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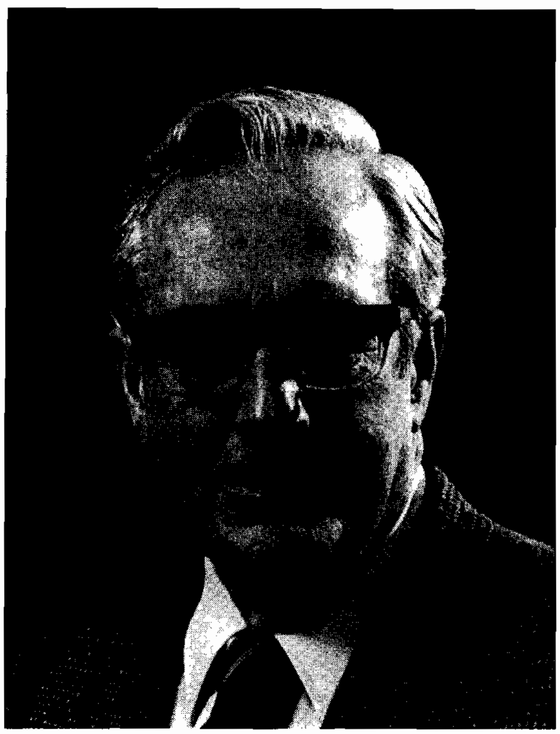
CONFERENCE DEDICATION

DEDICATION OF THE 65TH ANNUAL MVCAC CONFERENCE TO RUSSELL E. FONTAINE

Bruce F. Eldridge

Director of Mosquito Research Program
University of California, Davis



Russell E. Fontaine 1914-1996

Dr. Russell E. Fontaine, who passed away late last year, will be remembered for his long time service to public health entomology and to the state of California. Russ was born October 9, 1914 in Worcester, Massachusetts, and attended schools there before entering the University of Toronto, where he received a B.Sc. in agricultural sciences in 1939. Russ went on to obtain an M.P.H. in epidemiology and public health from the University of California School of Public Health in 1953, and a D.Sc. degree in medical entomology and public health from Tulare in 1959. Russ served in the U.S. Army during World War II, and there met Richard Peters, with whom he would later address many California problems in mosquito biology and control. After the war he served as medical entomologist for the U.S. Army in Seoul, Korea (1947-1948) and an entomology advisor for the U. S. Army Caribbean Command in the Panama Canal Zone (1948-1950). After his service with the Army, Russ came to California and was a Vector Control Advisor with the California Department of Public Health, where he conducted many studies and assessments of insect-borne disease problems. He served in this capacity until 1958. After his service in California, Russ began a series of overseas assignments in medical entomology, as Senior Malaria Advisor in Ethiopia (1958-1960) and Regional Malaria Advisor for Latin America (1960-1964). These assignments were followed by a tour with the Centers for Disease Control in Atlanta, where he served as Assistant Chief of the *Aedes aegypti* eradication program. In 1969, Russ traveled to Geneva to serve as Scientist with the World Health

Organization. Finally, in 1976, Russ returned to California to become Coordinator of the University of California Mosquito Research Program. He served in this capacity until his retirement in 1986.

Russ will be remembered for his warm personality, his dry sense of humor, and his dignified manner. This latter quality earned for him the nickname "The Senator". While he was in California Russ called upon his vast experience in public health entomology to identify important problems faced by mosquito abatement agencies in California, and to marshal a diverse University of California faculty to address these problems.

Russ' career was one dedicated to service for public health on local, national and international fronts. In recognition of this career, and to his many contributions to the state of California, this sixty-fifth annual conference of the Mosquito and Vector Control Association of California is dedicated to his memory.

SAN JOAQUIN COUNTY MULTI-SPECIES HABITAT CONSERVATION AND OPEN SPACE PLAN

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The San Joaquin County Council of Governments has been overseeing preparation of a multi-species, multi-habitat, multi-purpose open space management program for all of San Joaquin County since January, 1994. The impetus for the Plan arose from conflicts between proposed development and habitat lands for the Swainson's hawk, a species protected by the California Endangered Species Act, and the San Joaquin kit fox, a species protected under both the California and Federal Endangered Species Acts. In addition to those species, San Joaquin County is home to 101 other fish, plant and wildlife species which are afforded varying degrees of protection under the California Environmental Quality Act, the California Endangered Species Act, the Federal Endangered Species Act, the Migratory Bird Treaty Act and other local, state and federal regulations. Because the habitats for these species span multiple jurisdictions, local jurisdictions recognized the need for a regional strategy for managing open space and enlisted the aid of the San Joaquin County Council of Governments to coordinate the effort.

The resulting Plan acknowledges that the fragmented approach currently being used to mitigate impacts to species on a project-by-project basis is not biologically reliable, does not meet long-term species preservation goals, frequently results in project delays and increases project costs. The Plan will replace these project-by-project reviews with legally binding agreements from state and federal agencies to permit development within identified growth areas, in exchange for the management of large tracts of land managed for species located outside of population centers. The approach eliminates redundant and costly project reviews in favor of an open space master plan providing long-term species protection, conservation of agricultural lands, a streamlined permitting process, and new opportunities for recreation, education, flood control and other beneficial open space uses.

This streamlined approach to open space management is expected to reduce project costs for private development interests and other agencies seeking coverage under the Plan for a wide range of activities including state and local transportation projects, school expansions, levee maintenance, residential, commercial and industrial construction, and numerous other activities including the conversion of vernal pool grasslands to orchards and vineyards. A cost-benefit analysis completed for the Plan indicates that cost-savings for project proponents, taxpayers and state and federal agencies under the Plan could total approximately $318,592,500 during the 50-year life of the Plan. In addition, the Plan will only purchase easements from willing sellers and would make available up to $160,000,000 to private land owners interested in selling easements to maintain land for wildlife. Liability protections are included in the Plan for landowners located near preserves to ensure that agricultural productivity in the County will not be adversely impacted.

Throughout the process, representatives from conservation, agricultural, business, local planning agencies, the California Department of Fish and Game, the U.S. Fish and Wildlife Service, the U.S. Army Corps of Engineers and other public interests have participated in Plan preparation. A Memorandum of Understanding (MOU), outlining the goals and planning process for the Plan, was adopted on October 10, 1994. Since that time, a biological analysis, an economic analysis and a habitat conservation plan have been completed. The Plan was approved on March 24, 1997, by the Habitat Policy Advisory Committee. The Council of Governments is currently coordinating the final Plan review through the legal departments of the state and federal regulatory agencies. The Council of Governments and the state and federal regulatory agencies have committed to completion of the planning process by December 31, 1997.

At present, the following agencies have indicated interest in participating in the Plan, once formally adopted: Caltrans - District 10, the San Joaquin County Council of Governments, East Bay Municipal Utilities District, Stockton East Water District, local reclamation districts, local school districts, the San Joaquin Area Flood Control Agency, South San Joaquin Irrigation District, San Joaquin County and the cities of Escalon, Lathrop, Lodi, Manteca, Ripon, Stockton and Tracy.

Participation in the Plan is voluntary for both local agencies and individuals undertaking covered activities. Preserves for the Plan are identified by description, rather than by mapping, to maintain private property rights. The Plan will be financed from the collection of development fees; state and federal monies; leases and re-sales of preserve lands and the sale of conservation banking credits. Currently, proposed fees under the Plan are $750/acre for lower value wildlife habitat (primarily orchards and vineyards) and $1,500 per acre for row and field crop lands and natural lands including oak woodlands, creeks, and native grasslands. Vernal pool mitigation is anticipated to cost $30,000 per acre for wetted surface area and $5,000 per acre for upland grasslands (for an average cost of $8,000 per acre). The Plan will be implemented by a joint powers authority composed of elected officials from the County and cities opting to adopt the Plan.

LEGISLATIVE POLICY: A TEAM APPROACH

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The past few years have shown us, that in self defense, we must be active participants in the legislative process. The Legislative Committee ("Committee") of the Mosquito and Vector Control Association of California ("Association") has been working toward the development of a policy that will provide guidelines to assist the Committee in accomplishing our goals now and in the future. The policy has evolved over the past few years as the Committee has discovered what works and what does not work through trial and error. The policy format will allow the Legislative Committee and the Association Board to make changes as they become necessary while offering continuity to the Committee from year to year. We hope to have this policy in place by the end of this year. I am going to give you an overview of how this policy will work if we all work together and make it a team effort.

The first step in the development of the policy was to identify the objectives of the Legislative Committee. We have come up with five.

1. Analysis of proposed legislation as to its impact on mosquito and vector control, public health and special districts.

2. Keeping the Association membership informed about those pieces of legislation that could affect them, throughout the process from proposal to passage or defeat.

3. Organizing "grass roots" letter writing or telephone campaigns to lobby for or against particularly important legislation making its way through the process.

4. Developing relationships with our legislators, their staff and consultants. Particularly those who author legislation that has an impact on our services and those who sit on the committees that hear such bills.

5. To be a resource to the Association membership and our elected officials, their staff and consultants. To offer assistance and make contacts as frequently as possible. To stay in touch.

Our next task was to develop the Legislative Tracking system. This is the system that we use to analyze and track proposed legislation.

The Mosquito and Vector Control Association has a paid Legislative Advocate or Lobbyist. Our present Legislative Advocate, Ralph Heim, is amazing. He reads every piece of legislation introduced each year and identifies proposed legislation (or bills) that are of importance to this Association. To assist him in this, we have developed a list of topics that are of importance to us. Proposed legislation that fits into this list of topics, or that he identifies as relevant, would be included in the bills that he sends to the Committee Chair. Because legislators introduce new bills throughout the year, the Legislative Advocate sends bills to the Legislative Committee Chair continuously throughout the year with most of the bills being received and analyzed early. To give you an idea of the magnitude of the Legislative Advocate's job, there are thousands of bills introduced in the State Legislature every year. After he reads them all, he sends well over one hundred of them to the Legislative Committee for analysis and tracking.

The Committee Chair assigns each bill to a team of two committee members for analysis and tracking. The Chair may also submit bills to other Association committees for review and analysis.

The Committee meets in early to mid-March with the Legislative Advocate and the Executive Director to establish the Association position on all bills received to date. The Committee then meets at least quarterly to discuss newly received bills, developments in bills that are being tracked, to develop or revise Association positions on bills and to plan any action needed to promote or oppose bills. The Committee also stays in close contact with the Chair in between meetings to insure that the Association position and actions reflect the most recent amendments to each bill.

To assist the committee members with the tracking of their assigned bills, the Chair continuously sends amended bills and other relevant information received from the Legislative Advocate and other sources to the committee members.

We have developed a Legislative Alert System for keeping the Association membership informed about legislative matters in a timely fashion. Most of you probably already know how it works. When the Legislative Advocate makes a request that information be distributed to the members or asks for "grass roots" type action; or when the Legislative Committee Chair determines that information should be distributed to the membership, then the Legislative Chair produces a Legislative Alert. The Alert is forwarded via modem to the Association's central office for immediate distribution by fax to all member agencies.

When Legislative Alerts are received by the member agencies, they should be distributed to key personnel and to the board of trustees. In this way, all members are informed and can act or react accordingly.

Another important aspect of the Legislative Tracking System is the Association's Official Position. When we distribute information to the membership, we refer to the official position and level of priority that has been established for each bill. The positions and their meaning are:

"Support": we are in favor of the passage of the bill.

"Oppose": we want to see the bill defeated.

"Neutral": we are not taking a position on this bill because it would have minimal affect on our member agencies.

"Watch": we are uncertain of the affect of this bill and/or we expect the bill to be changed.

Very often a bill is introduced as a "spot bill". A spot bill is one that is never intended to be passed as introduced. Instead, it is simply taking up space as a viable bill that can be totally amended to introduce legislation after the normal date for introducing bills. These bills are often easily identified and would definitely be given a watch position.

The Priority Levels indicate the type of action we intend to take on each bill. They are:

"Level 1": We will ask the Legislative Advocate to launch a full-blown lobby effort in accordance with our stated position.

"Level 2": Important bill. We will ask the Legislative Advocate to undertake a modified (selective) lobby effort in accordance with our position.

"Level 3": We care enough about this bill to take a position but not enough to lobby for or against it. We will simply ask our Legislative Advocate to draft a letter declaring our position to the author and other interested parties.

So, if we designate a bill as a Support Level 1, that means we will ask our Legislative Advocate to lobby vigorously for this bill's passage. A bill with a position of Oppose Level 3 would only warrant letters to the author of the bill and other interested parties stating our opposition.

The final step in the development of the Legislative Policy was to establish who would be responsible for carrying it out. The final written policy will summarize the responsibilities of each of the principal players as I have described them to you. Those responsible parties will be:

The Legislative Committee
The Legislative Committee Chair
The Legislative Advocate
The Executive Director of the Association
The member agencies of the Association

There are several other vital areas of responsibility included in the policy which should be mentioned. The policy designates the Executive Director as the official spokesman for the Association. Shared responsibility is given to the Legislative Chair, the Legislative Advocate and the Executive Director for establishing legislative contacts, testifying before the Legislature and its committees and developing bills to be introduced on the Association's behalf.

This Legislative Tracking System and Policy will be reviewed by the Ways and Means Committee and then the Association Membership.

Once it becomes an established policy for the Association, it will certainly be subject to revision in order to adapt to the legislative climate of the day. Our hope is that this policy will serve as a model for other groups with similar needs and provide the guidelines that the Mosquito and Vector Control Association of California will need to keep us ahead of the game in California politics into the future.

THE *AEDES (OCHLEROTATUS) DORSALIS* GROUP IN NORTH AMERICA

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ABSTRACT

The *Aedes* (*Ochlerotatus*) *dorsalis* group was examined for genetic and morphological variation within and among species. Of eighteen loci examined, three were diagnostic for all four members of the *dorsalis* group that are present in western North America. At least nine loci were diagnostic for each pair of species within the *dorsalis* group. There was little intraspecific variation in both *Ae. melanimon* and *Ae. dorsalis*. Coastal and inland forms of *Ae. dorsalis* were essentially identical in their allozyme patterns. An examination of wing scale patterns revealed considerable variation within and among all three species. The wings of *Ae. melanimon* specimens were essentially completely dark scaled whereas the wings of *Ae. campestris* specimens were nearly all pale scaled. The wings of *Ae. dorsalis* were extremely variable. Coastal specimens tended to be mostly dark scaled while inland specimens tended to be mostly pale scaled. Scaling patterns of the abdominal tergites proved to be reliable in separating adult females of *Ae. melanimon* from those of *Ae. dorsalis* end *Ae. campestris*. Coastal females of *Ae. dorsalis* were easily distinguished from *Ae. campestris* by the scaling pattern on the wings. However, inland *Ae. dorsalis* were essentially indistinguishable from *Ae. campestris*. Larval specimens of *Ae. melanimon* could be identified by seta 1-M being much shorter than the antenna, whereas it is equal to or longer than the antenna in both *Ae. dorsalis* and *Ae. melanimon*. Larvae of *Ae. dorsalis* could be separated from *Ae. campestris* by the branching pattern of the seta 5-C and the detached pecten spines.

The *Aedes* (*Ochlerotatus*) *dorsalis* group consists of nine species which are characterized by basal and apical banding of the tarsi. Three of the four species found in North America are common in the western United States, and all four have been implicated as vectors or potential vectors of western equine encephalomyelitis virus (Reeves 1990). *Aedes campestris* Dyar & Knab is a rare mosquito found throughout the Great Basin and as far north as the Hudson Bay. *Aedes melanimon* Dyar is a common mosquito found in irrigated areas throughout the Central Valley of California and much of the Great Basin. *Aedes dorsalis* (Meigen) is a widespread mosquito that is commonly found in fresh or alkaline water throughout western North America. In addition, there has been an intrusion into brackish water on the Pacific Coast. *Aedes canadensis* (Theobald) is found in the eastern United States and throughout much of Canada.

With the exception of *Ae. canadensis*, accurate identification of these mosquitoes is difficult, and some of the characters used in identification may not be reliable. Adult females are generally distinguished by the scaling pattern of the wings. These scales can easily be rubbed off; therefore, may not be a reliable character except in the case of newly emerged females. Also, there has been considerable morphological variation observed in at least one member of this group. *Aedes dorsalis* has often been described as having a coastal form and an inland form which differ in the shape of the comb scales (Bohart 1956) and the distribution of dark scales on the wings (Chapman & Grodhaus 1963). Coquillett (1906) suggested that the coastal form is distinct from the inland form and gave it the specific name of *lativittatus*. In addition, there have been reports of a melanistic form within the coastal populations of *Ae. dorsalis* (Chapman & Grodhaus 1963, Bohart & Washino 1978). These adults resemble *Ae. melanimon*, but the associated larval skins were identified as *Ae. dorsalis*. Identification of larvae is also difficult, and some characters may be more variable than first believed. The considerable morphological variation suggests the presence of cryptic species which would further complicate species identification. Until reliable identifications can be made, virus-vector relationships among these mosquitoes will remain in doubt.

An excellent tool for studying the taxonomy and systematics of an organism is allozyme electrophoresis. Biochemical and molecular methods can be used to: (1) aid in identification and determine geographic distribution, (2) determine the amount of gene flow between populations, (3) help explain variation in vector competence, and (4) aid in the reconstruction of phylogenies and evolutionary histories. Biochemical methods provide a means to clarify species identification and phylogeny reconstruction of the *dorsalis* group. Therefore, the specific aims of this study were 1) to determine morphological and biochemical characters for the separation of the members of the *dorsalis* group present in California and 2) to determine the degree of gene flow among the coastal and inland populations of *Ae. dorsalis.*

MATERIALS AND METHODS

Mosquitoes were collected by various individuals as fourth instars or pupae using dippers and aquatic nets. Collecting localities of *Ae. dorsalis*, *Ae. melanimon* and *Ae. campestris* are shown in Fig. 1. *Aedes canadensis* was collected from northern Indiana. The specimens were brought back to the laboratory and reared to adulthood. Upon emergence, adults were identified and stored in Bio-Freeze Vials (Costar Corporation, Cambridge, MA) at -70°C until the specimens were used for electrophoresis. Mosquitoes were then homogenized and proteins separated by polyacrylamide electrophoresis on a 6% slab gel matrix. The specimens were assayed for eighteen loci using standard histochemical staining techniques (Steiner and Joslyn 1979).

Some morphological characters used to distinguish *Ae. campestris*, *Ae. dorsalis* and *Ae. melanimon* were also examined. Ten to twenty specimens from each collection were reared individually for voucher material. In some populations, mosquitoes were collected as adult females and allowed to bloodfeed and deposit eggs. The eggs were hatched and reared to adulthood. Larvae, male genetalia and adult females were obtained from each isofemale line. The specimens were examined for variation in wing and abdominal scale patterns in the adult females and setal counts for the larvae. Characters were chosen based upon those found in the literature and dichotomous keys that have been used to identify these mosquitoes.

RESULTS AND DISCUSSION

Of the eighteen allozyme loci examined in this study, three were diagnostic for all four members of the *dorsalis* group. Criteria for diagnostic loci were based upon the methods of Ayala & Powell (1972). In addition, at least 9 loci were capable of distinguishing each species pair. *Aedes melanimon* and *Ae.* canadensis were very distinct, with 13 loci being diagnostic for this species pair. While *Ae. dorsalis*, *Ae. melanimon* and *Ae. campestris* are very similar in external morphology, these results indicate that all four members of the *dorsalis* group in North America are genetically very distinct.

No diagnostic loci were found to separate coastal versus inland populations of *Ae. dorsalis.* In addition, there was little or no population structuring according to geographic location. The only population which was somewhat distinct from all other was the Salton Sea population However, all other inland populations were genetically very similar to coastal populations. Chapman & Grodhaus (1963) and Bohart & Washino (1978) have described melanistic forms of *Ae. dorsalis* in coastal California. Coquillett (1906) considered this coastal form to be a separate species, *Ae. iativittatus*, although this was later synonomized with *Ae. dorsalis*. The genetic data show little or no evidence of restricted gene flow or differentiation among populations of *Ae. dorsalis*. This would confirm that *Ae. iativittatus* should remain a synonym of *Ae. dorsalis*.

An examination of wing scale variation confirms that coastal populations of *Ae. dorsalis* tend to be darker than inland populations. Inland populations have pale scales over most of the costa whereas coastal populations generally have more dark scales than pale scales. In some coastal specimens, the wings are completely dark scaled. According to the current taxonomic status of these mosquitoes, these specimens should be considered to be *Ae. melanimon*. However, the allozyme analysis indicate that *Ae. melanimon* was found along the coast in only one location (Bolinas, CA). It was collected along with a few specimens of *Ae. dorsalis* from which it was easily distinguished. *Aedes dorsalis* could be separated from *Ae. campestris* by the R4+s vein being mostly dark scaled in *Ae. dorsalis* and mostly pale scaled in *Ae. campestris*.

The scaling pattern of the abdominal segments of these mosquitoes is a more reliable character for the separation of *Ae. dorsalis* and *Ae. melanimon.* Both mosquitoes possess a dorsal stripe of pale scales, but *Ae. melanimon* has more dark scales on the abdomen than *Ae. dorsalis. Ae*des *dorsalis* has a small patch of dark scales on either side of the dorsal stripe. This patch is usually in the shape of a rectangle or an oval. It tends to be smaller in inland specimens and may be completely absent on some segments. In *Ae. melanimon*, the patch is larger and irregular in shape and extends laterally and posteriorly. The abdomen of *Ae. campestris* is almost completely covered with pale scales on most segments. However, this character may not be useful in the separation of *Ae. campestris* from *Ae. dorsalis* as the abdomens resemble inland *Ae. dorsalis* in many specimens.

The larvae of *Ae. dorsalis* and *Ae. campestris* can be separated from *Ae. melanimon* by the length of mesonotal seta 1 (1-M). In *Ae. dorsalis*, seta 1-M is about equal in length to the antenna, whereas in *Ae. melanimon*, it is much shorter. *Aedes dorsalis* and *Ae. campestris* are distinguished by the distal pecten spines being detached and head hair 5-C being triple branched in *Ae. campestris*. However, these characters are known to be variable and specimens examined in the present study exhibited some variation. Further studies incorporating both morphological and molecular evidence are required to clarify species relationships of these mosquitoes.

REFERENCES CITED

Bohart, R.M. 1956. Identification and distribution of *Aedes melanimon* and *Aedes dorsalis* in California. Proc. Calif: Mosq. Contr. Assoc. 24: 81-83.

