wintered colonies (Edwards 1980). At this time, the long-term effect of IGR on the queens or larvae is unknown. The application of methoprene in *V. vulgaris* nests resulted in no queen cells being constructed. Therefore, the effect of methoprene in controlling yellowjacket population in a given area probably would be more cost-effective in the long run. To incorporate methoprene baiting into an integrated pest management (IPM) control program in parks or campgrounds should be investigated further before final conclusions can be drawn.

Acknowledgments

The authors wish to thank Franklin Ennik, for his valuable comments on reviewing the manuscript; David Sullivan, for his technical support with Altosid Liquid Larvicide (ALL®); and the staff of Samuel P. Taylor State Park and Huddart County Park for providing the test sites for this study.

REFERENCES

Akre, R. D., W. B. Hill, J. F. MacDonald, and W. B. Garnett. 1975. Foraging Distances of *Vespula pensylvanica* Workers (Hymenoptera: Vespidae). J. Kans. Entomol. Soc. 48:12-16.

Akre, R. D., W. B. Garnett, J. F. MacDonald, A. Green, and P. Landolt. 1976. Behavior and Colony Development of *Vespula pensylvanica* and *V. atropilosa* (Hymenoptera: Vespidae). J. Kans. Entomol. Soc. 49:63-84.

Akre, R. D., A. Greene, J. F. MacDonald, P. Landolt, and H. G. Davis. 1980. Yellowjackets of America North of Mexico, U.S. Department of Agriculture, Agricultural Handbook No. 552. 102 pp.

Chang, V. 1988. Toxic Baiting of the Western Yellowjacket (Hymenoptera: Vespidae) in Hawaii. J. Econ. Entomol. 81(1):228-234.

Edwards, J. P. 1975. The Effects of a Juvenile Hormone Analogue on Laboratory Colonies of Pharaoh's Ant, *Monomorium pharaonis* (L.) (Hymenoptera, Formicidae) Bull. Ent. Res. 65:75-80.

Edwards, R. 1980. Social Wasps: Their Biology and Control. Rentokil Limited, Felcourt, East Grinstead, W. Sussex RH192JY. 357 pp.

Ennik, F. 1973. Abatement of Yellowjackets Using Encapsulated Formulations of Diazinon and Rabon. J. Econ. Entomol. 66(5):1097-1098.

Grant, D.C., C. J. Rogers, and T. H. Lauret. 1968. Control of Ground-nesting Yellowjackets with Toxic Baits A Five-Year Testing Program. J. Econ. Entomol. 61(6):1653 1656.

Hrdy, I., J. Krecek, J. Zdarek, J. Chimela, and J. Ledvinka. 1977. Control of the Pharaoh's Ant (*Monomorium pharaonis*) with Juvenoids in Baits. Proc. VIIIth Int. Congr. Int. Union for the Study of the Social Insects, Wageningen. 83-84.

Jewell, D. J. 1986. An Experimental Yellowjacket Control Program. Proc. Calif. Mosq. Control Assoc. 54:168.

Keh, B., N. T. Brownfield, and M. E. Persons. 1968. Experimental Use of Bait with Mirex Lethal to Both Adult and Immature *Vespula pensylvanica* (Hymenotoptera: Vespidae). Calif. Vector Views 15:115-118.

MacDonald, J. F., R. D. Ackre, and R. W. Matthews. 1976. Evaluation of Yellowjacket Abatement in the United States. Bull. Entomol. Soc. Am. 22(4):397-401.

Parrish, M. D. and R. B. Roberts. 1983. Insect Growth Regulators in Baits: Methoprene Acceptability to Foragers and Effect on Larval Eastern Yellowjackets (Hymenoptera: Vespidae). J. Econ. Entomol. 76(1)109-112.

Queistad, G. B., L. E. Staiger, and D. A. Schooley. 1975. Environmental Degradation of the Insect Growth Regulator Methoprene: V. Metabolism by Houseflies and Mosquitoes. Pest Biochem. Physiol. 5:233-241.

Rohe, D. L. and M. B. Madon. 1969. A Field Evaluation of Mirex and Chlordane Poisoned Baits for the Control of the Ground-nesting Yellowjacket *Vespula pensylvanica* (Saussure) on Santa Catalina Island, California. State of California., Dept. Publ. Health, Bureau of Vector Control. 11pp.

Rogers, C. J. 1972 A. Flight and Foraging Patterns of Ground-nesting Yellowjackets Affecting Toxic Baiting Control Programs. Proc. Calif. Mosq. Control Assoc. 40:130-132.

Rogers, C. J. 1972 B. Field Testing of "Yellowjacket Stoppers"[a] and Population Depletion Trapping for the Control of Ground-nesting Yellowjackets. Proc. Calif. Mosq. Control Assoc. 40:132-134.

Rogers, C. J. and T. H. Lauret. 1968. A Standard Yellowjacket Trap for Population Sampling and Control Evaluation. J. Econ. Entomol. 61(6): 1739- 1740.

Vinson, S. B. and R. Robeau. 1974. Insect Growth Regulator Effects on Colonies of the Imported Fire Ant. J. Econ. Entomol. 67:584-587.

Wagner, R. E. and Reierson, D. A., 1971. Yellowjacket Control with a Specific Mirex-Protein Bait. Calif. Agric. 25:8-10.

Wilson, G. R. and M. J. Booth. 1981. Pharaoh Ant Control with IGR in Hospitals. Pest Control 49: 14-15.

Mosquito Abundance and Arbovirus Surveillance in Northwestern Riverside County in 2001

Lal S. Mian[1] and Cecillia Reed[2]

[1]*Department of Health Science and Human Ecology, California State University, San Bernardino, CA 92407-2397*
[2]*Northwest Mosquito and Vector Control District, 1966 Compton Avenue, CA 92881-3318*

ABSTRACT: Of the 29,642 adult mosquitoes collected from various areas served by the Northwest Mosquito and Vector Control District (NWMVCD), 3178 (10.7%) were from New Jersey light traps (NJLTs) and 26,464 (89.3%) from CO_2-baited CDC traps. Mosquito distribution by habitat was the highest, 8.28 per trap-night (45.4%) for rural, 7.15 (39.2%) for suburban and 2.82 (15.45%) for urban sites. The overall mosquito composition by species showed *Culex quinquefasciatus* as the most abundant species comprising 53.9% of the total mosquitoes collected, followed by *Culex stigmatosoma* (18.2%), *Culex tarsalis* (15.7%), *Culex erythrothorax* (8.8%), *Anopheles hermsi* (1.5%), *Anopheles punctipennis* (0.6%), *Culiseta inornata* (0.6%), *Culiseta incidens* (0.4%), *Culiseta particeps* (0.3%) and *Ochlerotatus sierrensis* (<0.1%). Species composition differed between NJLTs and CO_2-baited traps; the latter attracting significantly higher numbers of *Culex* species. Based on NJLT data, mosquito populations in 2001were lower than the 5- and 15-year figures.

During the 2001 season, 291 pools (12,003 female mosquitoes) were tested for encephalitis viruses. These included 185 pools (8103 females) of *Cx quinquefasciatus*, 71 pools (2476 females) of *Cx. stigmatosoma* and 35 pools (1424 females) of *Cx. tarsalis*. None of the pools tested positive for either St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) viruses.

Biweekly blood samples from six sentinel chicken flocks from May through October, tested negative for antibodies to encephalitis viruses.

INTRODUCTION

The Northwest Mosquito and Vector Control District has provided vector control services to the residents of northwestern Riverside County for over four decades. The District's service area of approximately 200 square miles that includes the cities of Corona, Norco, Lake Elsinore, part of Riverside and the adjoining unincorporated communities of the northwestern Riverside County, has a population of approximately 400,000.