Bohart, R.M. and Washino. RK. 1978. Mosquitoes of California. Third Edition. University of California Press, Berkeley.

Chapman, H.W. and Grodhaus, G. 1963. The separation of adult females of *Aedes dorsalis* (Meigen) and *Aedes melanimon* Dyar in California. Calif. Vector Views 10: 53-56.

Coquillett, D.W. 1906. A classification of the mosquitoes of North and Middle America. U. S. Bull. Ent. Tech. Ser. 11: 1-31.

Reeves, W.C. 1990. Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987. Calif. Mosq. Vect. Contr. Assoc., Sacramento, CA.

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

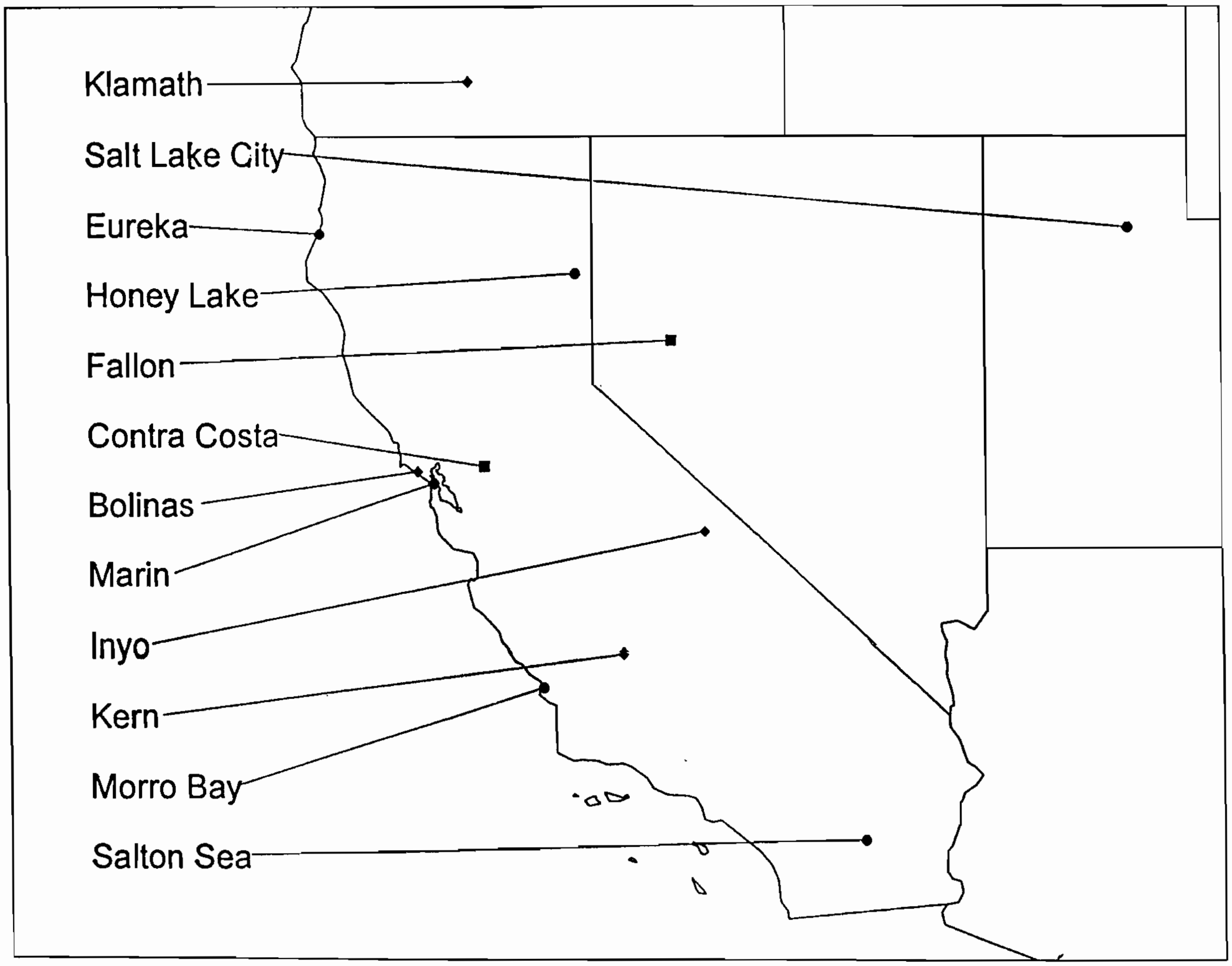

Figure 1. Locations of *Aedes dorsalis* (circles), *Ae. melanimon* (diamonds), and *Ae. campestris* populations examined in present study. The two sites marked with squares are locations where more than one species was collected. *Aedes dorsalis* and *Ae. melanimon* were collected from the Contra Costa, CA site and *Ae. dorsalis*, *Ae. melanimon* and *Ae. campestris* were collected from the Fallon, NV site.

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MOSQUITO CONTROL AND WATERFOWL HABITAT ENHANCEMENT BY VEGETATION MANIPULATION AND WATER MANAGEMENT: A TWO YEAR STUDY

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ABSTRACT

Management of wetlands for the control of mosquitoes and the enhancement of waterfowl habitat are sometimes viewed as incompatible objectives. This research examined the effects of burning or discing in large-scale plots of saltgrass (*Distichlis spicata*) on mosquito densities and waterfowl food resources, one and two years post-treatment. Dry season burning and discing, followed by flooding to prevent the regrowth of saltgrass, lowered populations of the three most common mosquitoes at Suisun Marsh, *Aedes melanimon*, *Culex tarsalis*, and *Culiseta inornata* during both years. Burning increased plants and invertebrates important in waterfowl diets, including brass buttons (*Cotula coronopifolia*) and *Chironomus stigmaterus* midge larvae (Chironomidae). This research demonstrates that burning and discing can achieve long-term control of mosquitoes and enhancement of waterfowl habitat.

Wetlands are highly productive areas that are important as sources of food and habitat for fish and other wildlife. Until recently, however, these values were not always appreciated or understood, and many wetlands were converted to agricultural land or drained for mosquito control. In California over 95% of the state's wetlands have been lost or heavily impacted (Mitsch and Gosselink 1993, Heitmeyer et al. 1989).

Wetland habitats have received increasing attention directed towards preservation and increasing their productivity for waterfowl and other wildlife. Management of wetlands for the control of mosquitoes and the enhancement of waterfowl habitat are sometimes viewed as incompatible objectives. This study expands upon work by Batzer, de Szalay and Resh at Suisun Marsh designed to adapt practices that are commonly used to enhance waterfowl habitat for purposes of mosquito control (Batzer and Resh 1992, de Szalay and Resh In press).

MATERIALS AND METHODS

Site Description. The studies were conducted at the California Department of Fish and Game's Grizzly Island Wildlife Area (GIWA) in Suisun Marsh (Solano Co., CA), which is located in the Sacramento-San Joaquin River Delta. Suisun Marsh contains 135,905 hectares of wetlands with an additional 71,659 hectares of bays and sloughs and is an important migratory area for waterfowl and shorebirds (California Department of Fish and Game 1987). This seasonally flooded brackish-water wetland is subdivided into diked cells, the majority of which are flooded in late summer and drawn down in early spring. Saltgrass (*Distichlis spicata)* and pickleweed (*Salicornia virginica*) are the dominant vegetation in most wetland cells (Rollins 1981). Three major species of mosquitoes are found at Suisun Marsh: *Aedes melanimon*, *Culex tarsalis*, and *Culiseta inornata*.

Experimental Design. Four experimental plots were constructed in large stands of *D. spicata*. Each plot consisted of two randomly assigned treatment areas (600 m^2) and an unmanipulated control area (600 m^2). In one treatment area, a 5-m wide firebreak was created around the perimeter, and the vegetation inside the firebreak was burned. In the other treatment area, the vegetation was disced using tractor-pulled machinery. The third area was left unmanipulated and served as the control. Discing and burning were conducted in mid-September of 1994.

Flooding began within two weeks of treatment to prevent the regrowth of saltgrass and create large areas of open water. The wetlands remained flooded until they were drawn down in April of 1995. The plots remained dry during the summer allowing new vegetation to colonize the plots. In October the ponds were reflooded to depths ranging from 0 to 45 cm.

Sampling Methods. Mosquito larvae were sampled with a dip sampler (147 ml). Fifty random dips were taken in each treatment area and were pooled for a site. Because *Ae. melanimon* eggs hatch immediately after flooding and develop rapidly in the warm water occurring at the beginning of the flooding season, we sampled our plots every 5-7 days at the start of the season. We continued sampling the plots every two to four weeks, throughout the 1994-95 field season, until the plots were drawn down in late spring and allowed to dry. In October of 1995, the plots were once again flooded. Mosquito dips were taken weekly through October and November 1995 and then every two to four weeks until April 1996. Other invertebrates were sampled using a benthic corer and d-frame sweep net, and vegetative regrowth (as percent cover) was also measured during the first and second years following treatment.

Statistical Analysis. Our analyses followed a repeated measures, randomized block design (blocked by plot); the plots were sampled multiple times throughout the season and each plot contained both control and treatment areas. The ANOVA model used to analyze the data had treatments as fixed effects and plots as random effects. Date, plot, treatment, and interactions between these factors were examined. If the ANOVA model indicated a significant difference, and there was no significant interaction between date and treatment, then Tukey's Multiple Comparison tests were used to compare the individual treatments means averaged over the season (Zar 1984). If there was an interaction between date and treatment, then both the Tukey's Multiple Comparison test and Bonferroni Multiple Comparison procedure were used to compare the individual treatment means on each date. The smallest interval from these two tests was chosen for comparison between the means on each date, as recommended by Neter et al. (1985).

RESULTS AND DISCUSSION

Mosquitoes. During the first year following treatment, densities of *Ae. melanimon* and *Cx. tarsalis* were significantly lower in the burned and disced areas than in the control (Table 1; $F_{2,6}$=13.44, P<0.006; $F_{2,6}$=8.47, P<0.02, respectively; ANOVA test). No mosquitoes were ever found in the burned area. In the disced area, *Cs. inornata* was never found, *Cx. tarsalis* was present in only one of the four plots on one date, and *Ae. melanimon* was present in two of the four plots on only one date. While no *Cs. inornata* mosquitoes were found in the burned or disced treatment areas, the difference from control was statistically significant only early in the season when densities were greater than 0.1 mosquitoes per dip. This was reflected in the Date-Treatment interaction for *Cs. inornata* densities (Table 1; P<0.0001; ANOVA test, P<0.05, Bonferonni Multiple Comparisons).

During the second year following treatment *Ae. melanimon*, *Cx. tarsalis*, and *Cs. inornata* were found in all three treatment areas. *Ae. melanimon* had lower densities in both burned and disced areas (Table 1; P<0.019; ANOVA test) and *Cx. tarsalis* densities were significantly lower in the burned areas than in the control areas (Table 1; P<0.038; ANOVA test). *Cx. tarsalis* densities also showed a significant date effect; high densities were found during the first three dates with lower densities later in the season ($F_{5,15}$=4.43, P<0.01; ANOVA test). There was a significant Date-Treatment interaction for *Cs. inornata* densities (P<0.01; ANOVA test); this time *Cs. inornata* was present at low levels early in the season but in February and March densities increased significantly in the control area while remaining low in the burned and disced areas (Table 1; Bonferonni Multiple Comparisons P<0.05).

One possible cause for the reduction of *Ae. melanimon* during the first year was the destruction of the eggs that were oviposited in moist soil as the water level receded. Eggs may be destroyed during the discing and burning process itself or by desiccation caused by the removal of this protective vegetation layer. Other studies have shown successful reduction of *Aedes* mosquitoes with vegetation burning (Wallace et al. 1990, de Szalay et al. 1995).

Reduction of *Cx. tarsalis* and *Cs. inornata* may have been caused by the large areas of open water in the burned and disced treatment areas. Open water discourages oviposition by *Cx. tarsalis* and *Cs. inornata* and exposes mosquito larvae to wave action and increased predation (Balling and Resh 1983). In our study, early flooding of ponds prevented the regrowth of saltgrass and created open water. During the first and second year, the burned and disced areas had higher percent open water than found in the control plots (P<0.016; ANOVA test). This contrasts with similar experiments in which flooding was not conducted until six weeks after discing; this allowed the regrowth of saltgrass and did not reduce mosquito densities (de Szalay et al. 1995). Therefore, rapid flooding is an important component in these mosquito control techniques.

not heavily consumed at Suisun Marsh (Batzer et al. 1993), waterfowl in other areas of California have been found to consume them in areas with high densities of *T. verticalis*. (Euliss et al. 1991).

Other species that increased due to treatments were *Cricotopus sylvestris*, *Limnophyes sp.*, and *Berosus ingeminatus*. Densities of *Cricotopus sylvestris* were significantly higher on the first date (March 7, 1996) in burned areas than in the disced and control areas ($F_{2,6}$=5.78, P<0.04; ANOVA test). *Limnophyes sp.* had a different pattern; more were found in both burned and control areas than in disced areas (P<0.04; ANOVA test). *Berosus ingeminatus* were more numerous in the disced area than in the burned and control areas over all dates ($F_{2,6}$=2.15, P<0.0148; ANOVA test).

Table 1. Effects of burning and discing saltgrass on the mean numbers of mosquitoes per dip (± S.E.) during the first and second years post-treatment.

SPECIES	CONTROL	DISC	BURN
YEAR ONE			
Ae. melanimon	0.104 (± 0.038)	0.105 (± 0.088)*	0 (± 0)*
Cx. tarsalis	0.12 (± 0.026)	0.007 (± 0.066)*	0 (± 0)*
Cs. inornata	0.345 (± 0.121)	0 (± 0) †	0 (± 0) †
YEAR TWO			
Ae. melanimon	0.183 (± 0.034)	0.047 (± 0.027)*	0.06 (± 0.029)*
Cx. tarsalis	0.372 (± 0.988)	0.193 (± 0.055)*	0.0367 (± 0.012)**
Cs. inornata	0.86 (± 0.419)	0.197 (± 0.119 ‡	0.0175 (± 0.012) ‡

* Significant decrease from control over entire season (P<0.05). ** Significant decrease from control and disc over entire season (P<0.05).
† Date-treatment interaction; significant decrease from control in November. ‡ Date-treatment interaction; significant decrease from control in February and March..

Other Invertebrates. Twenty-seven species of other invertebrates were collected and were numerically dominated by midge larvae, water boatmen, copepods, and ostracods. Densities of *Chironomus stigmaterus* midge larvae (Chironomidae) were higher in burned areas than either the disced or control areas ($F_{2,6}$=8.01, P<0.02; ANOVA test) and there was also a significant date effect ($F_{1,3}$=11.76, P<0.04; ANOVA test). Densities of *Trichocorixa verticalis* (Corixidae) were higher in disced areas than the control or burned areas ($F_{2,6}$=14.01, P<0.0055; ANOVA test). These increases are important for waterfowl management because *C. stigmaterus* was the dominant invertebrate consumed by waterfowl at Suisun Marsh (Batzer et al. 1993), and Chironomidae were widely consumed by waterfowl throughout California (Euliss and Grodhaus 1987, Connelly and Chesemore. 1980, Euliss and Harris 1987). Although *T. verticalis* were

Vegetation. After the first year of flooding (September 1994-April 1995), the ponds were drawn down permitting new vegetation to colonize the burned and disced areas, including the following plants that are important in the diets of waterfowl: western sea-purslane (*Sesuvium verrucosum*), brass buttons (*Cotula coronopifolia*), and alkali bulrush (*Scirpus robustus*) (Rollins 1981, Dave Feliz California Department of Fish and Game, pers. comm.). During the first year the percent cover of *C. coronopifolia* was statistically higher in the burned areas than the control or disced areas (P<0.01; ANOVA test).

During the second year species richness was higher in the burned and disced areas than in the control areas ($F_{2,6}$=15.06, P<0.0046, ANOVA test). *D. spicata* cover was highest in the control area and lowest in the burned area. Although *D. spicata* had grown back in parts of the disced area, the percent cover was still less than in the control (P<0.0002; ANOVA test). Percent cover of *S. robustus* was higher in the burned areas than in the control or disced areas (P<0.0033; ANOVA test). Cover of *S. verrucosum* was higher in the burn than the control areas (P<0.025; ANOVA test, P<0.05 Tukey). Cover of *C. coronopifolia* was higher in the burn than the control areas (at alpha 0.1, P<0.068; ANOVA test, P<0.1 Tukey).

In addition to reducing mosquito densities, removal of vegetation created shallow open water areas, which is important because shallow water facilitates feeding by dabbling ducks and is more easily obtained by wetland managers (Euliss et al. 1991). In our study, depths of 15 to 30 cm were sufficient to create large areas of open water in the treatment areas.

Management implications. While complete elimination of pesticide use would be preferred by wildlife agencies, a more practical goal would be to reduce greatly the required frequency and extent of pesticide usage through combined vegetation and water management. Based on this research, vegetation and water manipulation can substantially contribute to mosquito control at GIWA. Furthermore, these techniques need only be applied to areas of high mosquito population, usually found in transition zones. By removing emergent vegetation in the transition zone, the area of open water is extended from the deep marsh to the higher dry areas (i.e., levees), thereby reducing mosquito habitat.

In summary, burning and discing as performed in our study, were effective for two years post-treatment in reducing mosquito populations, and in enhancing waterfowl food resources. Currently, the California Department of Fish and Game at GIWA and the Solano County Mosquito Abatement District are working together to implement these techniques at GIWA.

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REFERENCES CITED

Balling, S.S. and V.H. Resh. 1983. Mosquito control and salt marsh management: factors influencing the presence of *Aedes* larvae. Mosquito News 43: 212-218.

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

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

California Department of Fish and Game. 1987. Testimony on the Suisun Marsh (Exhibit 5). Submitted to the State Water Quality Control Board, Bay-Delta Hearing. Sacramento, California. 34 pp.

Connelly, D.P. and D.L. Chesemore. 1980. Food habitats of Pintails, *Anus acuta*, wintering on seasonally flooded wetlands in the Northern San Joaquin Valley, California. California Fish and Game. 66: 233-237.

de Szalay, F.A and V.H. Resh. In Press. Responses of wetland invertebrates and plants important in waterfowl diets to burning and mowing of emergent vegetation. Wetlands.

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. Calif. Mosq. and Vector Control Assoc. 63: 86-90.

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

Euliss, N.H. and S.W. Harris. 1987: Feeding ecology of northern pintails and green-winged teal wintering in California. Journal of Wildlife Management. 51: 724-732.

Euliss, N.H., Jr., R.L. Jarvis, and D.S. Gilmer. 1991. Feeding ecology of waterfowl wintering on evaporation ponds in California. Condor 93: 582-590.

Heitmeyer, M.E., D.P. Connelly, and R.L. Pederson. 1989. The Central, Imperial and Coachella Valleys of California. pp.475-505. *In*: L.M. Smith, R.L. Pederson and R.M. Kaminski (eds.).

Habitat management for migratory and wintering waterfowl in northern America. Texas Tech Univ. Press, Lubbock, Texas.

Mitsch, W.J. and J.G. Gosselink. 1993. Wetlands. 2nd edition. Van Nostrand Reinhold, New York, New York.

Neter, J. W., Wasserman, M., and M.H. Kutner. 1985. Applied linear statistical models. Second Edition. Richard D. Irwin, Inc. Homewood, Illinois.

Rollins, G.L. 1981. A guide to waterfowl habitat management in Suisun Marsh. California Department of Fish and Game Publication. Sacramento, California.

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

Zar, H.J. 1984. Biostatistical Analysis. Second Edition Prentice-Hall Inc., Englewood Cliffs, New Jersey.

1997 Proc. Mosq. and Vector Control Assoc. of CA **65:16-23**

SURVEILLANCE FOR MOSQUITO-BORNE ENCEPHALITIS VIRUS ACTIVITY AND HUMAN DISEASE IN CALIFORNIA, 1996

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The California Mosquito-Borne Encephalitis Surveillance Program is a cooperative effort conducted by the California Department of Health Services Division of Communicable Disease Control, the Arbovirus Research Unit of the University of California at Davis, the Mosquito and Vector Control Association of California, local mosquito and vector control agencies, local health departments, physicians and veterinarians, and other interested parties. The surveillance program is multifaceted and includes: 1) mosquito population monitoring and testing for St. Louis encephalitis (SLE) virus and western equine encephalomyelitis (WEE) virus, 2) serological monitoring of sentinel chickens in areas of California with historical evidence of encephalitis virus activity, 3) testing of domestic animal species that show clinical signs of possible SLE or WEE infection, and 4) serological testing of patients suffering from fever, neurological symptoms, and other signs of viral meningitis or encephalitis. The 1996 surveillance program began in April with the deployment of sentinel chicken flocks and the beginning of mosquito collection data for the adult mosquito occurrence report. On May 17, the first of 28 weekly bulletins and adult mosquito abundance reports was distributed to all surveillance program participants. Positive serology and mosquito pool results were communicated immediately to submitting agencies.

Human Disease Surveillance: In 1996, 73 human serum and/or cerebrospinal fluid specimens from patients presenting symptoms of viral meningitis or encephalitis were tested by the Department's Viral and Rickettsial Disease Laboratory (VRDL) for antibodies to SLE and WEE viruses. Neither elevated IgM antibody nor a four-fold rise in total antibody between paired sera was observed in specimens from any of the suspect patients.

Equine and Emu Surveillance: A total of 12 serum and brain tissue specimens from horses displaying neurological signs was submitted by practicing California veterinarians for arboviral testing at VRDL in 1996. None of these specimens was positive on arbovirus serology or antigen testing. The United States Department of Agriculture / Animal and Plant Health Inspection Service (USDA/APHIS) Veterinary Diagnostic Laboratory in Ames, Iowa isolated WEE from an emu from Orland, Glenn County. Serum and tissue were collected from the emu on July 16.

Mosquito testing: Twenty-seven local mosquito control agencies throughout the state submitted a total of 98,739 mosquitoes (2,300 pools) in 1996 (Table 1). Mosquitoes were pooled and tested for arboviruses by plaque assay using Vero cell culture. Of these, 16 pools were positive for WEE and none for SLE. Positive *Culex tarsalis* pools were collected between July 16 and September 17 in Butte (1), Kern (3), Lake (1), Sacramento (3), Sutter (2), and Yolo (6) Counties.

Chicken Serosurveillance: In 1996, a total of 176 sentinel chicken flocks was maintained by 49 local mosquito and vector control agencies. Fifteen

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of these flocks were involved in arbovirus research projects conducted by the Arbovirus Research Unit in Riverside and Imperial counties. Over 18,910 chicken sera were tested for WEE and SLE. Of these, 195 tested positive for WEE (Table 2), and 40 were positive for SLE (Table 3). The first seroconversions to WEE were detected in sera collected from Kern and Tulare counties during the week of June 16, and to SLE in sera collected from Los Angeles County on July 19. Locations of chicken flocks that contained one or more positive chicken in 1996 are shown in Figure 1. SLE activity was confined to southern California, primarily in the irrigated agricultural and salt marsh habitats of Imperial and Riverside Counties. WEE activity was found in Imperial County and throughout the Central Valley from Kern to Tehama County. Surveillance efforts outside of California detected WEE in Jackson County, Oregon and Washoe County, Nevada via testing conducted by the VRDL.

More WEE activity was detected in 1996 than in 1995; 50 flocks with 195 birds seroconverted in 1996 versus 29 flocks with 81 birds in 1995. SLE activity in 1996 was less than half of the activity recorded during each of the last four years. WEE and SLE activity detected by the encephalitis virus surveillance program from 1991-1996 is summarized in Figures 2 and 3.

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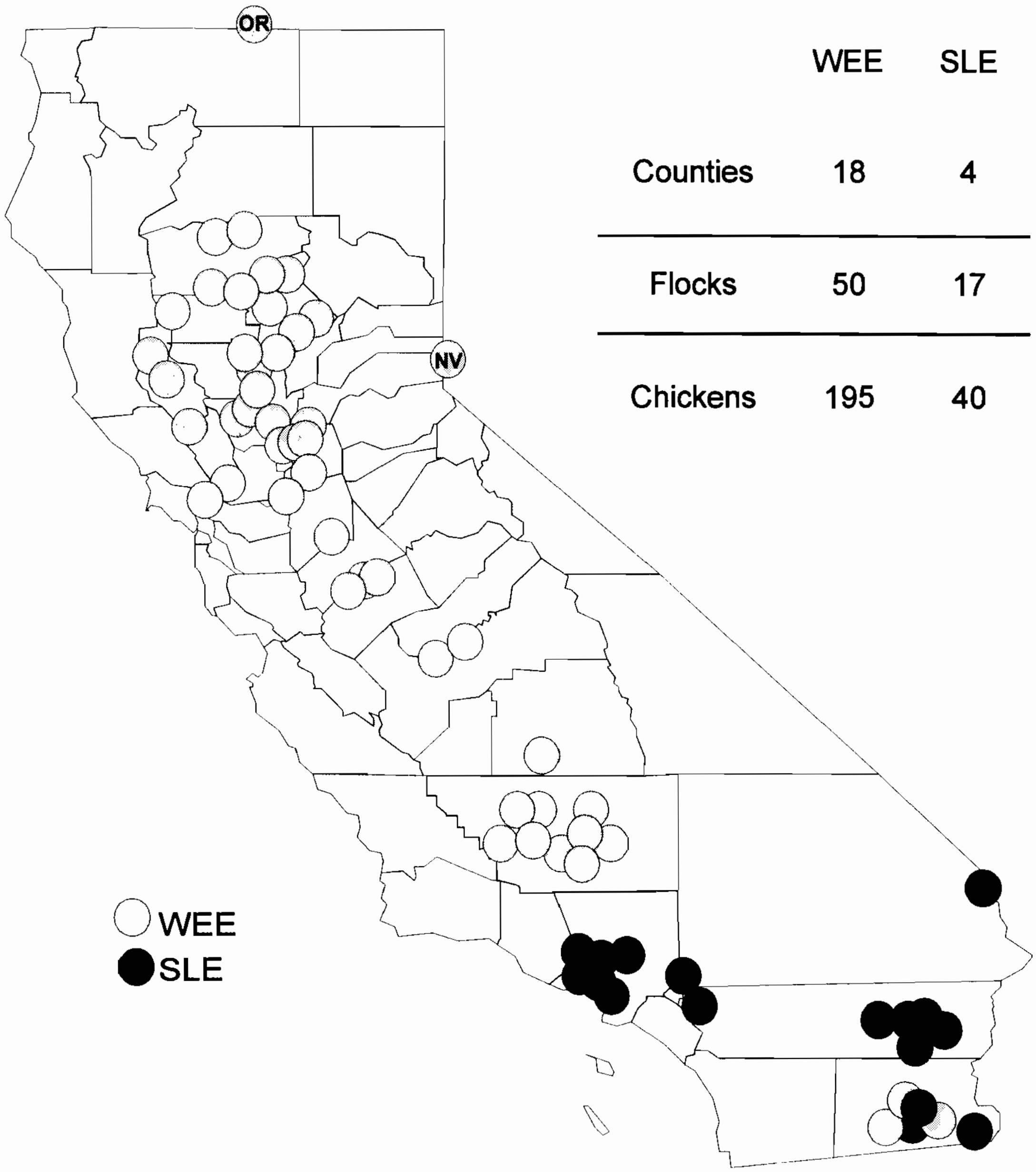

	WEE	SLE
Counties	18	4
Flocks	50	17
Chickens	195	40

Figure 1. Sentinel chicken flocks with at least one seroconversion to St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus, California. 1996.

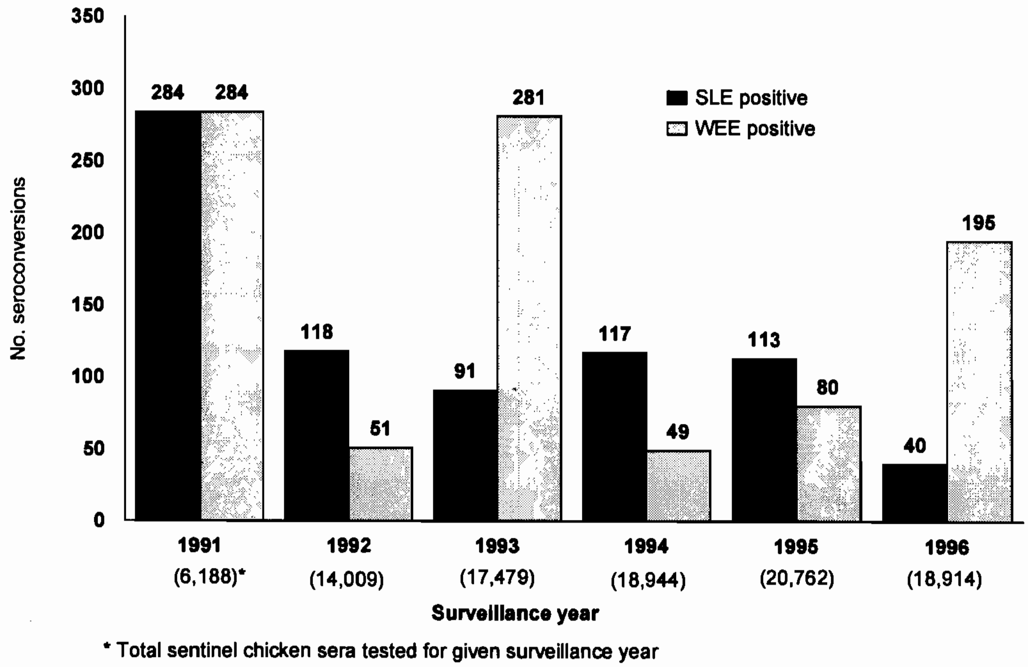

Figure 2. Seroconversions to St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus in sentinel chickens, 1991-1996.

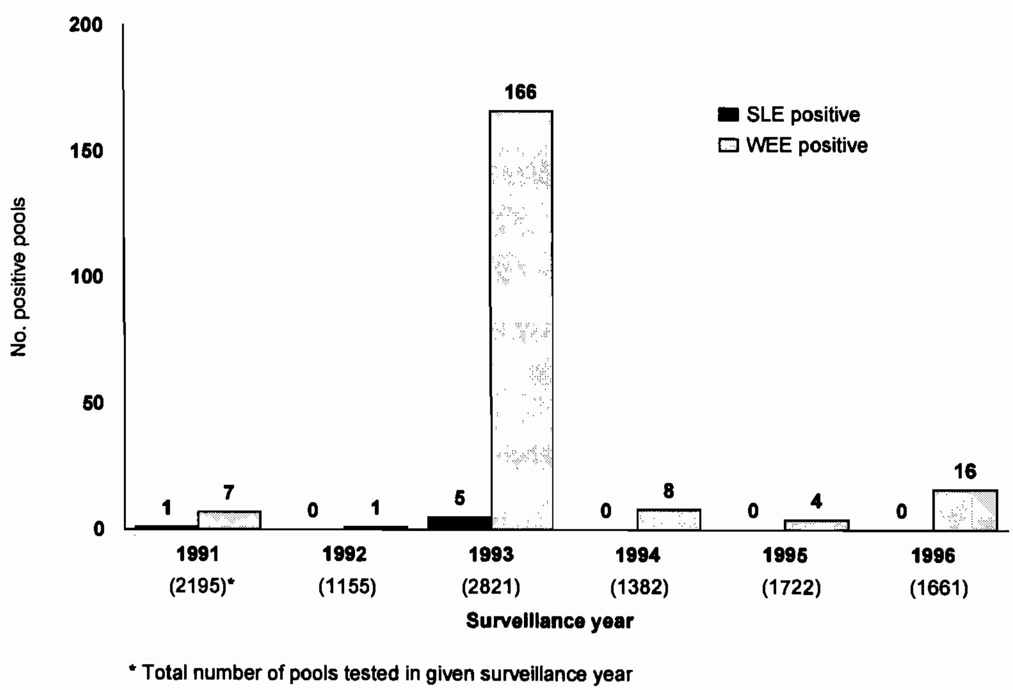

Figure 3. St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus activity in pooled *Culex tarsalis*, 1991-1996.

Table 1 Mosquitoes tested by VRDL for WEE, SLE and CE viruses in 1996, by submitting agency and mosquito species.

County*	Agency	*Cx. tarsalis*		*Cx. pipiens/ Cx. quinquefasciatus*		*Cx. stigmatostoma*		*Ae. melanimon*		Total	
		pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.	pool	mosq.
Butte	BUCO	2	100	1	35	0	0	0	0	3	135
Contra Costa	CNTR	118	5865	0	0	0	0	0	0	118	5865
Fresno	FRNO	5	120	0	0	0	0	0	0	5	120
Glenn	GLEN	28	1400	0	0	0	0	0	0	28	1400
Jackson/OR	JCVC	53	2334	11	550	0	0	0	0	64	2884
Kern	KERN	163	7467	0	0	0	0	5	250	168	7717
Kings	KNGS	26	1172	0	0	0	0	0	0	26	1172
Lake	LAKE	203	9781	0	0	3	68	5	120	211	9969
Los Angeles	LONG	112	3980	160	6700	0	0	0	0	272	10680
Los Angeles	LOSA	5	167	22	1060	2	45	0	0	29	1272
Los Angeles	SGVA	20	813	21	702	1	12	0	0	42	1527
Los Angeles	SOUE	51	1499	86	3367	18	271	0	0	155	5137
Madera	MADR	6	290	6	300	0	0	0	0	12	590
Merced	MERC	2	100	1	50	0	0	3	150	6	300
Orange	ORCO	15	278	62	1305	2	21	0	0	79	1604
Placer	PLCR	67	2861	0	0	0	0	0	0	67	2861
Riverside	NWST	71	3526	117	5754	44	2072	0	0	232	14941
Sacramento/Yolo	SAYO	316	14164	0	0	0	0	22	687	338	1639
San Bernardino	SANB	36	1407	25	971	7	111	0	0	68	2489
San Diego	SAND	24	1093	0	0	0	0	0	0	24	1093
San Joaquin	SJCM	28	1280	0	0	0	0	0	0	28	128
Santa Barbara	GLVY	5	183	6	223	0	0	0	0	11	406
Shasta	SHAS	28	1316	2	100	0	0	0	0	30	1416
Sutter/Yuba	SUYA	188	8469	0	0	0	0	2	50	190	8519
Tulare	DLTA	42	1605	0	0	0	0	0	0	42	1605
Stanislaus	TRLK	31	1476	1	50	0	0	2	100	34	1626
Ventura	VENT	16	769	2	100	0	0	0	0	18	869
	Total	**1661**	**73515**	**523**	**21267**	**77**	**2600**	**39**	**1357**	**2300**	**98739**

*Bold font indicates counties with WEE isolation from *Culex tarsalis*.

Table 2. Chicken Seroconversions to WEE by Location and Biweekly Sampling Date, 1996

County	Location	City	6/21	7/05	7/19	8/02	8/16	8/30	9/13	9/27	10/11	10/25	Total
Butte	Gray Lodge	Gridley	0	0	1	0	9	0	0	0	0	0	**10**
Butte	Honcut Road	Honcut	0	0	0	1	4	1	0	0	0	0	**6**
Butte	M & T Ranch	Chico	0	0	0	2	3	2	1	0	0	0	**8**
Butte	Paiva Ranch	Chico	0	0	0	3	2	2	0	0	0	0	**7**
Butte	Thebach Ranch	Biggs	0	0	0	3	3	5	0	0	0	0	**11**
Contra Costa	Rhone Poulenc	Martinez	0	0	0	0	0	0	1	0	0	0	**1**
Fresno	Firebaugh High	Firebaugh	0	0	0	1	1	0	0	0	0	0	**2**
Fresno	Kerman	Kerman	0	0	0	0	0	2	0	0	0	0	**2**
Glenn	County Road 20	Orland	0	0	1	4	0	1	0	0	0	0	**6**
Glenn	N.E. Willows	Willows	0	0	1	1	1	7	1	0	0	0	**11**
Imperial	Cady Road	Brawley	0	0	0	0	0	2	0	0	1	0	**3**
Imperial	Campbell	Holtville	0	0	0	0	0	1	0	0	0	0	**1**
Imperial	Nichols	El Centro	0	0	0	0	0	0	0	1	1	0	**2**
Jackson, OR	Anhorn Field	Central Point	0	0	0	0	1	0	0	0	0	0	**1**
Kern	Arvin	Arvin	0	0	1	1	4	1	2	1	0	0	**10**
Kern	Beldridge	Beldridge	0	0	0	1	0	0	0	0	0	0	**1**
Kern	Delano MAD	Delano	0	0	3	0	0	1	1	0	1	0	**6**
Kern	FC Tracy Ranch	Buttonwillow	0	0	1	0	1	1	0	0	0	0	**3**
Kern	Kern NWR	Lost Hills	1	0	6	1	0	0	0	0	0	0	**8**
Kern	Old River	Old River	0	0	3	0	0	0	0	0	0	0	**3**
Kern	Panama Lane	Bakersfield	0	0	2	0	1	0	0	0	0	0	**3**
Kern	Teviston	Delano	0	0	0	1	0	1	1	2	0	0	**5**
Kern	Wasco	Wasco	0	1	5	0	0	0	0	1	0	0	**7**
Lake	Dry Lake	Lower Lake	0	0	0	0	1	0	0	0	0	0	**1**
Lake	VCD Fish Ponds	Upper Lake	0	0	0	0	0	2	1	0	1	0	**4**
Merced	Koda Farms	Dos Palos	0	0	0	0	0	1	0	0	0	0	**1**
Merced	Newman Gun	Gustine	0	0	0	0	0	0	2	0	0	0	**2**
Merced	Valdhaus Dairy	Merced	0	0	0	0	0	0	0	0	1	0	**1**
Napa	Myrtledale	Calistoga	0	0	0	0	0	0	0	1	0	0	**1**

Table 2. Chicken Seroconversions to WEE by Location and Biweekly Sampling Date, 1996

County	Location	City	6/21	7/05	7/19	8/02	8/16	8/30	9/13	9/27	10/11	10/25	Total
Sac/Yolo	G. Whitney	Hood	0	0	0	0	5	1	2	0	0	0	**8**
Sac/Yolo	Herald	Herald	0	0	0	0	0	2	0	0	0	0	**2**
Sac/Yolo	Knights Landing	Knights Landing	0	0	0	0	2	0	0	0	0	0	**2**
Sac/Yolo	Merritt	Davis	0	0	0	0	0	1	0	0	0	0	**1**
Sac/Yolo	Natomas	Sacramento	0	0	0	0	0	1	1	0	0	0	**2**
Sac/Yolo	W. Sacramento	W. Sacramento	0	0	0	0	2	1	0	0	0	0	**3**
Sac/Yolo	Winters	Winters	0	0	0	0	2	0	0	0	0	0	**2**
San Joaquin	Bacon Is-Mantelli	Holt	0	0	0	1	0	2	1	0	0	0	**4**
San Joaquin	White Slough	Lodi	0	0	0	0	0	1	0	0	0	0	**1**
Solano	FP Smith Equip	Cordelia	0	0	0	0	1	2	2	1	0	0	**6**
Stanislaus	Modesto Sewer	Patterson	0	0	0	0	1	0	0	0	0	0	**1**
Sutter/Yuba	Barker	Rio Oso	0	0	0	0	6	0	0	0	0	0	**6**
Sutter/Yuba	Dean Ranch	Sutter	0	0	0	2	0	2	0	0	2	0	**6**
Sutter/Yuba	Ledbetter	Marysville	0	0	0	0	3	0	1	0	0	0	**4**
Sutter/Yuba	Robbins	Robbins	0	0	0	1	1	0	1	0	0	0	**3**
Sutter/Yuba	Sheppard	Live Oak	0	0	0	2	2	0	1	0	0	0	**5**
Sutter/Yuba	Sr. Citizen Center	Olivehurst	0	0	0	0	1	0	0	0	0	0	**1**
Tehama	Corning	Corning	0	0	0	1	0	0	0	0	0	0	**1**
Tehama	MAD Office	Red Bluff	0	0	0	1	3	4	0	0	0	0	**8**
Tulare	Alpaugh	Alpaugh	1	0	0	0	0	0	0	0	0	0	**1**
Washoe/NV	Rosewood Lakes	Reno	0	0	0	0	1	0	0	0	0	0	**1**
		WEE Totals	**2**	**1**	**24**	**27**	**61**	**47**	**19**	**7**	**7**	**0**	**195**

Table 3. Chicken seroconversions to SLE by location and biweekly sampling date, 1996.

County	Location	City	6/21	7/05	7/19	8/02	8/16	8/30	9/13	9/27	10/11	10/25	Total
Imperial	Bard	Bard	0	0	0	0	0	0	0	0	3	0	3
Imperial	Campbell	Holtville	0	0	0	0	0	0	0	0	3	0	3
Imperial	Nichols	El Centro	0	0	0	0	0	3	0	3	3	0	9
Los Angeles	Bernard Bio Stn.	Claremont	0	0	0	0	0	0	0	3	0	0	3
Los Angeles	El Dorado Park	Long Beach	0	0	0	0	0	1	0	0	0	1	2
Los Angeles	Griffith Park	Los Angeles	0	0	0	0	0	1	0	0	0	0	1
Los Angeles	Monterey Park	Monterey Park	0	0	1	0	0	0	0	0	0	0	1
Los Angeles	Oil Fields	Baldwin Hills	0	0	0	0	0	0	0	0	2	0	2
Los Angeles	Sepulveda Basin	Encino	0	0	0	0	0	0	0	0	1	1	2
Riverside	Adhor Farms	Mecca	0	0	0	0	0	0	1	0	0	0	1
Riverside	Desert	North Shore	0	0	0	0	1	0	0	0	0	4	5
Riverside	Gordon	Mecca	0	0	0	0	0	0	0	1	1	0	2
Riverside	Jessup	Valerie	0	0	0	0	1	0	0	0	0	0	1
Riverside	Mecca	Mecca	0	0	0	0	0	0	0	0	1	0	1
Riverside	Temescal Canyon	Corona	0	0	0	0	0	0	1	0	0	0	1
San Bernardino	Horse Ranch	Colton	0	0	0	0	0	0	1	0	0	0	1
San Bernardino	Treatment Plant	Needles	0	0	0	0	0	0	0	0	0	0	2*
		SLE Totals	**0**	**0**	**1**	**0**	**2**	**5**	**3**	**7**	**14**	**6**	**40**

* bled November 12, 1996

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RESEARCH TOWARDS THE DEVELOPMENT OF AN ENZYME IMMUNOASSAY TO DETECT ARBOVIRUS ANTIBODIES IN WILD BIRDS

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ABSTRACT

The hemagglutination inhibition and the plaque reduction neutralization tests are used widely, because they detect viral antibodies in sera from most vertebrate species. However, these tests are time consuming and expensive. Conversely, the enzyme-linked immunoassay (EIA) is a sensitive and rapid technique, but currently is limited by the species-specific nature of the detection antibody in most systems. During attempts to develop generalized assay to detect viral antibodies in a variety of wild bird species, we evaluated 3 EIA protocols with negative or equivocal results. A fourth EIA which used a polyvalent detector antibody in an indirect protocol currently is being developed. After thorough evaluation, this EIA should detect antibodies against any viral antigen in the sera of California bird species. This test will be useful in assessing the seroprevalence of antibodies in wild birds to focus vector control measures and research on virus transmission in time and space.

Western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses continue to be a public health and veterinary problem in California. Early epidemiological studies by Reeves and Hammon (1962) identified wild birds in several orders as reservoir hosts in the enzootic transmission cycle of both viruses. Although not critical to virus maintenance or amplification, chickens were found to be infected frequently in endemic areas, and a sentinel chicken surveillance program was designed to monitor the intensity of enzootic transmission. An enzyme-linked immunosorbent assay (EIA) was developed to allow the rapid, sensitive and cost effective detection of WEE and SLE antibodies in sentinel chickens (Reisen et al. 1993). However, this assay was not useful in testing sera from a variety of wild bird species, because of the species-specific antibody requirements of the test and the realization that serum proteins differ widely among bird species.

The detection of antibodies to WEE and SLE in sera from wild birds continues to be useful in surveillance and ecological studies on virus persistence and amplification. Currently the hemagglutination inhibition test (HI) or plaque reduction neutralization test (PRNT) are used widely to detect viral antibodies in wild bird sera (Gruwell et al. 1988, McLean et al. 1988). The HI test is cumbersome, time consuming, and lacks specificity, especially in low titered sera, that may lead to reporting false positives. The PRNT test is the sensitive and specific "gold standard" assay, but it is expensive and not suited for general use, because it requires virus growth in cell culture within BL3 level containment facilities.

The objective of the present report is to describe briefly our research efforts towards the development of an EIA protocol that will detect antibodies to WEE and SLE viruses in a variety of wild bird species.