As part of its mandate, the District conducts routine encephalitis virus surveillance (EVS) and needed mosquito control to safeguard the well being of its constituents from the ravages of mosquito bites and mosquito-borne pathogens. The District has reported virus activity in its service area at different times in the past. The last reported virus activity dates back to 1997 when two sentinel chickens at Rubidoux and one at Lake Elsinore tested seropositive for Saint Louis encephalitis (SLE) virus antibodies (Kramer et al. 1998). Based on routine surveillance for both SLE and (WEE) viruses carried out in 2001, the present paper highlights mosquito abundance and surveillance activities in the northwestern Riverside County.

MATERIALS AND METHODS

The general procedure described by Mian et al. (1999) was used as follows:

Adult mosquito population dynamics.

A total of 12 NJLT's was operated at different fixed sites in urban (3), suburban (6) and rural habitats (3). Urban trap sites were located one mile or more inside heavily populated areas; suburban from ¼ - <1 mile inside populated areas; and rural <1/4 mi inside to completely outside of populated areas. The trap lines were set in north-south—Corona to Lake Elsinore and east-west—High Grove to Prado wetland transects. Trap samples sorted out into mosquitoes were identified to sex and species. A report based on the weekly data was sent to the California Department of Health Services for the state-wide adult mosquito occurrence report.

Adult mosquito testing.

Carbon dioxide-baited CDC traps (8-10 per night) were used to collect overnight host-seeking female mosquitoes at various locations within the District's territory. Weekly collected mosquitoes were anesthetized with triethylamine prior to sorting them out by sex and species. Only females of *Culex quinquefasciatus* Say, *Culex. stigmatosoma* Dyar and *Culex tarsalis* Coquillett were pooled (10-50 mosquitoes per vial). Pools packed with dry ice in styrofoam containers were shipped overnight to the University of California Davis Arbovirus Research Unit (DARU) to test for SLE and WEE viruses.

Sentinel chicken flocks.

Six sentinel flocks each consisting of eight white leghorn chickens, were maintained at High Grove, Rancho Jurupa Park—

Rubidoux, Prado wetland, Glen Ivey, Woodcrest, and Lake Elsinore. Biweekly blood samples were collected from these birds. Using the comb prick method, filter paper strips soaked in a drop of blood sample were air-dried and mailed to DARU for serological evidence of SLE and WEE viral antibodies.

RESULTS AND DISCUSSION

Mosquito abundance.

During 2001, 29,642 mosquitoes were collected in NJLTs and CO_2-baited CED traps from various sites within the District's territory (Table 1). Of the total mosquitoes collected, 33.3% were from rural, 59.9% from suburban and 10.8% from urban sites. On a per trap-night basis, however, mosquito distribution was the highest (45.4%) at rural habitats, followed by suburban (39.2%) and urban milieus (15.4%). For the trap type, NJLTs accounted for 3,178 (10.7%) mosquitoes and CO_2-baited traps 26,464 (89.3%). Mosquito distribution by species showed *Cx. quinquefasciatus* as the most abundant species (53.9%), followed by *Cx. stigmatosoma* (18.2%), *Cx. tarsalis* (15.7%), *Culex erythrothorax* Dyar (8.8%), *Anopheles hermsi* Barr and Guptavanji (1.5%), *Anopheles punctipennis* (Say) (0.6%), *Culiseta inornata* (Williston) (0.6%), *Culiseta incidens* (Thomson) (0.4%), *Culiseta particeps* (Adams) (0.3%) and *Ochlerotatus sierrensis* Ludlow (<0.1%).

Species composition by trap showed some variations. For example, in the New Jersey traps, species composition per trap-night included *Cx. erythrothorax* (0.17), *Cx. stigmatosoma* (0.17), *Cx. tarsalis* (0.14), *An. hermsi* (0.07), *Cx. quinquefasciatus* (0.06), *Cs. inornata* (0.04), *An. punctipennis* (0.04), *Cs. incidens* (0.03), and *Cs. particeps* (0.02) (Fig. 1). As expected, CO_2-baited traps attracted more host-seeking culicine species with *Cx. quinquefasciatus* (58.62) as the most abundant, followed by *Cx. stigmatosoma* (17.39), *Cx. tarsalis* (15.03) and *Cx. erythrothorax* (6.97) (Fig. 2). Except for *An. hermsi* (0.62), all other species were <0.1%.