TEST PROCEDURES

Definitions: The indirect immunofluorescence assay (IFA) and indirect EIA are the most widely used serological methods for the detection of viral antibody in vertebrate sera. Both assays are considered "indirect", because the binding of the primary antibody to the known viral antigen is detected by a second antibody directed against the species that produced the primary antibody.

Therefore, indirect tests by definition are species-specific; e.g., the primary antibody may be unknown sera from chickens, and the secondary antibody, anti-chicken antibody raised in rabbits. The secondary antibody has attached to it, through the conjugation process, an enzyme such as horse radish peroxidase. Color is generated quantitatively by the reaction of the substrate and the enzyme conjugated to the detector antibody. The amount of conjugated secondary antibody bound to the primary antibody is measured by the amount of substrate that the enzyme degrades. The color change is quantified using an EIA plate reader that measures light absorption of solutions within individual wells. A sample is considered positive when the absorbance is ≥2X the optical density of the negative control. This method currently is used to detect WEE and SLE antibody in chicken blood spots by the Encephalitis Virus Surveillance (EVS) program.

Test Parameters: The optimum incubation times and temperatures for each test system are determined largely by trial and error. In our laboratory, we coat the plates overnight at 4C with tissue culture antigen, diluted in coating buffer at pH 9.6. Serum and conjugate incubations are for 1 hour, and 30 minutes, respectively, at 37C. Shorter times tend to yield less accurate results. Washing procedures also are critical, because it is important that all wells are treated in exactly the same way.

We use horse radish peroxidase as an enzyme marker because it has high activity with the chosen substrate (ABTS) that produces a dark green color reaction that may be assessed in a Bio-Tex EIA reader at 405 NM wave length. (The choice of enzyme-substrate system is largely a matter of personal preference). Standardization is the key to good EIAs, and controls consisting of known negative and positive sera should be used on each plate. In our laboratory, crude Vero cell tissue culture viral antigens are inactivated prior to use. Activity of the antigen is assessed by a "checker board" titration. Antigen titration is carried out against both positive and negative sera to determine the antigen dilution giving the optimum specific discrimination against the positive and negative sera.

EIA results are expressed as absorbance values. The range of "background" or "negative control" absorbance determined from sera from an uninfected animal. A value indicative of antibody presence is determined from sera from a known infected animal. Samples usually are classified as positive if they are 2-3X the negative control value, and negative if they are at or below this threshold.

ASSAYS EVALUATED

Indirect EIA: Our first experiments evaluated the indirect EIA used to detect WEE and SLE antibodies in sentinel chickens by the EVS program (Table 1). Unfortunately, the goat anti-chicken conjugated antibody did not cross react or "recognize" sera from pigeons or house sparrows known to have antibodies to WEE and SLE viruses. Clearly, if the an indirect EIA was going to be used, we would have to develop a broadly reactive or polyvalent detector antibody.

Blocking EIA: The blocking EIA protocol of Hall et al. (1995) as modified by E.D. Walker (Michigan State University, personal communication) measures quantitatively the inhibition of the mouse monoclonal-viral antigen reaction by positive sera from any vertebrate species (Table 1). Hall et al. (1995) suggested that a threshold of 20% inhibition of monoclonal binding was indicative of a positive reaction. Therefore, positive WEE or SLE sera of any vertebrate species should inhibit binding between specific monoclonal antibody and its homologous antigen. However, our available monoclonals were not strongly competitive and this procedure did not work well in our hands. We did not see consistent and appreciable increase from 0% inhibition for negative sera, when known positive serum was tested.. When we had marginal success, the difference from the negative controls was insufficient, and we concluded that this procedure was not adequate, even as a screening assay, using available monoclonals.

Modified antigen capture EIA: The antigen capture EIA of Tsai et al. (1987) uses a *Flavivirus*-specific monoclonal antibody to detect a SLE viral antigen in either vertebrate tissues or mosquito pools. In our modification of this procedure, plates initially were coated with unknown bird sera, followed by a specific WEE or SLE antigen (Table 1). If the unknown sera contained specific WEE or SLE antibody, then the "captured" antigen should be detected by anti-virus antibody produced in mice. Antibody conjugated with the enzyme horse radish peroxidase must be reactive with the detector antibody, but not with the unknown sera used to coat the plate. Results using this procedure were variable. Chickens, pigeons, house sparrows and house finches gave acceptable results, but other taxa such as black birds did not. In addition, we were not able to establish good agreement between the HI titers in known positive and negative bird sera and absorbance values in this EIA.

Generic indirect EIA: We have contracted with a Biotech company to produce a polyvalent IgG in rabbits from the sera of normal white crowned sparrows, ring-necked doves, domestic ducks and chickens. Hopefully, this antibody will be broadly reactive and cross-react with or "recognize" sera from any wild bird species. This rabbit anti-bird antibody will be affinity purified, conjugated with horse radish peroxidase, and biotinylated. This reagent should overcome the species specificity in the second or "detector" antibody in the indirect procedure currently used for chicken sera. When completed, this new procedure should detect antibodies to any viral antigen in any bird species.

The key to any good surveillance system is rapid and economical laboratory testing, using specific and sensitive methods to provide accurate results in a timely manner. Compared to the HI and the PRNT, our new EIA should be extremely valuable for rapidly screening large numbers of wild bird sera from mixed species. Serological methods have played a useful role in the diagnosis and epidemiological assessment of the encephalitidies. A method other than the more cumbersome HI for the large scale monitoring of wild bird seroprevalence by field workers without BL3 containment is needed urgently.

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LITERATURE CITED

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

Hall, R. A., A. K. Broom, A. C. Hartnett, M. J. Howard and J. S. Mackenzie. 1995. Immunodominant epitopes on the NS1 protein of MVE and KUN viruses serve as targets for a blocking ELISA to detect virus-specific antibodies in sentinel animal serum. J. Virol. Meth. 51 :201-210.

McLean, R. G., J. P. Webb, E. G. Campos, J. Gruwell, D. B. Francy, D. Womeldorf, C. M. Myers, T. H. Work and M. Jozan. 1988. Antibody prevalence of St. Louis encephalitis virus in avian hosts in Los Angeles, California, 1986. J. Am. Mosq. Control Assoc. 4 :524-528.

Reeves, W. C. and M. Hammon. 1962. Epidemiology of the arthropod-borne viral encephalitides in Kern County, California, 1943-1952. Univ. Calif. Berkeley Publ. Publ. Hlth. 4 :1-257.

Reisen, W. K., J. Lin, S. B. Presser, B. Enge and J. L. Hardy. 1993. Evaluation of new methods for sampling sentinel chickens for antibodies to WEE and SLE viruses. Proc. Calif. Mosq. Vector Contr. Assoc. 61 :33-36.

Tsai, T. F., R. A. Bolin, M. Montoya, R. E. Bailey, D. B. Francy, M. Jozan and J. T. Roehrig. 1987. Detection of St. Louis encephalitis virus antigen in mosquitoes by capture enzyme immunoassay. J. Clinical Microbiol. 25 :370-376.

Table 1. Summary of EIA test protocols evaluated for their ability to detect WEE and SLE antibodies in the sera of wild birds.

EIA test procedures

Chicken indirect	Blocking	Ag-Capture	General indirect
virus ag	virus ag	bird sera	virus ag
wash	wash	wash	wash
block	block	block	block
bird sera	bird sera	virus ag	bird sera
wash	mouse MAB	wash	wash
conjugated rabbit anti-chick	wash	mouse MAB	conjugated polyclonal rabbit anti-bird antibody
wash	conjugated goat anti-mouse	wash	wash
ABTS	wash	conjugated goat anti-mouse	ABTS
	ABTS	wash	
		ABTS	

reagent (boxed entries)

ag = viral antigen

MAB = monoclonal antibody in mice

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HOST SELECTION BY *CULEX TARSALIS* AROUND THE MARGIN OF THE SALTON SEA IN THE COACHELLA VALLEY

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ABSTRACT

Two years of sampling blood engorged *Culex tarsalis* from walk-in red boxes (WIRB) and CO_2 traps, resulted in data indicating a predominance of passeriform hosts (WIRB-65%; CO_2-56%) followed by rabbits (WIRB-23%; CO_2-38%). Remaining taxa included unknown birds, unknown mammals, canines, galliforms, and columbiforms.

Knowledge of host selection by vector mosquitoes is critical to understanding encephalitis virus infection rates among vertebrates as well as virus geographical distribution. These data are especially useful in our studies of avian infection in the Coachella Valley. This is because the bird fauna is diverse, but our sampling methodology is selective for bird species collected in mist nets and ground traps. Information on host selection by mosquitoes in the desert biome of southeastern California is limited to Gunstream et al.(1971) who tested blood engorged females collected by New Jersey light traps near ranches in Imperial County. The purpose of our research was to 1) survey the host selection patterns of *Culex tarsalis* at habitats along the margin of the Salton Sea, and 2) determine if these patterns changed over time similar to those recorded in the Central Valley (Reeves 1971).

MATERIALS AND METHODS

Blood engorged females were collected from February 1995 to October 1996 from 5 walk-in red boxes (WIRB) located within 150 meters of the shore of the Salton Sea. CO_2 baited CDC style traps were operated near each site as a part of a regular one mile sample grid of 63 traps. Blood meals from *Cx. tarsalis* were identified to order by a microprecipitin test (Tempelis 1975).

RESULTS

The total number of blood meals tested from all sites and methods was 645; 101 from CO_2 traps and 544 from WIRBs. Host selection by *Cx. tarsalis* collected by these methods was similar, with 56% passeriform bird and 38% rabbit from CO_2 traps and 65% passeriform bird and 23% rabbit from WIRBs.

Based on females collected from WIRBs at two sites, passeriform birds were the major host (68%), followed by rabbits (23%) and unknown birds, unknown mammals, and canines (3% each) and galliform and columbiform birds (<1%). These percentages were stable temporally when statistically relevant numbers of engorged females were collected.

There appeared to be an interesting negative temporal relationship between the percentage of resting females that were engorged and host seeking abundance, as measured by CO_2 trap. This may be the result of factors such as autogeny or mosquito avoidance by intolerant hosts.

We presently are conducting a survey of bird populations around the margin of the Salton Sea. Although our collection methods favor Passeriformes, Columbiformes and Galliformes, they do sample roughly the same habitats as the location of WIRBs. The predominance of Passeriformes in these habitats could explain the high proportion of

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passeriform blood meals in resting *Cx. tarsalis* in WIRBs.

Questions to be addressed by statistical examination of this data and further bird serosurveys include: 1) Does *Cx. tarsalis* actively seek a specific host group or do they opportunistically feed on hosts encountered within specific habitats? and 2) How do environmental factors, such as vegetative ecotones and topographical features such as waterways, influence the patterns of host selection?

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REFERENCES CITED

Gunstream, S.E., R.M. Chew, D.W. Hagstrum and C.H. Tempelis, 1971. Feeding patterns of six species of mosquitoes in arid southeastern California. Mosq. News, 31: 99-101.

Reeves, W.C. 1971. Mosquito vector and vertebrate interactions: the key to maintenance of certain arboviruses, 223-230 In: Fallis, A.M. (editor). The ecology and physiology of parasites. Toronto Univ. Toronto Press.

Tempelis, C.H. 1975. Host-feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. J. Med. Entomol. 11: 635-653.

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VERTICAL TRANSMISSION OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS IN *AEDES DORSALIS* MOSQUITOES IN CALIFORNIA

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The first evidence of natural vertical transmission of western equine encephalomyelitis virus (WEE) was found by Fulhorst and associates (1994) who isolated three strains of WEE from *Aedes dorsalis* collected as larvae from a salt marsh in Morro Bay, San Luis Obispo County, California, in 1991 and 1992. However, further evidence to support the hypothesis that WEE can be transmitted vertically by *Ae. dorsalis* is lacking. Field studies conducted in 1994 -1995 in Morro Bay failed to produce any WEE isolates from 1,023 pools of 44,089 *Ae. dorsalis* collected as immatures and 217 pools of 9961 *Ae. dorsalis* collected as adults. No WEE isolations were made from 17 other mosquito species, including *Culex tarsalis* collected in Morro Bay. In contrast, WEE was demonstrated to be active in the Coachella Valley during 1995 as evidenced by seroconversion in sentinel chickens; but virus was not isolated from 215 pools of 9,848 *Ae. dorsalis*, and two groups of a total of ten sentinel rabbits, upon which *Ae. dorsalis* predominantly feed, did not produce antibody to WEE. These data indicate that *Ae. dorsalis* was not involved in WEE transmission in either location. Therefore, we conducted a series of laboratory studies to evaluate the vertical and horizontal vector competence of *Ae. dorsalis* for WEE. Field-collected *Ae. dorsalis* from Morro Bay were intrathoracicly inoculated with either DAV 3340 or DAV 5875, isolated in Morro Bay in 1991 and 1992, respectively. As a control, *Ae. dorsalis* females from the Ft. Baker colony, a long established laboratory colony, were inoculated with DAV 5875. One hundred percent of the inoculated parents were infected, and contained $\geq 10^{7.0}$ plaque forming units (PFU) per mosquito at the time of oviposition. None of 141 pools of 2,166 individual progeny was infected with DAV 3340, as was none of 276 pools of 3,713 individual progeny with DAV 5875. Similarly, none of 120 pools of 1606 individual progeny of control *Ae. dorsalis* colony mosquitoes infected with DAV 5875 was infected. Therefore, we were unable to achieve vertical transmission of WEE in the laboratory.

To evaluate horizontal transmission of WEE, *Ae. dorsalis* reared from larvae collected in Morro Bay were fed on ten-fold dilutions of virus on pledgets soaked in a mixture of virus-defibrinated rabbit blood-2.5% sucrose. After 11 days extrinsic incubation at 25°C, individual mosquitoes were tested for their ability to transmit virus using the *in vitro* capillary transmission assay, then frozen for subsequent determination of the median infectious dose (ID_{50}). The ID_{50} for DAV 3340 was 2.4 $\log_{10}$ PFU per 0.1ml. Following feeding on 4.2 and 3.2 $\log_{10}$ PFU WEE, 3/18 (17%) and 4/6 (67%), respectively, infected *Ae. dorsalis* transmitted virus perorally. The transmission rates increased with decreasing dosage, and mosquitoes which fed on the highest concentrations of virus well above the ID_{50} were unable to transmit virus. The ID_{50} for DAV5875 was <2.6 PFU per 0.1 ml (an endpoint was not reached). Only 1/20 (5%) of *Ae. dorsalis* infected with high concentrations of DAV 5875 transmitted virus perorally; mosquitoes infected with lower concentrations of virus, i.e., closer to the ID_{50}, were not tested for ability to transmit. Therefore, *Aedes dorsalis* from Morro Bay were competent vectors of sympatric strains of WEE, but further studies need to be done to clarify the transmission results.

We are currently in the process of sequencing the E2 portion of the viral genome to determine genetic relatedness between the vertically transmitted isolates from Morro Bay, and two viral strains isolated from *Cx. tarsalis* in the Coachella Valley.

REFERENCES CITED

Fulhorst CF, Hardy JL, Eldridge BF, Presser SB, Reeves WC, 1994. Natural vertical transmission of western equine encephalomyelitis virus in mosquitoes. Science 263:676-678

TIME OF HOST-SEEKING BY *CULEX TARSALIS* IN THE COACHELLA VALLEY[1]

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ABSTRACT

A time-segregated sampler that divided a 14 hour catch period into one hour intervals and was baited with bottled CO_2 gas released at the rate of 1.0 or 0.5 liters per minute was operated biweekly at the Dex-O-Tex duck club on the north shore of the Salton Sea, Riverside County, California, from May 1994 through November 1995. The trap was operated on nights without rain or excessive wind. Temperature and relative humidity were recorded hourly by a temperature logger. Regardless of temperature, humidity or population relative abundance (total females collected per trap night), *Culex tarsalis* Coquillett always commenced host-seeking at sunset, continued for 1-3 hours, declined during the midnight and morning hours, and then ceased just after sunrise. During August and September host-seeking commenced at sunset, but continued at the same level until dawn, perhaps indicating that females were not able to readily locate vertebrate blood-meal hosts during this time of the year when bird abundance was low along the Salton Sea. These data indicated that adulticides would be most effective if applied during the first 3 hours after sunset, a time period when the potential risk of encephalitis virus transmission was greatest.

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REFERENCES CITED

Reisen, W.K., H.D. Lothrop and R.P. Meyer. 1997. Time of host-seeking by *Culex tarsalis* (Diptera: Culicidae) in California. J. Med. Entomol. 34:430-437.

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TALE OF TWO SPECIES: THE INTERDEPENDENCE OF MOLECULAR GENETIC AND BIOLOGICAL STUDIES FOR CHARACTERIZING MEMBERS OF THE *ANOPHELES QUADRIMACULATUS* AND *ANOPHELES CRUCIANS* SPECIES COMPLEXES

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Characterizing the role of *Anopheles* sibling species as vectors of malaria requires both molecular genetic and ecological studies. Many apparent morphological *Anopheles* mosquito species, including *Anopheles quadrimaculatus*, the principle vector of malaria in eastern North America, are species complexes and molecular genetic techniques provide the methods for identifying and characterizing species complexes and distinguishing sibling species in ecological studies. Field based biological and ecological studies are required because biological and ecological differences between sibling species cause them to differ in their capacity to participate in the transmission of malaria and other mosquito-borne pathogens. Field studies of species complexes also provide the opportunity to study how closely related species coexist and can help to elucidate mechanisms involved in the evolution of mosquitoes and other organisms.

Previous genetic studies were the framework for studying the biology and ecology of sympatric sibling *An. quadrimaculatus* species in northern Florida. In addition, we used biochemical and molecular methods to identify mosquitoes to species because *An. quadrimaculatus* sibling species are not distinguishable using the characters in currently available taxonomic keys. While surveying aquatic habitats for *An. quadrimaculatus* larvae, we found larvae to be abundant in many habitat types and observed differences in the larval ecology of sibling species A, B and C1. Species A larvae occurred in a wide variety of habitats but was most abundant in permanent aquatic habitats in association with mats of floating and emergent vegetation. Species B was most abundant in semi-permanent cypress ponds in association with small floating plants, and species C1 occurs most often in temporary ground pools in intermittently flooded swamps. Species C1 was collected exclusively from intermittently flooded swamps and has adapted to this habitat by persisting in the soil as fully embryonated eggs during dry periods, and then hatching in response to a compound that occurs in this habitat, shortly after ground pools form.

Ecological studies can also form the basis for conducting molecular studies of *Anopheles* mosquitoes. *Anopheles crucians* Weideman, like *An. quadrimaculatus*, has a broad geographic and ecologic distribution. In our larval surveys, we collected large numbers of *An. crucians* sensu stricto from many habitat types. High abundance and the diversity of larval habitats utilized by this mosquito were similar to the conditions observed for *An. quadrimaculatus* complex mosquitoes and suggest that *An. crucians* s. s. also is a species complex. Further ecological evidence for an *An. cruscians* s.s. species complex was obtained when we determined that *An. crucians* populations in intermittently flooded swamps, like *An quadrimaculatus* species C1, have adapted to the habitat by surviving as fully embryonated eggs in the soil during dry periods. This adaptation suggests that this swamp habitat population is genetically distinct from *An. crucians* populations in other habitats. We tested this hypothesis by comparing mt DNA and r DNA restriction fragment polymorphisms (RFLPs) of *An.*

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crucians collected as larvae from swamps and other habitats. The RFLPs for larvae from swamps were distinct, indicating a degree of DNA sequence divergence consistent with the swamp population being a separate *An. crucians* species.

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AN UPDATE OF THE COACHELLA VALLEY MOSQUITO AND VECTOR CONTROL DISTRICT AFRICANIZED HONEY BEE PROGRAM

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The African Honey Bee (AHB) has made minimal migration into the Coachella Valley with only two finds (October 1995 and March 1996). The District has been active in forming procedures for an effective public awareness and control program for AHB through the following: 1) an extensive trapping and monitoring program, 2) identification for AHB, 3) seven-days a week response to bee calls, and 4) public education.

The Africanized Honey Bee (AHB), is anticipated to be a permanent resident and has considered the Coachella Valley "home" for over a year. The bees were first trapped in October 1994 by Riverside County Agriculture officials. AHB were found and screened by Coachella Valley MVCD (the District) again in March 1996. To date, AHB have been identified 39 times in the state, seven times in Riverside County, and twice in the Coachella Valley. The District has been forming and maintaining the following programs aimed at reducing the risk of AHB: 1) maintain an extensive trapping and monitoring program, 2) provide bee identifications, 3) respond seven days a week for the bee calls, and 4) create public awareness through education.

Trapping and Monitoring. The AHB trapping program was acquired from the Riverside County Agricultural Commissioner's Office on July 1, 1996. Since the transition, the number of traps in service was increased from 30 to a total of 53 throughout the 2400 square miles the district services. Trap locations have been changed and new sites were established to be more suitable for trapping the bees. The traps are placed in discreet areas, normally unseen by people to prevent any human contact with bees, as well as, securing the traps from being tampered with or destroyed. Twenty of the 53 trap sites encompass the north and west portion of the Salton Sea. Thirty traps are located within the Northwestern (mainly urban areas) areas of the Valley, and three traps at the eastern area of the Riverside County, along Interstate 10, near the Joshua Tree National Monument entrance. The distribution of the traps in the eastern border were selected due to the minimal inhabitation and limitation of shade trees in the area. Trap surveillance continues on a bi-weekly basis throughout the year with each trap indicating the District's name and phone number.

Bee Identification. District personnel were trained by state/county officials in morphometric identification (wing length). The District was able to utilize these skills in March 1996, when a feral colony was detected by park rangers at the Salton Sea State Park and reported to the District for abatement. While screening the sample, it was determined that the average wing lengths of ten selected bees was abnormally small, and the sample was immediately forwarded to County Ag, then to California Department of Food and Agriculture, where genetic tests later concluded that the bees were Africanized.

District identification is also offered to the local pest control industry or others who service residential and industrial areas for bees in the Coachella Valley.

Seven Day Response to Bee Calls: District personnel respond to bee calls seven days a week, i.e., the response to the Salton Sea State Park occurred on a Saturday morning. A paging system and a schedule of personnel during nights and weekends allows for a specialist to respond and evaluate any bee situation. The District spray team responds to bee swarm calls on public property, outside of structures. Control of swarms on public property is carried out by at least two staff. Use of a power sprayer located on the responding vehicle or hand can sprayers greatly depends on the size of the swarm, using M-Pede, a registered insecticidal soap, to spray on and subdue the bees. Remnant traps are used to collect the remaining straggler bees.

Courtesy inspections are made to all requesting private and public properties. District personnel, along with the business or home owner, evaluate the structures and perimeter of the property and offer advice in "bee proofing" any entry point by using exclusion methods (ex: caulking, screening openings).

On-site risk analysis is also provided (especially for residents who want information prior to calling a pest control company) to evaluate any bee situation and determine what course of action may be needed. In most cases, people are unaware of the differences between a colony, swarm, or "foraging" bees, and the dangers each situation may pose, if any.

Public Awareness: In November 1996, the District initiated an Africanized Honey Bee Working Group for the Coachella Valley to establish a network between local governmental agencies and to develop a coordinated information and response plan between these agencies for AHB. The first meeting for the working group had 14 agencies attend. School districts, police depts., fire depts., and water district personnel attended. A quarterly newsletter has maintained information between agencies when meetings are not needed. Communication between work group members is mostly dependent upon phone, fax, and the newsletter.

The District provides AHB English and Spanish presentations to any group or organization requesting information. Last February, over 21,000 people visited the District display booth at the Riverside County Fair and National Date Festival.

Seven brochures are distributed by the District focused at AHB. A European honey bee information sheet was recently added, defining the differences between a swarm and colony of bees. To date, all of the calls received from the public with the exception of the find at the State Park, have been related to European bees. Another relatively new brochure focuses at the resort gardeners or the hobbyist gardener, both high-risk populations because of their exposure to ideal nesting sites. Both brochures will be translated into Spanish. The brochures can also be found on a local cable station-advertising channel.

The District can be found on the Internet. Brochures and updated AHB find information can be obtained by viewing the district's "homepage" at http://www.cvmosquito.org. The e-mail address is cvmosquito@cvmosquito.org.

SUMMARY

The District does the following:

(1) Maintains a trapping and monitoring program, to keep District aware of any new AHB migration from the east (Colorado River) and south from Imperial County.

(2) Provides bee identifications for any requesting public or private industry.

(3) Responds seven days a week for any bee call made to the district and suppress bees, where needed, on public property.

(4) Educates public on AHB, to provide facts and information through group presentations, brochures, courtesy inspections, on-site evaluations, and the working group.

It is the District's belief, that by following and providing these services to the public the impact which AHB may pose to the community can be reduced as the bees continue to migrate throughout California.

ACKNOWLEDGMENTS

The authors thank the staff of Coachella Valley MVCD, especially Mario Montez and Branka B. Lothrop, Ph.D. Thanks also to Kriss Costa, Santa Clara Co. VCD, for her continued assistance.

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REMNANT TRAPS AND THEIR USE IN THE ORANGE COUNTY VECTOR CONTROL DISTRICT AFRICANIZED HONEY BEE PROGRAM

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ABSTRACT

A total of 65 pheromone-baited remnant traps were placed following the removal of 59 feral swarms and 6 feral colonies of European Honey Bees (*Apis mellifera linguistica*). Plastic trap containers baited with a combination of Queen Mandibular Pheromone (QMP) and Nasinov Gland Pheromone (NGP) were the most effective traps used to collect straggler bees following treatment of feral swarms and colonies.

As the Africanized Honey Bee (*Apis mellifera scutellata*-AHB) expands its range in California, the removal of feral swarms and colonies will become increasingly important to public safety. Visscher and Khan (1994 unpublished data) used controlled experiments to demonstrate that pheromone-baited remnant traps were an effective method for collecting straggler bees that usually escaped death when either a swarm or colony was destroyed. Using a similarly designed remnant trap as part of the Orange County Vector Control District's AHB Management Program, it was observed that these traps substantially reduced the time to retreat clusters of straggler bees. The pheromone baits (e.g., QMP and NGP) supplied to the traps provided a 2-fold function by 1) effectively calming the bees, and 2) reducing the likelihood of a possible stinging incident. This paper briefly describes the trap designs used in our evaluations and their application to honey bee control. Data also are included on the comparative efficiency of several types of pheromone baits and bait combinations along with observations on various trap placement parameters.

MATERIALS AND METHODS

TRAP DESIGN: The remnant traps used in our evaluations had to be inexpensive, simple to manufacture and deploy, retain a reservoir of M-Pede insecticidal soap, and provide easy attachment of attractant pheromone baits. Two different containers were evaluated in this study. One was a simplified version of Visscher's & Khan's design (1994) of a trap fabricated from a takeout food container. Our trap consisted of the standard white waterproof cardboard container minus the screen ingress (entry) cones. Entry holes were made in the containers either by cutting round 3/4" dia. holes 1" below the lip of the lid on the opposite sides of the container or by removing two of the flaps from the top of the trap (Fig. 1A). Traps were suspended by the wire bails supplied with the containers. The second type was made from 16 oz. white plastic tub, similar to those used for takeout food (Dart R 16-oz. plastic deli cups and lids). Entry holes were made by cutting 1 1/2" "V-shaped" slots into the removable clear plastic lid of the container (Fig. 1B). Plastic traps were suspended by wire coat hangers cut and bent so that the ends fit easily into holes punched into the sides of the containers 1/2" below the top of the container.

Appropriate caution labels were attached to each trap. Pheromone baits were attached to the inside flap of the trap lid with staples. Approximately 6 oz. (to a depth of 1 1/4") of 4% M-Pede solution was supplied to the reservoir of each trap. For each treatment, the trap was hung as close as possible (e.g., within 3 feet) to the original landing site of the swarm.

Both trap designs were inexpensive to produce and easily assembled in less than 5 minutes. The reusable plastic traps were less expensive than cardboard traps ($6.98 for 50 16-oz. plastic deli containers with lids vs. $9.59 for 50 1-qt. cardboard take-out boxes. Furthermore, the use of cardboard traps was limited to a single placement because the M-Pede tended to soak through the cardboard shell, rendering the trap too weak for subsequent reuse. A distinct advantage of the cylindrical plastic traps was the removable lid. This feature facilitated easy

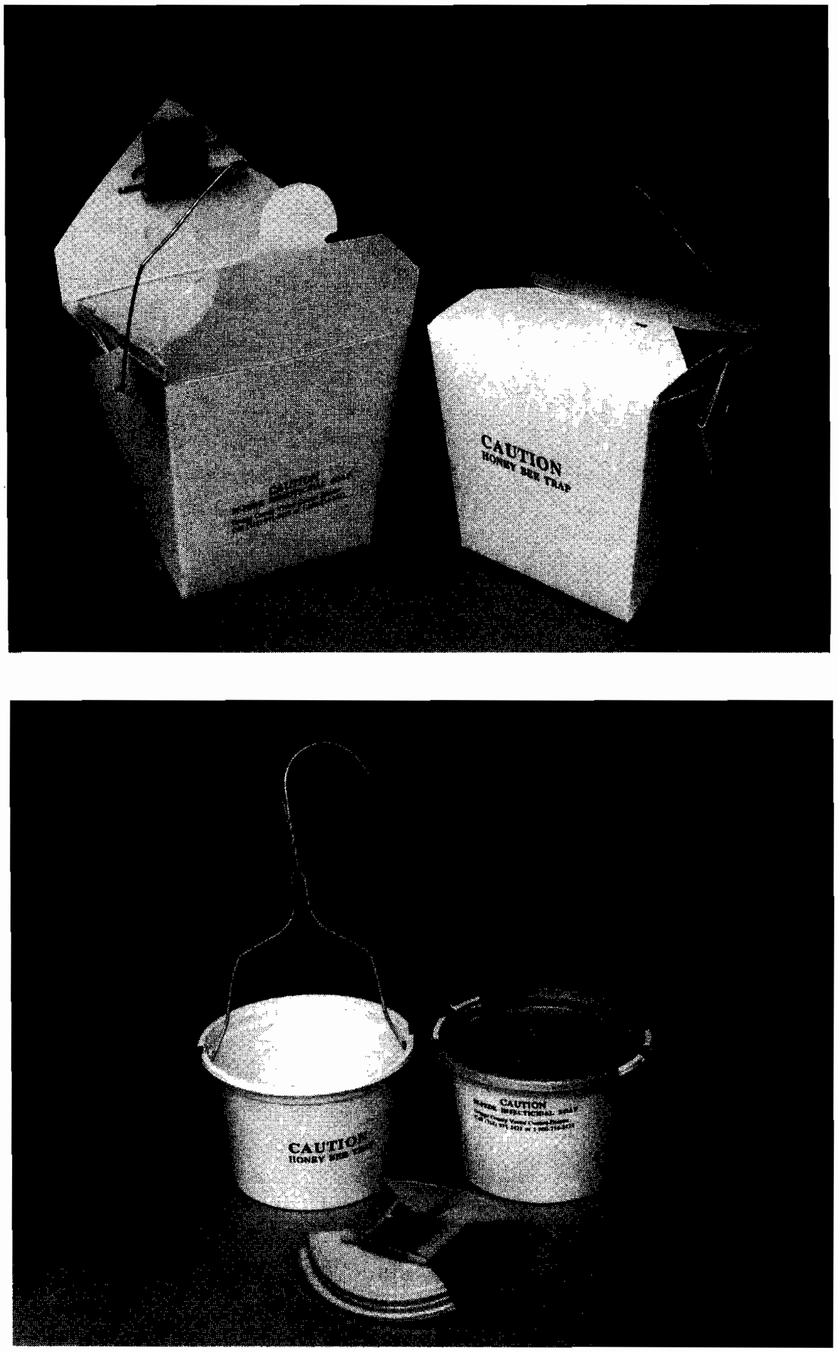

Fig. 1. A: Cardboard "take-out" remnant trap, showing entry holes and attached pheromones. B: Plastic deli carton remnant trap, showing pheromones stapled to removable lid and attached wire hanger.take-out boxes.

attachment of the pheromone baits and the lids could either be removed, stored, or discarded. Prepared traps were stored in rigid closed containers (e.g., empty 3-pound metal coffee cans) to protect them from damage and exposure.

Pheromones: The two attractant pheromones used to bait remnant traps in this study included Queen Mandibular Pheromone (QMP) and Nasinov Gland Pheromone (NGP). QMP is produced by the mandibular glands of queen honey bees and attracts worker bees. NGP is produced in the NGP gland of worker honey bees and functions as an aggregation pheromone. Both of these pheromones were obtained from Phero Tech Incorporated (7572 Progress Way, Delta, B.C. Canada V4G 1E9; Tel: (604) 940-9944, Fax: (604) 940-9944).

QMP was originally impregnated into 2" cotton wicks that released the QMP more quickly, but at a rate that rapidly exhausted the residual pheromone. No attempt was made to standardize the amount of pheromone in queen equivalents (e.g., 1 cotton wick = "x" queen equivalents). QMP currently is available at a strength of 10 Queen equivalents impregnated into 2" lengths of flexible clear plastic tubing (Flex Tube R). This formulation releases pheromone more slowly over a longer period of time.

QMP was stored at -4 C in quantities that likely were to be used within a month (e.g., from 2 to 4 QMP wicks/Flex Tubes) and were transported to the swarm/colony site in airtight empty 35 mm plastic film canisters. NGP pheromone (ca. 0.5 ml/treatment) was stored in 0.5-ml snap-top polypropylene-type vials, kept sealed until attachment to a trap, and transported into the field in a plastic 35mm film canister. NGP Combined QMP/NGP formulations were used in 26 of 65 trap placements.

Swarm/Colony Size: A small swarm is described as softball size or smaller and estimated to contain 5,000 or fewer bees. These were to most often observed in early summer and late fall. A medium swarm was considered to about the size of a football or volleyball and has between 5,000 and 20,000 bees. Large swarms were described as basketball-sized and contained upwards of 20,000 bees. Predominately large swarms were encountered in May and June at the peak of the swarm season (Ross, et al. in press.).

Trap Placement and Retrieval: Over a 6-month period between April and October 1996, 65 remnant traps were deployed following the removal of 59 feral swarms and 6 feral colonies of honey bees treated as needed with M-Pede insecticidal soap applied as a 4% solution (5 fluid-oz. M-Pede/gallon of water). Remnant traps were placed within 3 feet (ca. 1M) of a treated swarm site in 58 of the 59 replicates and more than 50 feet (ca. 16M) from a single swarm site. Traps were left overnight and retrieved the following morning. Collections of trapped bees were returned to the District where the number of trapped bees was documented. Counts included bees collected both on the inside and outside of the trap (Fig. 2).

Fig. 2. Remnant trap with bees.

RESULTS AND DISCUSSION

Swarm/Colony Size and Trap Efficiency: Over the six-month period, the 65 traps collected an average of 367 bees per trap with a low of no bees collected to a high of nearly 2,500 individuals. Within each of the swarm size categories evaluated there was considerable variation in the total number of bee trapped between replicates (Table 1). Overall, remnant traps collected more bees from large versus small swarms. Remnant traps set out after removal of large swarms collected a mean of 441 bees per trap (N=21). Traps placed after removal of medium- and small-sized swarms collected a mean of 429 (N=20) and 216 (N=18) per trap, respectively.

Remnant traps also were effective for collecting foraging bees following removal of their colony (Table 2). By placing a remnant trap close to the site of their colony before beginning the post-treatment clean-up, the trap appeared to "calm" (casual observation) the bees which expedited subsequent trapping success. The largest numbers of bees (627-712) were collected when an entire colony was removed (e.g., exposed comb colonies found in trees) and a trap placed nearby to provide an alternative aggregation site.

Table 1. Effect of trap type on the number of bees collected from each swarm size and colony.

Trap Type	Swarm Size or Colony	N	Mean # Bees/ Trap (Range)
Plastic Pint	L	9	593.67 (703-2434)
Cardboard	L	11	329.82 (0-1191)
Plastic Pint	M	7	342.57 (134-742)
Cardboard	M	13	476.15 (32-1178)
Plastic Pint	S	2	371.00 (285-457)
Cardboard	S	16	197.10 (5-425)
Plastic Pint	C	3	398.67 (8-627)
Cardboard	C	3	306.67 (75-712)

Pheromone Treatments: The most striking results we observed were the attractiveness produced by the various pheromone treatments and combinations supplied to the remnant traps. All of the pheromone formulations were effective in attracting bees with some treatments clearly more efficacious than others (Table 2). QMP was more effective when presented in the cotton wick than in the plastic Flex Tube form. The cotton wick releases QMP more quickly than the Flex Tube, and is thus

Table 2. Remnant Trap results following treatment and removal of 6 colonies.

Trap placed (date + 24-hr time)	Deployment time (hrs)	Trap Type	Qty of QMP (whole or half wick)	Qty of NGP	# of Bees in Trap	Was original site removed?
9-05-96 1300	20.25	PLASTIC	0	1	8	N
5-29-96 1100	20.5	CARDBOARD	1	0	75	N
5-20-96 1100	22	CARDBOARD	0.5	1	133	N
8-23-96 0930	71	PLASTIC	1 FLEX TUBE	1	561	N
10-10-96 1200	119	PLASTIC	1 FLEX TUBE	0	627	Y
6-26-96 1100	21.5	CARDBOARD	1 FLEX TUBE	1	712	Y

more potent over a shorter period of time. The cotton wick, if kept dry and stored in an airtight container, may be used for 1-2 trap placements, whereas the Flex Tube handled similarly, may be used in as many as 3 placements.

Both QMP and NGP used alone were less effective than in combination (Table 3). The 1/2 cotton wick of QMP collected a mean of 218 bees/trap, but when NGP was added, collection size increased dramatically to a mean of 419 bees/trap. A sizable increase in the number of bees trapped also occurred when full cotton wicks or QMP Flex Tubes were used in combination with NGP.

Table 3. Effect of varying pheromone combinations on the number of bees collected.

Quantity of QMP	Qty of NGP	# of Traps	Avg. # Bees/ Trap	Range
0.5 cotton wick	none	5	218.2	0-322
1 cotton wick	none	19	313.74	5-1191
1 Flex Tube	none	10	312.7	101-703
none	1	4	211.75	8-285
0.5 cotton wick	1	3	418.67	133-970
1 cotton wick	1	4	694	147-1178
1 Flex Tube	1	18	450.33	37-2434
2 Flex Tubes	1	2	344.5	283-406

Trap Type: Plastic traps were used in 21 of the 65 placements and collected a mean of 460 bees/trap while the cardboard type traps used in 43 of the 65 placements only collected a mean of 345 bees/trap. Excluding medium-sized swarms, the plastic traps routinely collected more bees than cardboard traps independent of pheromone treatment (Tables 1 & 4). This difference may be attributed to a variety of factors, however the more slippery sides of the plastic traps may have cased more bees to loose their grip and fall into the M-Pede.

Table 4. Effect of trap type and pheromone on the number of bees collected.

QMP	NGP	Trap Type	N	Mean
Cotton Wick	none	Plastic	0	N/A
Cotton Wick	none	Cardboard	23	303
Cotton Wick	1	Plastic	1	147
Cotton Wick	1	Cardboard	5	750
Flex Tube	none	Plastic	5	324
Flex Tube	none	Cardboard	4	220
Flex Tube	1	Plastic	11	584
Flex Tube	1	Cardboard	7	156
None	1	Plastic	1	285
None	1	Cardboard	1	272

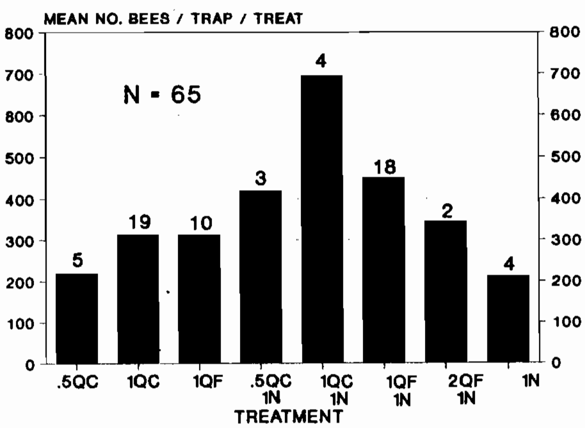

Fig. 3. Effectiveness of varying combinations of QMP and NGP in remnant traps. Numbers above each bar represent the number of replicates for that treatment.

SUMMARY

The purpose of remnant trapping is to reduce the likelihood of a stinging incident following insecticidal treatments and removal of honey bee swarms and colonies. Remnant traps accomplish this function by "calming" the remaining bees, aggregating the bees for easy removal, and minimizing the necessity of retreating clusters of straggler bees the day following treatment. In addition, placing the traps immediately after treatment and before clean up activities begin tended to increase the acceptance of the trap plus effectively reduced the chances of the bees aggregating in a less desirable location.

When placing remnant traps, we discovered that it was not critical for the bees to enter the trap. In most instances, a majority of the trapped bees did not actively enter the trap, but remained outside clinging to all exposed surfaces. These bees were easily collected by simply placing the entire collection (trap) into a plastic bag, sealing the bag, and then shaking the bag vigorously to assure that all the bees became coated with the M-Pede supplied to the inside reservoir of the trap.

The simplicity of our trap facilitates its use in a wide variety of locations away from busy locations where it is less likely to be disturbed, but still easily retrievable without dislodging the bees that aggregate on the outside of the trap. After placing the trap, residents should be informed of the trap's location to keep children away and to allow the bees to quietly cluster on the trap. Early morning retrieval of traps is recommended because cool morning temperatures reduce activity and decrease the likelihood of provoking the bees when the trap is being removed.

REFERENCES CITED

Ross, C.L., Meyer R.P., Loughner D.D., Shaw S.L., Tapper J.J. 1966. Africanized Honey Bee Program of the Orange County Vector Control District and Experiences with European Honey Bees in Preparation for the Africanized Honey Bee Control. Proc. Mosq. Vector Control Assoc. of Calif: (in press).

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THE DISTRIBUTION OF TICKS IN FOUR DISTINCT HABITATS IN A NATURE PRESERVE IN SONOMA COUNTY, CALIFORNIA

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ABSTRACT

Abundance in populations of adult and nymphal ticks were determined in four distinct habitat types (oak woodland, chaparral, douglas fir, and grassland) at a private preserve in Sonoma County, California. Ticks were collected semi-monthly over a period of one year by standard flagging techniques along two 50 meter transects in each habitat type. Adults of *Ixodes pacificus* and *Dermacentor occidentalis* along with *Dermacentor* spp. nymphs were collected predominantly in chaparral habitat, whereas *Ixodes* spp. nymphs were most prevalent in the oak woodland habitat.

The northern coastal counties of California are a prime habitat for many tick species (Arthur and Snow 1968, Furman and Loomis 1984). Cases of Lyme disease and other emerging tick-borne human diseases, such as babesiosis and ehrlichiosis, have been reported in these counties (Burgdorfer et al. 1985, Jerant and Arline 1993, California Department of Health Services 1994, Vugia et al. 1996). Previous studies on tick ecology in California have primarily focused on only one or two habitat types, on trail, habitat edges or both (Lane et al. 1985, Lane and Loye 1989, Lane and Stubbs 1990, Lane 1990, Kramer and Beesley 1993, Clover and Lane 1995). The present study was conducted to determine the prevalence and seasonal variation of tick species within four distinct habitats typical of the northern coastal counties of California.

MATERIALS AND METHODS

Study Site. Pepperwood Ranch consists of 3,117 acres located 14.5 kilometers northeast of the city of Santa Rosa in Sonoma County, California. Pepperwood is owned by the California Academy of Sciences and offers a diversity of undisturbed ecological communities. The following four habitat types were chosen: (1) oak woodland, (2) chaparral, (3) douglas fir forest, and (4) open grassland. All four habitat types had similar south facing slopes.

The habitat characteristics are summarized in Table 1. Additional detail on these specific habitat types can be found in de Nevers (1985). Due to the presence of serpentine soil in the chaparral site, no manzanita was present. The douglas fir habitat is at the edge of its range and trees were not as tall and thick as a mature stand normally would be.

Tick Collection. Each habitat was divided into two 50 meter transects. Stakes were used to indicate the beginning and end points and every 10 meters of the transect. Tick collection was conducted twice monthly from October 1995 through October 1996. Ticks were collected by sweeping a one-meter square flannel flag through an arc of 180 degrees while walking along the transects. Flags were checked at each 10-meter segment and the number of adult and nymphal ticks flagged were recorded. Adults were identified to species and returned to the same 10-meter section from which they were collected. Nymphs were identified to genus only and returned. Larval ticks were noted but not routinely cataloged. At the time of collection a general description of the weather was noted. At each site the temperature and relative humidity were taken with a Bacharach sling psychrometer (model 12-7011). To assess the

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association between tick abundance and weather factors. Microsoft Excel was used to calculate Pearson correlation coefficients.