The data from NJLTs also showed a relatively even seasonal distribution at all habitats except for the peak months, June and July, when the suburban peak was 4X the urban peak and twice as much as the rural peak. (Fig. 3). In comparison with the District's 5- and 15-year NJLT data, the mosquito populations in 2001 were lower (Fig. 4). Peak mosquito activity in 2001 also showed a month long delay, occurring in July rather than in June. This might be attributed to warmer temperatures and less precipitation in the area.

Arbovirus surveillance.

During 2001, a total of 12,003 (291 pools) culicine mosquitoes was tested for arboviral activity. These included 8103 (185 pools) *Cx. quinquefasciatus*, 2476 (71 pools) *Cx. stigmatosoma* and 1424 (35 pools) *Cx. tarsalis*. None of the pools tested positive for either SLE or WEE virus.

During the same period, 576 biweekly blood samples from sentinel chicken flocks tested negative for antibodies to SLE and WEE viruses. The 2001 season appeared to be relatively calm both in terms of mosquito abundance and viral activity in the area. The closest viral activity occurred in the Coachella Valley of Riverside County. The last time arboviral activity detected in the District was in 1997 when two sentinel chickens had seroconversion to SLE in Rubidoux in September/October and one in Lake Elsinore in October.

Table 1. Species composition by habitat of adult mosquitoes caught in New Jersey light traps and CO2-baited traps in northwestern Riverside County in 2001.

	Mosquito distribution by habitat						Total		
	Rural		Suburban		Urban				
Mosquitoes	**#**	**1/**	**#**	**#/tn 2/**	**#**	**#/tn 3/**	**Total**	**%**	**#/tn 4/**
Anopheles hermsi	397	0.33	52	0.02	9	0.01	458	1.5	0.10
Anopheles punctipennis	133	0.11	41	0.02	0	0.00	174	0.6	0.04
Culex erythrothorax	1779	1.49	772	0.33	57	0.05	2608	8.8	**0.56**
Culex quinquefasciatus	5592	4.69	9531	4.11	867	0.76	15990	53.9	3.44
Culex stigmatosoma	522	0.44	3104	1.34	1757	1.54	5383	18.2	1.16
Culex tarsalis	1292	1.08	2933	1.27	428	0.38	4653	15.7	1.00
Culiseta incidens	28	0.02	26	0.01	65	0.06	119	0.4	0.03
Culiseta inornata	55	0.05	95	0.04	21	0.02	171	0.6	0.04
Culiseta particeps	69	0.06	6	0.00	7	0.01	82	0.3	0.02
Ochlerotatus sierrensis	2	0.00	2	0.00	0	0.00	4	<0.1	0.00
Total number caught	9869	8.28	16562	7.15	3211	2.82	29642	100	***6.39***
Percent	**33.3**	**45.4**	**55.9**	**39.2**	**10.8**	**15.4**	**100**		

1/ number by 1139 trap-nights
2/ number by 2317 trap-nights
3/ number by 1192 trap-nights
4/ number by 4648 trap-nights

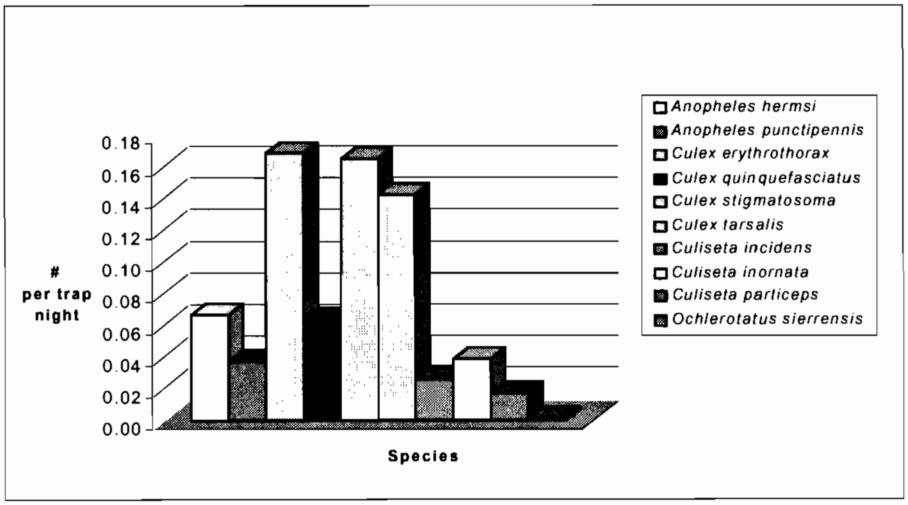


Fig. 1. Species composition of adult mosquitoes caught in New Jersey light traps in Northwestern Riverside County, 2001.

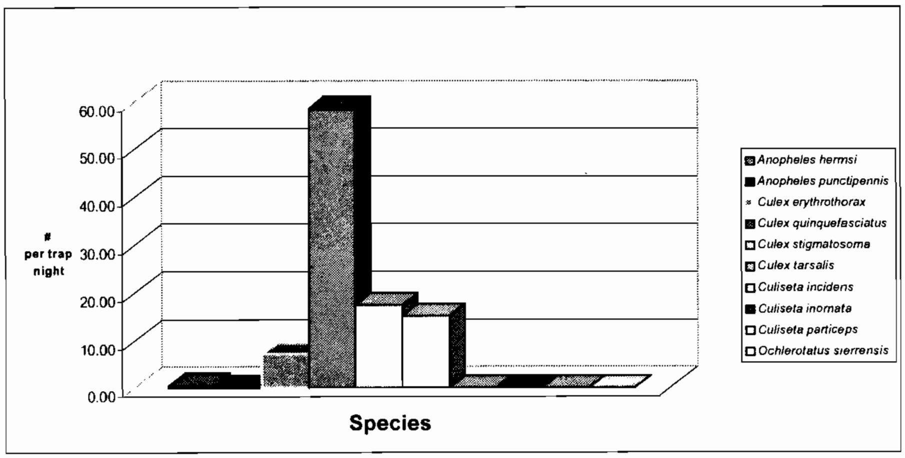


Fig. 2. Species composition of adult mosquitoes caught in CO_2-baited traps in Northwestern Riverside County, 2001.

Acknowledgment

The authors acknowledge with thanks Dr. Major S. Dhillon, District Manager, for his logistic support and Dr. Jeff Beehler, former vector ecologist, for his contribution during the early part of the program. Tanya Sahagun, Jared Dever, Heather Atamian, and Brian Baharie are acknowledged for their field assistance.

REFERENCES

Kramer, V.L., M.S. Ascher, E. Baylis, B. Enge, C.L. Fritz, W.K. Reisen, D.A. Eliason, B.F. Eldridge and R. Chiles. 1998. Surveillance for mosquito-borne encephalitis virus activity and human disease in California, 1997. Proc. & Papers, Mosq. Vector Control Assoc. Calif. 66: 37-44.

Mian, L.S., C.N. Nwadike, L. Allsup and J.H. Hitchcock. 1999. Mosquito abundance and arboviral activity in San Bernardino County during 1998. Proc. & Papers, Mosq. Vector Control Asoc. Calif. 67: 12-14.

GUIDELINES FOR CONTRIBUTORS

Proceedings & Papers of the Mosquito and Vector Control Association of California

The Proceedings is the *Proceedings and Papers of the Mosquito and Vector Control Association of California*. One volume is printed each year and includes the presentations given at the Association's annual conference. Publication of submitted papers is also encouraged.