RESULTS

Ixodid ticks were collected in all four habitat types. The ticks collected included *Ixodes*, *Dermacentor*, and *Haemaphysalis*, three of the four hard tick (*Ixodidae*) genera established in California (Furman and Loomis 1984). The only established genera not collected was *Rhipicephalus*. A total of 56 *Ixodes pacificus* Cooley & Kohls adults and 163 *Ixodes* ssp. nymphs were collected. The adults were collected between mid-November and mid-May (Figs. 1 and 2). Although *Ixodes* nymphs were collected in every season (Fig. 3), 99% of them were collected between mid-February and early August with greatest activity between early March and early July (Fig. 4).

Table 1. Habitat characteristics at Pepperwood Ranch, Sonoma County, CA.

Habitat	Predominant vegetation	Site orientation	Elevation (meters)
Oak woodland	Live oak California Bay Madrone Poison oak Annual grasses	Southeast	366
Chaparral	Leather oak Chamise Toyon Annual grasses	Southeast	348
Douglas fir	Douglas fir Ferns Annual grasses	Southeast	351
Grassland	Harding Grass Woodland wild rye Soft Chess	South to Southeast	366

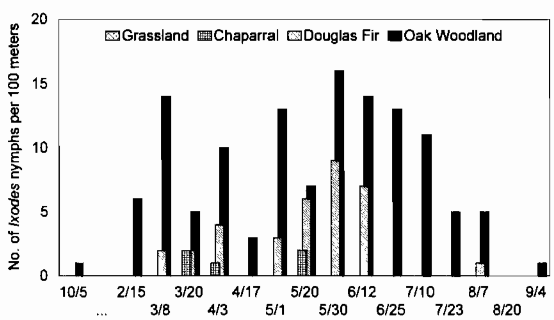

Figure 1. Distribution of *Ixodes pacificus* adults collected at Pepperwood Ranch, Sonoma County, CA, during 1995-96.

A total of 14 adult *Dermacentor occidentalis* Marx and 23 nymphal *Dermacentor* spp. were collected. Adults were collected between February and June (Fig. 5). *Dermacentor* spp. nymphs were collected between early April and early October (Fig. 6).

Oak Woodland. Of the 163 *Ixodes* spp. nymphs collected during the study, 124 (76%) were collected in the oak woodland habitat (Table 2). Of 56 *I. pacificus* adults collected, eight (14%) were from oak woodland. No *D. occidentalis* adults were collected from the oak woodland. Of 23 *Dermacentor* spp. nymphs collected, two (9%) were from this habitat.

Chaparral. Of 70 adult ticks collected, 52 (74%) were from the chaparral habitat. These included 43 *I. pacificus* and nine *D. occidentalis*. Of the 23 *Dermacentor* spp. nymphs collected, 20 (87%) were from this habitat. Only five of the 163

(3.1%) *Ixodes* spp. nymphs recorded during the study were found here.

Douglas fir. Of the 70 adult ticks collected, five (7%) were from the douglas fir habitat. Thirty-four (21%) of the 163 *Ixodes* spp. nymphs were collected here. Only one (4%) of the 23 *Dermacentor* spp. nymphs were collected in this habitat.

Grassland. No nymphal ticks were collected in this habitat type. Only two *I. pacificus* adults and

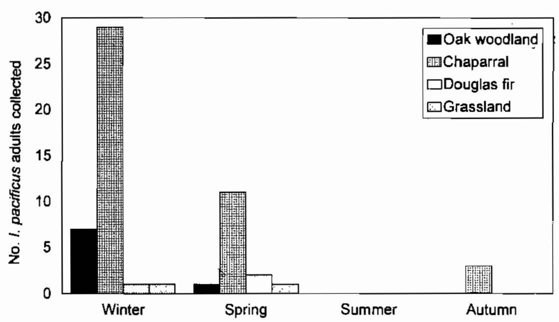

Figure 2. *Ixodes pacificus* adults collected by season at Pepperwood Ranch, Sonoma County, CA, during 1995-96.

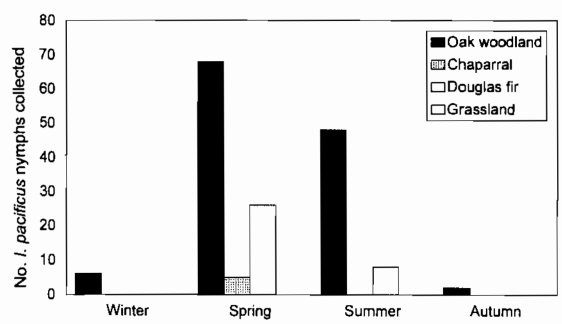

Figure 3. *Ixodes pacificus* nymphs collected by season at Pepperwood Ranch, Sonoma County, CA, during 1995-96.

three *D. occidentalis* adults were collected here.

Although not catalogued, larvae were commonly noted in chaparral and oak woodland sites, but were rare in the grass and douglas fir habitats. At a single collection in October 1995, over 400 larvae were collected in the chaparral habitat. A random sample of 100 was selected for identification; all larvae were *Dermacentor* sp. A single nymph of the rabbit tick, *Haemaphysalis leporispalustris* Packard, was flagged from the chaparral. *H. leporispalustris* is a non-human biting tick that feeds primarily on

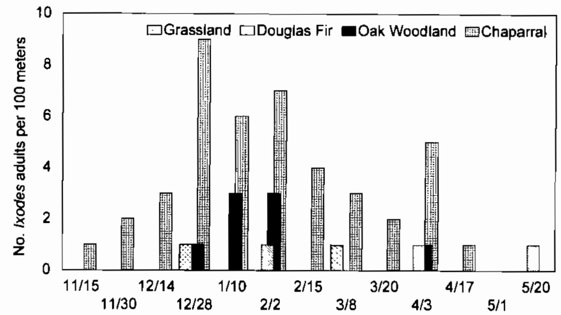

Figure 4. Distribution of *Ixodes* nymphs collected at Pepperwood Ranch, Sonoma County, CA, during 1995-96.

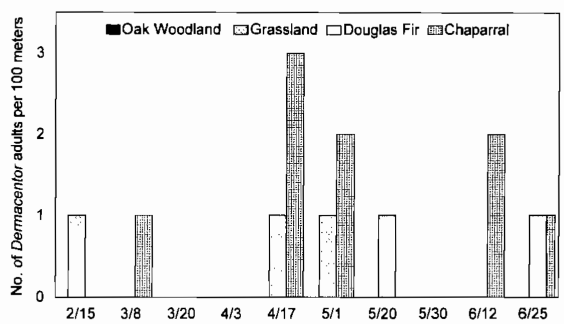

Figure 5. Distribution of *Dermacentor occidentalis* adults collected at Pepperwood Ranch, Sonoma County, CA, during 1995-96.

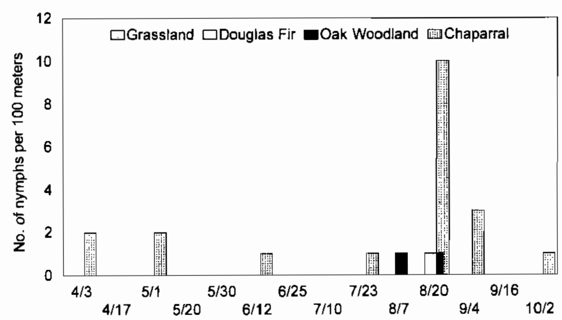

Figure 6. Distribution of *Dermacentor* nymphs collected at Pepperwood Ranch, Sonoma County, CA, during 1995-96.

jackrabbits and other lagomorphs (Furman and Loomis 1984).

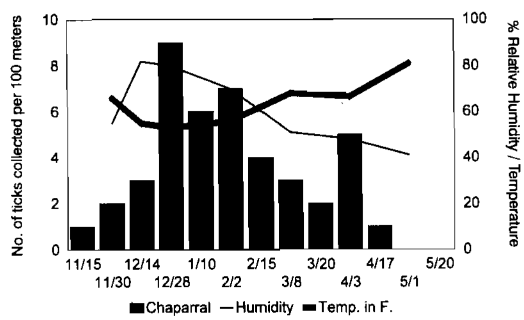

Figure 7. Distribution of *Ixodes pacificus* adults collected in chaparral. Percent relative humidity and temperature in degrees Fahrenheit are also shown.

This was not unexpected as the prime abundance period for both *Ixodes* and *Dermacentor* nymphs is from early April to early September when the grass has become so tall as to not be a questing source. Late in the year (Oct.17 & Dec. 14) when the grasses were still short unidentified larvae were flagged.

Based on our personal experiences and previous studies (Clover and Lane 1995) the oak woodland was expected to exhibit the highest numbers of nymphal *Ixodes*. This was borne out as 76.1% of all *Ixodes* nymphs were found here. The oak woodland habitat was not expected to yield many adult ticks, because the heavy canopy cover existing in this habitat results in an understory with very few

Table 2. Number of adult and nympal stage ticks collected in four habitat types, Pepperwood Ranch, Sonoma County, California, October 1995- October 1996.

	Ixodes pacificus adults	*Ixodes* spp. nymphs	*Dermacentor occidentalis* adults	*Dermacentor* spp. nymphs	Total
Oak woodland	8	124	0	2	134
Chaparral	43	5	9	20	77
Douglas fir	3	34	2	1	40
Grassland	2	0	3	0	5
total	56	163	14	23	256

DISCUSSION

Site orientation is noted in Table 1. Previous studies have shown that tick abundance is significantly higher on south versus north facing slopes. This is true for *D. occidentalis* (Lane et al. 1985) as well as *I. pacificus* (Lane and Stubbs 1990). Our study sites had similar south facing slopes and as such increased our probability for collecting ticks.

The low number of adult ticks collected in this study is likely due to not sampling trail edges or ecotones (Kramer and Beesley 1993). Nevertheless, some trends were apparent. Adult and nymphal stages of *D. occidentalis* and adult *I. pacificus* were collected in greatest numbers from the chaparral habitat. Few *Ixodes* adults and no nymphs from either genera were recorded from the grassland. questing sources for adults. No *D. occidentalis* adults and only eight *I. pacificus* adults were collected in this habitat.

According to Furman and Loomis (1984), only four of the 49 tick species present in California are acquired by humans or domestic pets through contact with infested grass, brush or leaf litter. These are *I. pacificus*, *D. occidentalis*, *D. andersoni* Stiles, and *D. variabilis* Say. Of these, *D. andersoni*, is restricted to a range of northeastern and central eastern portions of the state. Although *D. variabilis* is present in the coastal ranges from northern to southern California, we were unsuccessful in collecting this species. The reason for its absence is uncertain but may be due to our lack of flagging along ecotones or trail edges.

Although the climatic data remain to be thoroughly analyzed, adult *I. pacificus* collections in chaparral were positively associated with humidity (n = 7, r = 0.63) and negatively associated with temperature(n = 7, r = -0.79) (Fig. 7). These data agree with previous studies on questing behavior (Loye and Lane 1988, Lane 1990).

A tick encounter risk factor was tabulated for each habitat by dividing the total ticks collected by the number of flagging dates (i.e., 26). The highest tick encounter risk was calculated for oak woodland at 5.2 per visit, followed by chaparral, Douglas fir and grassland with 3, 1.5, and 0.2 ticks per visit, respectively.

Ixodes nymphs were collected in greater numbers than any other specimen type and were most abundant in oak woodland habitat. This resulted in oak woodland having the highest encounter risk among the habitats studied. Recent studies (Clover and Lane 1995, Lane 1996) have shown that in some areas the proportion of *I. pacificus* nymphs infected with *Borrelia burgdorferi* are three to fourfold the proportion of infected adults. Thus, visitors frequenting oak woodland habitat, especially during the spring and summer (Fig. 3), appear to be at greatest risk for acquiring a tick- borne disease.

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REFERENCES CITED

Arthur, D.R. and K.R. Snow. 1968. *Ixodes pacificus* Cooley and Kohls, 1943: Its life history and occurrence. Parasitology 58:893-906.

Burgdorfer, W., R.S. Lane, A.G. Barbour, R.A. Gresbrink, and J.R. Anderson. 1985. The western black-legged tick, *Ixodes pacificus*: a vector of Borrelia burgdorferi. Am. J. Trop. Med. Hyg. 34:925-930.

California Department of Health Services, 1994. Babesiosis in California: a two-part series. California Morb. 7/8.

Clover, J.R. and R.S. Lane. 1995. Evidence implicating nymphal *Ixodes pacificus* (Acari: Ixodidae) in the epidemiology of Lyme disease in California. Am. J. Trop. Med. Hyg. 53:237-240.

de Nevers, G. 1985. Pepperwood flora. California Academy of Sciences. Golden Gate Park, San Francisco.

Furman, D.P. and E.C. Loomis. 1984. The ticks of California (Acari: Ixodida). Bull. Calif. Insect Surv. 25:1-239.

Jerant, A.F. and A.D. Arline. 1993. Babesiosis in California. West. J. Med. 158:622-625.

Kramer, V.L. and C. Beesley. 1993. Temporal and spatial distribution of *Ixodes pacificus* and *Dermacentor occidentalis* (Acari: Ixodidae) and prevalence of *Borrelia burgdorferi* in Contra Costa County, California. J. Med. Entomol. 30(3):549-554.

Lane, R.S. 1996. Risk of human exposure to vector ticks (Acari: Ixodidae) in a heavily used recreational area in northern California. Am. J. Med. Hyg. 55(2): 165-173

Lane, R.S. 1990. Seasonal activity of two human-biting ticks. Calif. Ag. 44(2):23-25.

Lane, R.S., J.R. Anderson, J.S. Yaninek, and W. Burgdorfer. 1985. Diurnal host seeking of adult Pacific Coast ticks, *Dermacentor occidentalis* (Acari: Ixodidae), in relation to vegetational type, meteorological factors, and rickettsial infection rates in California, USA. J. Med. Entomol. 22(5):558-571.

Lane, R.S. and H.A. Stubbs. 1990. Host-seeking behavior of adult *Ixodes pacificus* (Acari: Ixodidae) as determined by flagging vegetation. J. Med. Entomol. 27(3):282-287.

Lane, R.S. and J.E. Loye. 1989. Lyme disease in California: Interrelationship of *Ixodes pacificus* (Acari: Ixodidae), the Western Fence Lizard (*Sceloporus occidentalis*), and Borrelia burgdorferi. J. Med. Entomol. 26(4):272-278.

Loye, J.E. and R.S. Lane. 1988. Questing behavior of *Ixodes pacificus* (Acari: Ixodidae) in relation to meteorological and seasonal factors. J. Med. Entomol. 25(5):391-398.

Vugia, D.J., E.H. Holmberg, E.M. Steffe, M.S. Ascher, and D. Gallo. 1996. A human case of monocytic ehrlichiosis with Adult Respiratory Distress Syndrome in northern California. West. J. Med. 164:525-528.

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PRELIMINARY RESULTS OF STUDIES OF ATTRACTANTS FOR *AEDES* MOSQUITOES

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ABSTRACT

Towels contaminated with soil from South Carolina saltmarshes were tested for attractancy to gravid female *Aedes* mosquitoes from California. Tests showed that colonized *Aedes dorsalis* and field-collected *Ae. tahoensis* and *Ae. melanimon* were attracted to these substrates for oviposition. In paired and multiple choice tests using towels contaminated with soil from California sites, females from a colonized strain of *Ae. dorsalis* also preferred contaminated towels to moist, uncontaminated controls. Towels contaminated with soils from irrigated pastures were most attractive, while towels contaminated with soil from alpine snowpools were the least attractive. In spite of these differences, evidence of site specificity was not definitive. Field-collected *Aedes melanimon* were attracted to towels contaminated with soil from South Carolina saltmarshes, but no more than to moist soil from California irrigated pastures. Attempts to collect *Aedes* eggs in traps in the field were unsuccessful.

Considerable research has been conducted on factors that attract ovipositing female mosquitoes, but most of this research has involved *Culex* mosquitoes that deposit egg rafts directly on water surfaces or container-breeding mosquitoes such as *Aedes aegypti*. Recently, Wallace (1996) discovered that towels that had been draped over laboratory mosquito cages ("contaminated towels") were highly attractive as oviposition substrates for *Aedes taeniorhynchus*, and further, that such towels could be used as the attractive principle in oviposition traps in nature. We conducted preliminary studies in California between June of 1996 and January of 1997 which were designed to answer the following questions: (1) Are contaminated towels attractive to various species of ovipositing California *Aedes* mosquitoes? (2) Are towels contaminated from California substrates effective as oviposition attractants? and (3) Is there any site specificity for various species of mosquitoes?

MATERIALS AND METHODS

Tests were conducted in the laboratory and field with towels which had been contaminated in South Carolina using the method described in Wallace (1996). In California, towels were contaminated using the following procedure: Soil samples were collected from various sites in California known to contain eggs of some species of *Aedes*. The sites represented were alpine snowpools, irrigated pastures, and coastal saltmarshes. New white bath towels (25 cm by 40 cm) were contaminated with portions of each type of soil sample by placing the towels in a pan containing a water-soil slurry. The slurry consisted of ca. 800 cc of soil mixed with ca. 1.5 l of distilled water. Towels were thoroughly saturated in the slurry, squeezed to remove excess water, then placed in covered plastic trays and stored in an insectary at a temperature of about 28°C. Tests were performed when there was obvious growth of bacteria and fungi on the towels, usually after 2-3 months.

Laboratory tests were performed at the Mosquito Control Research Laboratory at the Kearny Agricultural Center, Parlier, California. by placing gravid female mosquitoes in 61 cm^3 or 30.5 cm^3 cages and providing choices of towels contaminated with various substrates, and towels that had been stored in the insectary but had not been soil-contaminated. In some tests, clean, moist towels were compared with contaminated towels. For testing, sections of towels 25 x 33 cm were placed in 14 cm plastic Petri dishes. In all instances, 50 gravid

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females were placed in cages and the cages placed in incubators at 28°C overnight. In the morning, towels were removed and eggs were removed by rinsing with water into 24, 42 and 100 mesh sieves followed by filtration through paper. Eggs were counted by direct examination of the filter paper under a dissecting microscope. Tests were performed using a colonized strain of *Aedes dorsalis* fed on mice, and females of *Aedes tahoensis* and *Aedes melanimon* that had been collected in the field, then provided human blood. In one test, field-collected *Ae. melanimon* were offered a choice between towels contaminated with soil from a South Carolina saltmarsh and a substrate of moist soil from a California irrigated pasture.

Field tests were done by placing sections of contaminated towels in large plastic trays, and covering the towels with leaves or pine needles to prevent rapid evaporation from the towels. After 24-48 hours of exposure, towels were brought to the laboratory, and eggs were removed and counted as described above.

RESULTS AND DISCUSSION

Tests using South Carolina substrates. Tests with towels that had been contaminated in South Carolina were tested in the laboratory against two *Aedes* species. The results are shown in Table 1. When given a choice for oviposition between a contaminated towel and a moist clean towel, there was a clear preference shown by both *Ae. dorsalis* and *Ae. tahoensis*. In a separate test, field-collected *Ae. melanimon* deposited eggs on contaminated towels, but also deposited approximately equal numbers on a substrate of moist soil from a California irrigated pasture (395 and 329, respectively).

Table 1. Numbers of eggs recovered from South Carolina-contaminated and control towels in laboratory choice tests. Tests for *Ae. dorsalis* and *Ae. tahoensis* represent totals for two replications; *Ae. melanimon* for a single trial.

Species	Contaminated	Control
Ae. dorsalis	9,935	184
Ae. tahoensis	571	45

Field tests using traps containing South Carolina substrates were unsuccessful in trapping large numbers of *Aedes* mosquito eggs. The principal reason for this may have been our inability to locate traps at the time substantial populations of gravid females were present. At no time were large populations of adult mosquitoes noted at the time traps were placed.

Tests using California substrates. Gravid, colonized *Ae. dorsalis* females were presented with a choice of two towels contaminated with three combinations of substrates. These paired tests were performed twice, on January 8 and January 9, in 30.5 cm^2 cages. The results are shown in Table 2.

We also performed multiple choice tests using the same general protocol as the paired tests, but in larger cages (61 cm^3). The results of these tests are shown in Table 3. Among the three substrates, there was a clear preference for towels contaminated with soil from irrigated pastures, with no preference shown between alpine snowpool and coastal saltmarsh substrates. Contaminated towels of any kind were more attractive than moist clean towels or dry clean towels.

These results suggest that gravid female *Ae. dorsalis* will oviposit on towels contaminated by soil from a variety of sites. Irrigated pasture soils were always more attractive than coastal saltmarsh soils, and these, in turn, were always more attractive than soils from alpine snowpools. This may be an effect of the richness of the flora involved, rather than habitat specificity by *Aedes dorsalis*. This strain of mosquito has been in culture for many years, but originated from a coastal saltmarsh. Nevertheless, it preferred towels contaminated with irrigated pasture soils for oviposition.

In summary, we conclude that California *Aedes* mosquitoes are attracted to towels contaminated from a variety of sources, both from California sites or sites in South Carolina. This suggests that the attractive factor is a general one. Although there were differences in attractancy among the different kinds of substrates, it is difficult to argue for specificity based on these preliminary results.

Table 2. Numbers of eggs recovered from towels contaminated with California soils in paired choice tests using colonized *Aedes dorsalis*. Results are from identical trials conducted on two consecutive days, January 8-9, 1997.

Substrate (Contaminated with soil from)	**January 8**	**January 9**
Irrigated pasture vs.	1,670	925
Alpine snowpool	398	412
Coastal saltmarsh vs.	1,361	890
Alpine snowpool	257	472
Irrigated pasture vs.	1,157	1,439
Coastal saltmarsh	458	632

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REFERENCES CITED

Wallace, F.L. 1996. Construction of a field trap for initiating an ovipositional response in *Aedes taeniorhynchus*. J. Am. Mosq. Contr. Assoc. 12:491-493.

Table 3. Eggs recovered from towels in multiple choice tests conducted on December 17, 1996, and January 8, 1997, using colonized *Aedes dorsalis*.