CONTRIBUTIONS: A manuscript for publication in the Proceedings is encouraged from every speaker. Articles should be original contributions in the field of mosquito and vector ecology and control and provide information to benefit the diverse interests in technical development, operations and programs, and management documentation. Please do not submit papers that have been previously published or are being considered for publication elsewhere. An excessive number of papers on one subject or by any one author are generally discouraged. Although preference is given to papers accepted on the program agenda, 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.

MANUSCRIPT FORMAT: Manuscripts must be typed double-spaced only on one side of the page with one-inch margins on all sides. A 3-1/2" computer diskette should also be submitted which includes your manuscript and images of all tables, figures or photographs. Common IBM compatible word processing programs such as Microsoft Word or WordPerfect is preferred. One hard copy plus two copies of the tables, figures and/or photographs should accompany the diskette. These should be submitted to the Proceedings Editor within 60 days following the end of the conference. Articles received after that time may be returned for resubmission for the next *Proceedings*. Authors should refer to recent issues of the *Proceedings and Papers of the Mosquito and Vector Control Association of California* and the *Journal of the American Mosquito Control Association* for guidance. Authors of technical papers should follow the basic recommendations as presented in the *Scientific Style and Format: The Council of Biology Editors Manual for Authors, Editors and Publishers (Latest 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 are discussed in the Journal of the American Mosquito Control Association, 5:485 (1989). Bi-letter generic abbreviations are used for Culicidae. Common Abbreviations (et al, e.g., i.e., etc.) are not italicized. Use of the metric system (with English measurements in parenthesis) is encouraged. Avoid footnotes in text.

The papers in the Proceedings will appear, for the most part, as submitted. Editorial liberties will be exercised in those instances where improved clarity is needed and where style is incorrect. Articles requiring extensive editing and not conforming to style and instructions will be returned to the author for correction.

SUBMITTED PAPERS: Manuscripts (other than presentations at the conference) submitted for publication in the *Proceedings* will be treated as "Refereed or Peer Reviewed Articles." These will be sent for review to at least two or more scientists proficient in the subject. Following their comments and advice, the Editor will determine whether these should be published as Peer Reviewed articles.

TITLE: The title, author's name(s), organization, mailing address, e-mail address, and telephone number should appear at the top of the first page.

ABSTRACT: An Abstract is required, and should provide a brief summary of the paper. The Editor may refuse to publish Abstracts or Summaries alone.

PAGE NUMBERING: Number pages consecutively, including tables and figures. Insert the tables and figures as separate pages following the first place they are referenced in the text.

TABLES: Tables should be 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 (6-1/4"). Each table should be referenced at some point within the text. Avoid long and complex tables.

ILLUSTRATIONS: 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 a one or two column format. 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, or photocopies reproduced poorly will not be acceptable for publication. Since most figures may be reduced to one column in width, the original lines and printing must be legible when reduction becomes necessary.

LITERATURE/REFERENCES CITED: Alphabetize references by the author's surnames. Within the alphabetical order, sequence should be by year beginning with the most recent publication date. Include only publications that are cited in the text, and the style of citations should conform to the format in the latest issue of the *Proceedings*.

PROOF AND REPRINTS: Authors will receive a galley proof, as well as order forms for reprints. Major revisions at this stage will not be acceptable. Proofs with corrections, if any, and reprint order forms should be returned within 10 days to the MVCAC office:

Mosquito and Vector Control Association of California (MVCAC)
Attention: Emily Young
660 J Street, Suite 480
Sacramento, CA 95814
(916) 440-0826 • (916) 442-4182 FAX
e-mail: eyoung@mvcac.org

Editor: Minoo B. Madon
Greater L.A. County VCD
12545 Florence Avenue, Santa Fe Springs, CA 90670
(562) 944-9656 • (562) 944-7976 FAX
e-mail: mmadon@glacvcd.org