Substrate (Contaminated with soil from)	**Number of eggs**
Alpine snowpool	1,265
Coastal saltmarsh	1,348
Irrigated pasture	2,694
Moist towel (control)	88
Dry towel (control)	0

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SURVIVAL OF TWO LARVIVOROUS FISHES IN A MULTIPURPOSE CONSTRUCTED WETLAND IN SOUTHERN CALIFORNIA

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ABSTRACT

The survival of two species of larvivorous fish was monitored in a multipurpose constructed wetland in southern California. Survival of caged young-of-the-year stickleback (*Gasterosteus aculeatus*) and mosquitofish (*Gambusia affinis*) in two inlet marshes receiving approximately 8-16 X 10^5 liters of secondary-treated effluent day^{-1} differed appreciably; survival in Inlet Marsh 1 was lower than in Inlet Marsh 3. The less stressful conditions in the Outlet Marsh A enhanced the survival of the stickleback. Cage effects attributed to the rapid proliferation of a filamentous green alga, *Hydrodictyon* sp., caused mortality in three of the stickleback cages where prior survival of fish had been nearly 100%. The alga presumably interfered with the ability of the stickleback to surface in response to diel fluctuations in dissolved oxygen concentrations. Diel fluctuations in dissolved oxygen concentrations may limit stickleback reproduction to the large central pond in the wetlands where dissolved oxygen concentrations typically remain above 2 ppm for much of the diel period. Potentially stressful levels of ammonia-derived compounds may occur in the inlet marshes, particularly un-ionized ammonia which increases concomitantly with pH as a function of algal photosynthesis.

Larvivorous fish can greatly aid mosquito abatement efforts by reducing the need for, or frequency of, larviciding activities (Walton and Mulla 1991). Adding larvivorous fish to mosquito developmental sites may reduce the cost of pesticide application/larviciding, lessen the potential for the evolution of resistance by mosquitoes resulting from frequent applications of larvicides, reduce the chance that nontarget species might be adversely affected by larvicide applications, and lessen the chance of disease transmission and nuisance biting by mosquitoes for humans and livestock. However, a recent (Rupp 1996, Gratz and others 1996) and ongoing debate about the merits of using mosquitofish, (*Gambusia affinis*), for mosquito control has focused on a number of issues such as the effectiveness of mosquitofish as a mosquito control agent, the relative effectiveness of the mosquitofish in different habitats, the impact of the non-indigenous mosquitofish on native fishes and ecosystems, whether mosquitofish (or any larvivorous fish) reduces the numbers of host-seeking adult mosquitoes and consequently disease transmission, and alternative larvivorous fishes to the mosquitofish. The use of native larvivorous fishes should be considered as an alternative strategy to releasing mosquitofish, particularly in situations where the conservation of local biodiversity is an issue.

Multipurpose constructed wetlands are likely to play an increasing role in water reclamation strategies in arid regions of the U. S., such as southern California. The uses intended for multipurpose constructed wetlands include the removal of nutrients from reclaimed water, public education, and wildlife conservation (USBR, NBS and EMWD 1994). These wetlands also serve as developmental sites for disease-transmitting and pestiferous mosquitoes and, consequently, effective and environmentally-friendly methods of mosquito control need to be developed. Because multipurpose wetlands are intended to conserve local biodiversity of fauna, the use of exotic fishes for mosquito control is currently discouraged.

The stickleback, (*Gasterosteus aculeatus*), has been suggested as a viable alternative to the mosquitofish because of behavioral, morphological, and physiological factors that are favorable for mosquito control efforts (Walton et al. 1996). Some of the endemic species of fishes that have been identified as candidates for introduction into constructed wetlands (Kadlec and Knight 1996) or as potential candidates for mosquito control in constructed wetlands (USBR, NBS and EMWD 1994) may fail to proliferate under certain conditions associated with constructed wetlands, such as low dissolved oxygen concentrations, occasional high levels of un-ionized ammonia, etc. Results from preliminary studies suggest that the stickleback might not be an effective mosquito control agent in constructed wetlands (Walton et al. 1996); however, the effectiveness of the stickleback as a mosquito control agent in such wetlands needs to be compared to that of the mosquitofish. A joint introduction of both fish might achieve two goals: effective mosquito control and the enhancement of a native fish species.

Here, we examine the suitability of constructed wetlands for two species of larvivorous fish. We report on the survival of sentinel stickleback and mosquitofish in cages and on the physical and chemical factors potentially affecting larvivorous fish populations in a multipurpose constructed wetland located in southern California.

MATERIALS AND METHODS

Water Quality: Water quality parameters in the open water and in the vegetation near the sentinel cages were measured continuously for five day intervals using two recording water quality sensor arrays. The probes recorded dissolved oxygen concentration, pH, conductivity, and temperature. One probe was placed in the open water near one of the sentinel fish cages and the second probe was placed at either 2 m or 10 m into the vegetation. The sensor array was situated in the middle of the water column at approximately 25 cm below the water surface. This procedure was carried out for Inlet 1, Inlet 3 and Outlet A in July and August. Water quality was also monitored at two sites (water depth approximately 33 cm) along the west and southwest (near the vegetation of Inlet 4) sides on the central pond in September.

Nutrient concentrations and phytoplankton biomass were measured biweekly from July through September and approximately monthly thereafter. Samples were taken at 15 stations in the Demonstration Wetlands during July and at 17 stations from August through October (Fig. 1). Total phosphorus, total dissolved phosphorus, particulate phosphorus, total alkalinity, total inorganic carbon, ammonia-nitrogen, nitrate-nitrogen, nitrite-nitrogen, total nitrogen, and Kjeldahl nitrogen were determined using standard wet chemistry methods (Wetzel and Likens 1991, APHA 1995) or by ion-specific electrodes. Phytoplankton biomass was measured spectrophotometrically as chlorophyll *a*. The detailed results of the nutrient analyses will be published elsewhere. Here, we discuss the results that are particularly relevant to the physical-chemical factors affecting survivorship of the fish.

Fish: Survivorship of sticklebacks and mosquitofish was monitored by placing sentinel fish of each species in duplicate cages at three locations in a 10 ha Demonstration Wetland in San Jacinto, CA (Fig. 1). The wetland receives between 4 to 8 million liters of secondary-treated effluent daily from a water treatment plant located adjacent to the wetland. The effluent flow is divided among 5 inlet marshes, flows subsequently through a central pond, and then through two outlet marshes. Eleven fish were placed into each cage in Inlet Marsh 1 and Inlet Marsh 3; nine fish were placed into replicate cages positioned in Outlet Marsh A. Cages were 1.25 m^3 (length X width X height dimensions: 122 cm X 113 cm X 91 cm) and were constructed of fiberglass window screen affixed to a PVC frame. Cages were surrounded by an external cage (approximate dimensions: 130 cm X 120 cm X 94 cm). In order to limit the handling of the fish, census of the fish was carried out by lifting the inner cage out of the water and counting the number of surviving fish. Survivorship of sticklebacks was monitored approximately biweekly from June 14 through October 3, 1996. Survivorship of mosquitofish was monitored from August 2 through October 3, 1996. In order to prevent predation of the fishes by ardeids and other piscivorous birds residing in the wetlands, the cages were covered with chicken wire or plastic netting (BirdBlock™: Easy Gardener, Waco, TX; mesh opening: 1.6 cm).

RESULTS AND DISCUSSION

Water Chemistry: Dissolved oxygen concentrations in the open water of inlet marshes fluctuated between 0 and 15 to 20 ppm over a diel period (Fig. 2). Dissolved oxygen concentrations were nearly zero for much of the night and increased in the late morning as algal-derived oxygen was added to the water. The importance of photosynthesis vs. heterotrophic activity by bacteria is evident by the close association of dissolved oxygen concentrations with daylight, the noticeable impact of cloudy days on the oxygen dynamics, and the gradient of dissolved oxygen in the vegetation.

The diel fluctuations in dissolved oxygen concentrations were similar in both Inlet 1 and Inlet 3. Dissolved oxygen concentrations at 2 m into the vegetation were only slightly lower than in the open water; however, dissolved oxygen concentrations at 10 m into the vegetation of the inlet marshes were nearly zero for the diel period.

Diel fluctuations in dissolved oxygen concentrations in Outlet Marsh A were appreciably smaller than in Inlet Marshes 1 and 3 (Fig. 2). The maximum dissolved oxygen concentration in the open water rarely exceeded 7 ppm. In contrast to the inlet marshes, dissolved oxygen concentrations at 2 m and 10 m into the vegetation in Outlet Marsh A were appreciably higher and closely followed those observed in the open water. Even though the water quality in the outlet marshes was generally better than in the inlet marshes, dissolved oxygen concentration in the outlet marsh could still be extremely low (Fig. 2).

Dissolved oxygen concentration in the water above the shallow berm on the southwest and west sides of the central pond rarely dropped below 2-3 ppm (Fig. 3). Daily maximum dissolved oxygen concentration was typically 14 ppm.

Concomitant increases of pH and dissolved oxygen concentration could potentially result in toxic or stressful concentrations of un-ionized ammonia (Kadlec and Knight 1996) in the inlet marshes. Toxic levels of chloramines or trihalomethane might also occur in the inlet marshes (D. Nimmo, Colorado State University, personal communication). The pH of the open water in the inlet marshes can reach 8.7 and, given the concentration of ammonia in the marshes (total ammonia: mean $\pm$ SE, 15.7 $\pm$ 2.3 ppm; range, 9-23 ppm), potentially stressful or toxic levels of ammonia-derived compounds might occur as pH increases resulting from algal photosynthesis. As a consequence of lower algal biomass and reduced photosynthesis in the outlet marshes, the pH levels in Outlet Marsh A remained comparatively stable and were approximately pH 7 for much of the day (WEW, unpublished data). This trend was also true for the vegetated regions of the marsh. At these pH levels and at the lower ammonia concentrations typically observed in the outlet marshes (total ammonia: mean $\pm$ SE, 6.6 $\pm$ 2.8 ppm; range, 1-9 ppm), the fraction of total ammonia in the un-ionized form is low (Kadlec and Knight 1996).

Table 1. Percent of stickleback (*Gasterosteus aculeatus*) and mosquitofish (*Gambusia affinis*) surviving on October 3, 1996 in replicate cages positioned at different locations in a multipurpose constructed wetland in San Jacinto, CA.

		Location		
		Inlet Marsh 1	Inlet Marsh 3	Outlet Marsh A
		Survival (percent)		
Species	Period			
G. aculeatus	June 14-October 3	0*,0	0†, 25	78, 0★
G. affinis	August 2 - October 3	0, 39	100, 100	100,100

* = catastrophic mortality associated with a bloom of *Hydrodictyon* sp. on 26 June.
† = catastrophic mortality associated with a bloom of *Hydrodictyon* sp. on 11 August.
★ = catastrophic mortality associated with a bloom of *Hydrodictyon* sp. on 17 August.

Fish survival: Survival of both fish species in Outlet Marsh A and Inlet Marsh 3 was very good; however, survivorship of fish was much lower in Inlet Marsh 1 (Table 1). Individuals survived best in the outlet marsh. Mortality of sticklebacks in one cage in Inlet Marsh 3 and one cage in Outlet Marsh A was catastrophic and was probably the result of cage effects: nearly all fish in a particular cage were dead on a particular date and mortality was associated with

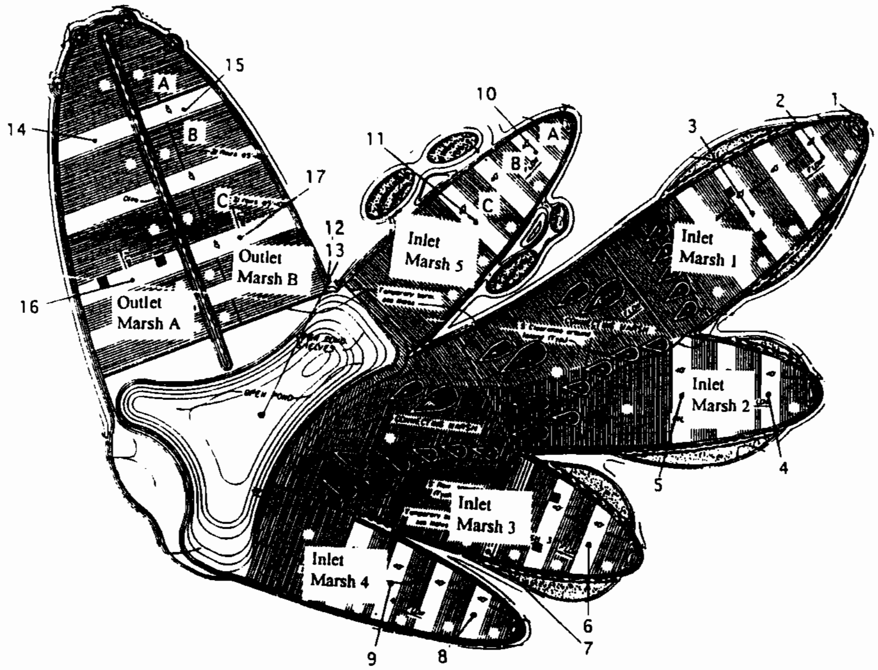

Fig. 1. Schematic illustration of the 10 ha Demonstration Wetland, Eastern Municipal Water District, San Jacinto, CA. *Scirpus* spp. is illustrated by cross-hatching. Chemistry sampling stations are indicated by numbers 1 through 17. The location of fish cages are indicated by filled squares in Outlet Marsh A (near site 16), Inlet Marsh 1 (near site 3) and Inlet Marsh 3 (between sites 6 and 7). Two cages (one holding sticklebacks and one holding mosquitofish) were positioned at each square.

the proliferation of a green alga, the water net, *Hydrodictyon* sp., within the cage. Following catastrophic mortality in some of the cages, the dead sticklebacks were found throughout the water column entangled in the net-like algae; yet, the fish appeared to be very robust suggesting that the onset of mortality was rapid. Large blooms of this alga were never observed outside of the cages. Herbivory, water movement or shading by dense planktonic algal populations in the inlet marshes may limit *Hydrodictyon* from proliferating in the open water outside the cages.

Surfacing and mid-water activities of the stickleback were restricted severely by dense blooms of *Hydrodictyon*. Continuous-recording water quality monitors indicated that dissolved oxygen concentrations in the three arms were severely depressed at night and in the early morning. In contrast to mosquitofish which naturally swim at the water surface, under hypoxic conditions sticklebacks were observed to gulp air/water at the air-water interface using a comparatively shallow angle between the longitudinal body axis and the water surface (essentially swimming at the water surface) or to exhibit an air-gulping behavior in which the body was maintained at a comparatively steep angle (25-35°) with respect to the water surface and the open mouth was thrust into the air (Fig. 4). The sticklebacks reside deeper in the water column when dissolved oxygen concentrations are not limiting. Dissolved oxygen concentrations in the cage probably remained low throughout day as the large mass of algae was degraded; algal blooms persisted for approximately two weeks before disappearing quickly.

The central pond may provide the only site at which stickleback males could successfully build a nest and tend the developing eggs. Unlike the mosquitofish which internally broods the eggs and then bears live young, male sticklebacks make and guard nests, and then tend the eggs and newly-hatched young. Low dissolved oxygen concentrations will force the male stickleback to abandon the nest in order to meet his metabolic needs. In contrast to individuals residing in cool, well-oxygenated lakes or streams, sticklebacks living

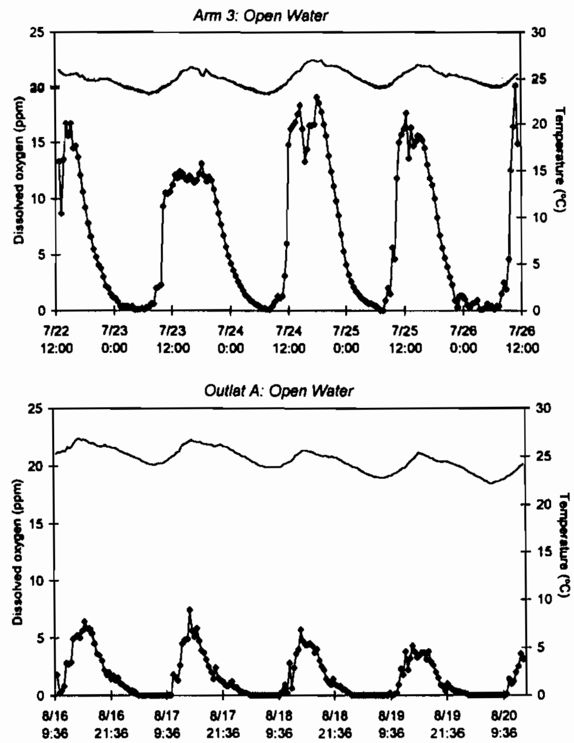

Fig. 2. Dissolved oxygen concentration (◆) and water temperature in the open water near sentinel fish cages in Inlet Marsh 3 and Outlet Marsh A for the period July 22-26, 1996 and August 16-20, 1996, respectively.

in warm, hypoxic waters are also at a greater risk of bird predation (FitzGerald and Wootton 1993).

Our results to date indicate that (1) phytoplankton populations play a very important role in the diel fluctuations of dissolved oxygen concentrations in constructed wetland ecosystems, (2) potentially toxic or stressful levels of compounds may limit our options in the choice of biological control agents for mosquitoes inhabiting constructed wetlands, and (3) a relatively small area of wetland is probably suitable for nest-building fishes. These results underscore the potentially stressful conditions for fishes and other potential biological control agents caused by low dissolved oxygen concentrations and other toxicants such as ammonia, chloramines and trihalomethanes. It remains to be determined whether a large-scale introduction of sticklebacks into the Demonstration Marsh can result in a self-sustaining population that will spread throughout the wetlands. A combination of mosquitofish and stickleback might be more efficacious for assisting mosquito control efforts than the stickleback alone because the viviparous reproduction and the surface-dwelling behavior of the mosquitofish may be a more amenable to the low dissolved oxygen concentrations in man-made wetlands.

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REFERENCES CITED

American Public Health Association. 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed. American Public Health Association, American Water Works Association and Water Environment Federation. Washington, D.C.

FitzGerald, G. J. and R. J. Wootton. 1993. The behavioral ecology of sticklebacks. pp. 537-572. *In*: T. J. Pitcher. Behaviour of Teleost Fishes. 2nd ed. Chapman and Hall. London.

Gratz, N. S. and others. 1996. Comments on "Adverse Assessments of *Gambusia affinis*". J. Am. Mosq. Control Assoc. 12: 160-166.

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

Rupp, H. R. 1996. Adverse Assessments of *Gambusia affinis*: an alternate view for mosquito control practitioners. J. Am. Mosq. Control Assoc. 12: 155-159.

U. S. Bureau of Reclamation, National Biological Survey, and Eastern Municipal Water District.

1994. Multipurpose Wetlands. Phase II/III Report.

Walton, W. E. and M. S. Mulla. 1991. Integrated control of *Culex tarsalis* larvae using *Bacillus sphaericus* and *Gambusia affinis*: effects on mosquitoes and nontarget organisms in field mesocosms. Bull. Soc. Vector Ecol. 16: 203-221.

Walton, W. E., P. D. Workman and S. A. Pucko. 1996. Efficacy of larvivorous fish against *Culex* spp. in experimental wetlands. Proc. Mosq. Vector Control Assoc. CA. 64: 96-101.

Wetzel, R. G. and G. E. Likens. 1991. Limnological Analyses. 2nd ed. Springer-Verlag. New York, N.Y.

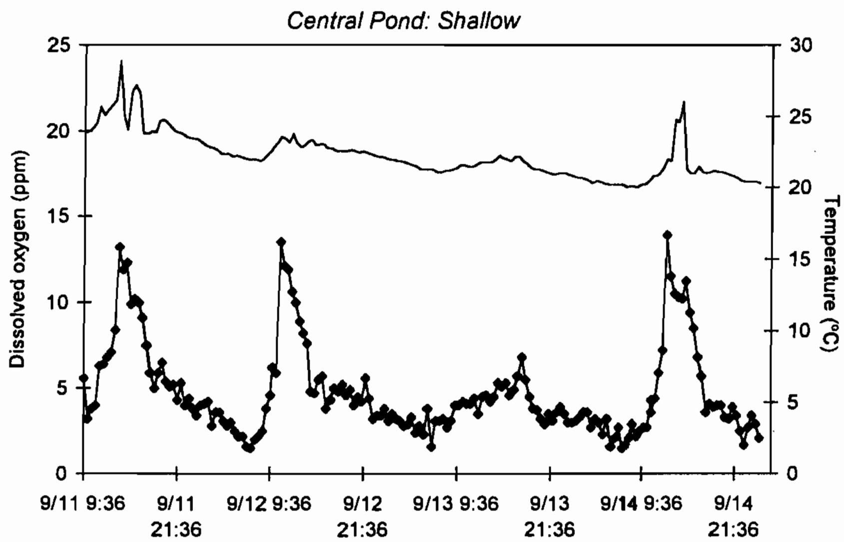

Fig. 3. Dissolved oxygen concentration (◆) and water temperature at the edge of the central pond for the period September 11-14, 1996.

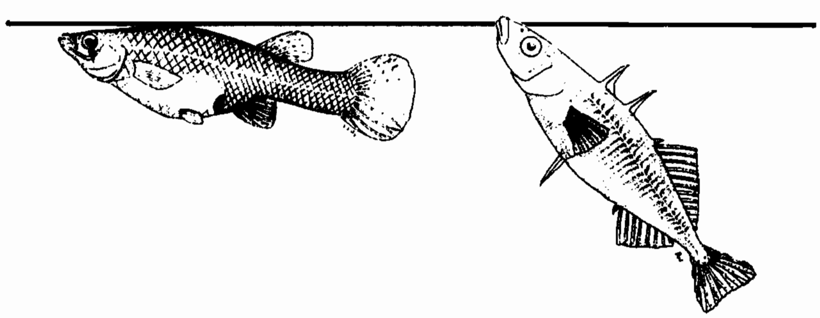

Fig. 4. Swimming orientation of the mosquitofish (left) and stickleback under low dissolved oxygen concentrations ($\leq$ 0.5 ppm). The mosquitofish swims with the longitudinal body axis horizontal just below the water surface. The stickleback either orients the longitudinal axis body at approximately 25-35° to the water surface and gulps air (illustrated) or swims with the mouth open at the air/water interface (not illustrated).

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TEMPERATURE EFFECTS ON METABOLISM: FOOD DEMANDS IN THREESPINE STICKLEBACKS, *GASTEROSTEUS ACULEATUS*

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Concerns have been raised about the continued use of exotic fish species (mosquitofish, *Gambusia affinis*) in California for biological mosquito control when appropriate native fish may be employed. The threespine stickleback, *Gasterosteus aculeatus*, is a native fish that may be used more widely for mosquito control in California (Downs 1991, Hubbs 1919). In an attempt to elucidate the possible effectiveness of these sticklebacks as a biological control agent of mosquitoes, we compared the food demands of these fish to that of mosquitofish.

Sticklebacks used in the study were collected on Jepson Prairie in Dixon, California and kept on the University of California, Davis campus where the experimental trials were conducted. Food demands of sticklebacks were obtained by converting measured respiratory metabolic rates (mg O_2/h/g; Cech 1990) to oxidative food demands (cal/d/g; Brett and Groves 1979) at three different acclimated temperatures (14.9,19.4 and 24.2 °C). The oxidative food demands were then calculated to "food demands" in terms of large and small mosquito, *Culex tarsalis*, larvae (mosquito larvae/d/g; Cech et al. 1980).Stickleback food demands in terms of mosquito larvae at 14.9, 19.4 and 24.2 °C are 168, 173 and 255 small mosquito larvae/d/g and 21, 22 and 32 large mosquito larvae/d/g respectively. A significant difference between the 24.2 °C temperature group and the other two temperature groups does exist (P-value <0.0005 by Bonferroni test). A Q_{10} value of 1.61 for the stickleback metabolic rate was calculated from the average oxygen consumption rates of temperature groups 14.9 and 24.2 °C. The food demands of threespine sticklebacks have been compared to published food demands of mosquitofish (Cech et al. 1980). Except for the coldest temperature, where food demands appear to be equal, stickleback food demands appear to be lower than that of the mosquitofish. Wooton (1976) has shown that sticklebacks do not reproduce readily in warm (>21 °C) waters that characterize shallow marsh habitats during the stickleback spawning season. Wooton's (1976) findings and the results of this study suggest that sticklebacks are less efficient than mosquitofish as a biological control agent of mosquito populations in California. In speculation, control of California mosquito populations and maintenance of an indigenous fauna may require higher stocking densities of sticklebacks than the densities of the presently used mosquitofish populations.

The authors thank J. Pedersen for his assistance in setting up and running experiments. Dr. T. Swanson and C. Crocker were also very helpful with experimental preparations. K. Williams is appreciated for his efforts in collecting the experimental fish. Research was supported by a UC Mosquito Research Program minigrant and the UC Agricultural Experiment Station, grant No. 3455-H, to J.J. Cech.

REFERENCES CITED

Brett, Jr. and T.D. Groves. 1979. Physiological Energetics. Pages 179-352 *in* W.S. Hoar, D.J.

Randall, and J.R. Brett, eds. *Fish Physiology*, Vol. 8: Bioenergetics and Growth. Academic Press. New York.

Cech, J.J. 1990. Respirometry. Pages 335-362 *in* C.B. Schreck and P.B. Moyle, eds. *Methods for fish biology*. Bethesda: Md.: American Fisheries Society.

Cech, J.J., Jr., M.J. Massingill, and T.E. Wragg. 1980. The food demands of mosquitofish,

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Gambusia affinis. *Proc. Calif. Mosq. Vector Control Assoc.* 48:45-47.

Downs, C. (ed.) 1991. Fishes in California Mosquito Control. *Calif. Mosquito and Vector Control Assoc. Sacramento.*

Hubbs, C.L. 1919. The stickleback: a fish eminently fitted by nature as a mosquito destroyer. *Calif. Fish and Game* 5(1):21-24.2.

Wootton, R.J. 1976. *The Biology of the stickleback.* Academic Press. London.

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INDOOR OVERWINTERING PROGRAM FOR MOSQUITOFISH, *GAMBUSIA AFFINIS*: A PROGRESS REPORT

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ABSTRACT

During the winter of 1989-90, Sacramento-Yolo MVCD began evaluating alternative methods for maintaining overwintering mosquitofish populations. Predation in our earthen ponds was significantly reducing our stocks of overwintered fish. Typically we were losing about 65% of the fish that were stocked in overwintering ponds in the fall. For this 1995-96 study, we used 8 aluminum raceways measuring 5.5 meters long, 63.4 cm deep, 81.2 cm wide, and which held 2,025 liters of water each. Raceways were stocked on October 18, 1995 with 16 kilograms (35 lbs) of mosquitofish each. During the final three weeks of this project we began sampling 40 fish from raceway # 2 each week and 40 fish from pond # 4 which is the pond that the raceway #2 fish came from. We compared total length, wet weight, fecundity and condition. Fultons' Condition Factor (Bagenal 1978) was used to compare the condition (amplitude) of a fish. Mosquitofish fecundity was determined by removing the gonads and counting the number of hydrated eggs. The data were averaged each week and the mean was used for comparison. In addition, the mean totals for the study period were also used in comparison. Mosquitofish samples were collected on March 27, April 2, and April 19, 1996. On each sampling date the raceway overwintered fish were significantly longer, heavier and in better condition than the fish overwintered in pond #4. On the first sample date the fish overwintered in pond #4 had a higher mean number of hydrated eggs (21.03), compared to the raceway fish (19.15). However mean fecundity for all three sampling dates was higher for the raceway overwintered fish (31.23) compared to 26.83 hydrated eggs from the fish overwintered in pond #4. The overall percent of fish overwintered in the raceways was 93.2% compared to 36.8% for the fish overwintered in our earthen ponds.

The development of more ponds and greater numbers of mosquitofish migratory predators like common mergansers (*Mergus merganser)* began to seriously deplete our overwintering fish stocks. During the fall of 1988 we contacted the manager of the Department of Fish and Games Central Valley Hatchery and requested the use of two of their cement raceways for overwintering mosquitofish. That winter we had a 66.1% survival rate in their cement raceways compared to a 41.3% survival rate in the Districts earthen ponds.

In August 1989 we purchased two (2,025 liter) aluminum raceways. These raceways were set up indoors and had a simple flow through water delivery system. That winter in the aluminum raceways we had a 60.7% survival rate compared to a 36.5% survival rate in our earthen ponds. During those early years we also did some production comparison studies and found that ponds stocked with these intensively cultured overwintered fish produced more fish than ponds stocked with fish that were overwintered in earthen ponds (Schon 1991).

During the following years we purchased one or two raceways per season and included them in our mosquitofish overwintering program. We have also learned to treat the raceway fish with paracides and antibiotics both prophylacticly and reactively for internal and external parasites and bacterial infections. These treatments have significantly increased mosquitofish overwintering success. Additionally we have installed a large (eight cubic foot) carbon filter as our Water District frequently injects our water supply with chlorine to control an iron/manganese bacterium that is found in wells throughout the area.

MATERIALS AND METHODS

For this study we used eight aluminum raceways 5.5 meters long, 63.4 cm deep, 81.2 cm wide, with a capacity of 2,025 liters, each. The water source was deep-well water from Elk Grove Water District and was run through a de-chlorinating unit to remove chlorine and sediments. Prior to entering the raceways the water was run through packed columns to increase dissolved oxygen levels. The raceway tail water was not circulated, but was discharged into eight of our overwintering ponds to maintain water levels throughout the winter. We preferred to run four gallons per minute per raceway, throughout this study and water pressure varied daily, and flow rates were often as low as two gallons per minute per raceway.

Water chemistry was taken twice per week (Mondays and Fridays) throughout this study. Temperature remained fairly constant at 18 °C, pH varied from a low of 7.2 to a high of 7.7. Nitrates and nitrites were constantly at 0 ppm. Dissolved oxygen levels at the base of the packed columns varied from 5 to 7 ppm. Measurements at the tail-race or water outlet were the same or 1 ppm less. Total Alkalinity (as $CaCO_3$) was constant at 120 ppm.

Raceways were stocked on October 18, 1995 with 16 kilograms (35 lbs) each. These fish came from ponds 1-4 that were drained, cleaned, and re-rocked in July. On August 4, 1995, ponds 1-4 were refilled and fertilized according to district protocol. These ponds were inculcated with plankton from several of our other production ponds and ten days later stocked with 56.8 kilograms (125 lbs) of mosquitofish from surrounding production ponds. The last week in April 1996 these ponds were pumped down, seined, and overwintering success was determined.

Three days after stocking, raceway fish were treated with Paracide-F® as a prophylactic treatment for internal and external parasites. Five days later propylactic treatments with Terramycin® were made to minimize bacterial infections.

During the final three weeks of this project we began sampling 40 female mosquitofish from raceway #2 each week and 40 female mosquitofish from pond #4 which was the pond that the raceway #2 fish had come from. We compared total length, wet weight, fecundity and condition. Fultons' Condition Factor was used to compare condition of the fish. This factor is based on the hypothesis that the heavier fish of a given length are in the best condition (Bagenal 1978). Mosquitofish fecundity was determined by removing the gonads and counting the number of hydrated eggs. The data were averaged each week and the mean was used for comparisons. In addition, the mean totals for the study period were compared. Mosquitofish samples were collected on March 27, April 2, and April 19, 1996.

RESULTS AND DISCUSSION

Of the 127 kilograms (280 lbs) of mosquitofish stocked in the raceways, 118.6 kilograms (261 lbs) or 93% were harvested during the third week of April, 1996. These data were recorded by weight or biomass and did not reflect the numbers of fish that were lost. Numerically we probably lost 30-35% of our original stocks, but because of the amplitude of the of the raceway fish the percent overwintered was much greater. Individual raceway overwintering success varied from a low of 80% (28 lbs) in raceway #1 to a high of 102.9% (36 lbs) in raceway #7. Combined overwintering success in the overwintering ponds 1-4 was 36.85% or 81 lbs.

The average total length of the raceway overwintered fish was 44.2 mm compared to pond #4 stocks at 38.2 mm. The greatest significant differences were seen when comparing the wet weight data. The average weight of the raceway overwintered fish was 1.24 g compared to the pond overwintered stocks at 0.68 g. Length and weight of the two different overwintering stocks were compared using Fulton's Condition Factor. The condition of the raceway overwintered fish was 1.41 compared to the pond #4 overwintered stocks at 1.19.

Fecundity data were determined by counting the number of hydrated eggs in each fish sampled. The mean number of hydrated eggs of the raceway overwintered fish was 31.23 compared to the sample of pond #4 overwintered fish 26.83. Although significantly different based on weight differences of the two stocks, we expected the raceway overwintered fishes fecundity to be much greater.

Overwintering *Gambusia* is the most difficult part of our extensive pond culturing program and the presence of migratory avian predators has required us to develop an alternative to overwintering all of our fish stocks in earthen ponds. Raceway overwintered fish provide our Mosquito Control Technicians with adequate numbers of fish for stocking into spring sources. Without these fish we would have had to take an excessive number of our brood stocks from our production ponds, thus reducing the number of fish available for stocking rice fields.

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The author would like to thank Randy Burkhalter and Jeff Fairbanks for the long hours spent in the lab counting hydrated eggs and recording all the length and weight data.

REFERENCES CITED

Bagenal, T. 1978. Methods for Assessment of Fish Production in Fresh Waters. Blackwell Scientific Publication.

Schon, W. 1991. Intensive Culture Techniques for Overwintering Mosquitofish, *Gambusia affinis.* Proc. California Mosq. and Vector Contr. Assoc. 59: 94.

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FIELD EVALUATION OF AQUA RESLIN 20-20 AND PERMANONE 31-66 AGAINST *AEDES NIGROMACULIS* IN MERCED COUNTY

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ABSTRACT

Water-based Aqua Reslin 20-20 and oil-based Permanone 31-66 were applied as ultra low volume ground adulticide treatments against *Aedes nigromaculis* at 1.96 and 1.97 grams AI/hectare. At 30m, both products achieved greater than 99% mortality at 24 hours. At distances of 60m and 90m, Aqua Reslin provided 55.4% and 49.4% mortality at 24 hours, while Permanone achieved 96.6 and 92.0% control. Windy conditions may have played a major role during the application of Aqua Reslin.

Adulticiding is still an important component of many mosquito abatement programs in California. According to the Vector Control Summary Tables published in the "Proceedings and Papers of the California Mosquito and Vector Control Association," in 1994 over 15,468 kg of mosquito adulticides were used by mosquito and vector control agencies in California (Eliason 1995). Natural pyrethrin and synthetic pyrethroids are commonly used as ultra low volume (ULV) ground adulticide treatments. Although some of these products can be applied neat, many require dilution. The diluent description on the chemical labels for these products include such statements:

1) this concentration is designed for dilution with light mineral oil,

2) this concentrate is formulated to be diluted with a suitable oil diluent, such as but not restricted to light mineral oil, deodorized kerosene or petroleum distillate,

3) mix product with refined soybean oil, light mineral oil of 54 second viscosity or other suitable solvent or diluent,

4) dilute with mineral oils (such as Orchex or Klearol), cottonseed oil or other suitable non phytotoxic ULV suitable oil.

Regulatory agencies routinely scrutinize, not only the active ingredients, but have shown increasing concern with the inert ingredients contained in mosquito control products. The diluents used for ULV adulticiding applications are not above this same scrutiny. AgrEvo Environmental Health has developed a water based permethrin product, Aqua Reslin 20-20. This field study was undertaken to evaluate Aqua Reslin 20-20 and the previously labeled Permanone 31-66 as ULV ground treatments against adult *Aedes nigromaculis*.

MATERIALS AND METHODS

Adult mosquitoes were collected during the morning of the test day with hand-held Insect Vacs (BioQuip Corporation) from a natural population of *Aedes nigromaculis* from an alfalfa field near Snelling, California. The mosquitoes were held in plastic collection chambers, placed in polystyrene ice chests, and transported to the laboratory facility of the Merced County Mosquito Abatement District. The mosquitoes were anesthetized with CO_2 and transferred to cylindrical screened test cages. The cages measured approximately 8.9 cm in diameter and 20.3 cm in length. The number of mosquitoes in each cage ranged from 9 to 98. Cages for each treatment and control were packed in separate ice chests for transportation to the test site.

Aqua Reslin 20-20 and Permanone 31-66 were evaluated as ground applied ULV treatments at rates of 1.968 g AI/ha and 1.979 g AI/ha, respectively, against caged adult female *Aedes nigromaculis* mosquitoes in a 8.11 hectare plowed fallow parcel of

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property located 4.8 km east of the Merced County MAD Headquarters. Testing was conducted on September 11 at 1945 h for Aqua Reslin and 2015 h for Permanone 31-66. Temperatures ranged from 22.2 to 23.4 Celsius degrees with a relative humidity of 45%. The winds were out of the northwest during the duration of the test. Wind velocity was measured with a hand-held Dwyer wind meter. Wind speeds of 11.26-16.09 km per hour with gusts of 20.91 km per hour were recorded during the Aqua Reslin application. Steady winds of 6.43-11.26 km per hour were recorded when the Permanone application was made.

To achieve the proper application rates, Aqua Reslin 20-20 was diluted at a ratio of 1:6.25 in water. Permanone 31-66 was mixed with Arcoprime Oil 90 at a ratio of 1:11.5. Two truck mounted LECO 800 cold aerosol generators were used to apply the materials. The trucks traveled in a south to north direction at a speed of 16.09 km per hour. Nozzle pressure was 246 g per cm^2 for Aqua-Reslin and 316 g per cm^2 for Permanone. Droplet size analysis and flow rate calibrations were completed prior to the treatments. Flow rates for both materials was 180 ml per minute. Droplet size, as measured with an AIMS (Army Insecticide Measuring System) machine, were 15.3 microns volume median diameter (VMD) for Aqua Reslin in water and 16.1 microns VMD for Permanone in oil.

Caged mosquitoes were placed in the treatment plots just before each test. The cages were hung approximately 1.52m above the ground on metal fence stakes. The stakes were arranged in three rows with 30m between each row. In each row, stakes were placed at 30, 60, and 90 meters perpendicular to the spray pattern of the truck. Approximately 15 minutes after each insecticide was applied, the caged mosquitoes were transported back to the Merced MAD lab facility, anesthetized with CO_2 , and transferred to 1.89 liter paper cans with screen lids. Control cages of mosquitoes were handled in a similar fashion, hung outside on metal stakes upwind of the test plot, and transferred in a separate area of the lab. Raisins were wrapped in water soaked cotton and placed on top of the screen lids. Initial knockdown was observed at 1 hour while mortality was recorded at 12 and 24 hours post-treatment. Data were corrected for control reductions using Abbott's formula. Means for each insecticide by distance and by row were analyzed using ANOVA. Treatment means were separated by Tukey's HSD test.

RESULTS AND DISCUSSION

Average 24-hour mortality for the Aqua Reslin and Permanone treatments by distance from the spray vehicle are presented in Table 1. Aqua Reslin

Table 1. Average 24-hour mortality of Aqua Reslin and Permanone ULV applications against caged *Aedes nigromaculis*.

		Distance*					
Treatment	Rate	30m	60m	90m	Mean**	F Value	P Value
Aqua Reslin	1.96 g/ha	99.7a	55.4b	49.4b	75.1B	16.05	.0099
Permanone	1.97 g/ha	99.6a	96.6b	92.0c	95.9A	28.34	.0034

* means for distance in the same row followed by a different lower case letter are significantly different ($P<0.05$) by Tukeys HSD test.

** comprehensive means followed by a different upper case letter are significantly different ($P<0.05$) by Tukey HSD test.

provided excellent control at 30m, but decreased significantly at the 60 and 90 meter distances. There was no significant difference in treatment mortality at 60 and 90 meters. Control mortality ranged between 1.99 and 10.6%. Permanone displayed excellent knockdown and mortality over time at all distances. The Permanone treatment by distance interaction was significant at all three distances; however, mortality was still acceptable for each distance. Control mortality ranged from 1.87 to 8.41%.

The results of these trials indicate that Aqua Reslin and Permanone are highly effective compounds against *Aedes nigromaculis* at the 30 meter distance. Permanone showed more activity than Aqua Reslin at distances of 60 and 90 meters from the spray path. No recovery from initial knockdown was observed for any treatments. The lower mortality observed in the Aqua Reslin treatment at the 60 and 90 meter distances could be attributed to gusty winds during application. Mortality by row for the Aqua Reslin application ranged from 61.3 to 93%. Aqua Reslin was highly effective at the 90 meter distance in one row. The results and the weather conditions prevalent during these trials warrants the further evaluation and development of water-based Aqua Reslin.

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REFERENCES CITED

Eliason, D.A. 1995. Vector Control Summary Tables from CMVCA Member Agencies, 1994. Proceedings and Papers of the California Mosquito and Vector Control Association. 63:71-75.

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CARBON DIOXIDE BAITED CDC STYLE TRAPS USED TO CONTROL *PSOROPHORA COLUMBIAE* (DYAR & KNAB) IN THE COACHELLA VALLEY

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Psorophora columbiae (Dyar & Knab) was the primary pest mosquito found on pastures, date gardens and fan palm nurseries during July, August, and September 1996 in the Coachella Valley, California.

Although excellent control of *Psorophora* larvae on pastures may be achieved with long term products, such as Altosid pellets, there were situations when timely applications were not possible. Often the reasons were a locked gate or absent owner. The main problem at fan palm nurseries was limited access caused by dense planting and sharp spines of the leaf stems of the fan palms. The problem with date gardens was a lack of available manpower to handle the acreage needed to be treated. Adults from such breeding sites were imposing a public nuisance, and the Coachella Valley Mosquito and Vector Control District took a classic approach to vector control and fogged in or around residential areas.

Experience from 1995 and 1996 fogging, indicated that fogging results varied, that costs were high and that public interference can have an impact on the fogging procedure. In 1995 flat sentinel cages were used to test the efficacy of ground fogging. Control varied from 20% to 100%, depending on the type of habitat, distance of cages from the fogging source, and wind direction. The high cost of ground fogging and variable results motivated the CVMVCD personnel to look for a different solution.

Carbon dioxide has long been known and used as an attractant for biting insects and for vector surveillance. Recently CO_2 was used alone or in combination with octanol for mosquito control by removal trapping or trapping out of mosquito populations from certain areas (Wood and Morris 1990; Day and Sjogren 1994; Kline and Lemire 1995; Kline and Bornard 1996).

In the summer of 1996, the CVMVCD initiated a pilot study to determine if using a series of CO_2 baited CDC style traps could replace fogging in small areas. The specific objectives of the study were to determine the effectiveness of so called "surrounding strategy" in limited locations and to compare the cost of such a method against ground fogging. The surrounding strategy involves placement of CO_2 traps around potential mosquito hosts, usually residential units.

During August 1996, massive breeding of *Psorophora columbiae* occurred at Two Palms Ranch and Cotton Patch Ranch pastures. The CVMVCD received a complaint from a resident at a trailer park located behind the Two Palms Ranch regarding a high mosquito population. The trailer park and the owner's house at the Cotton Patch Ranch were the only residential areas in the mile square section. Such a small area encouraged the use of the CO_2 traps. Four traps were placed around the trailer park and 4 around the owner's house. Each trap was baited with 0.5 kg of dry ice and placed on 1 m stands between 3:00 - 4:00 PM. Trapping continued for 7 nights. The first night over 10,000 mosquitoes were collected, while on the last night over 700. Residents reaction from the trailer park indicated a noticeable reduction in number of mosquitoes after the first night of trapping. When residents reported that they did not have a problem with mosquitoes, trapping was discontinued. The total cost of trapping for 7 days was $287.70, which included dry ice (0.5 kg of dry ice = $0.60), 7 hours of overtime for each day for setting the traps and 14 hours of regular time.

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At the beginning of September 1996, excessive breeding of *Psorophora columbiae* occurred in a fan palm nursery, near John Kelley School in Thermal. The CVMVCD received a complaint from the school Principal. The area around the school, a portion of Thermal, and a part of the nursery were fogged for 5 days, but the CVMVCD was still receiving complaints from the school. The main reason for the continuing complaints was that during the fogging time, residents were on the streets, so the fogger was off too often. The cost for 5 nights including chemicals and overtime for two operators came up to $1,200. At that time we decided to place traps around the school and at the north-west corner of the nursery. After the first night of trapping (Sept. 11, 1996) 8 traps collected over 8, 000 mosquitoes. The next morning we received a call from the school Principal that there was a noticeable reduction in the number of mosquitoes. After 3 days of trapping, the Principal informed us that there were no complaints from the students. After the 5th day of trapping, 8 traps collected over 250 mosquitoes, so we discontinue trapping. The total cost of trapping for 5 nights, including dry ice, 8 hours of overtime and 16 hours of regular time was $205.50.

The method of removal trapping with different attractants still has limited use. There are programs where removal trapping is very successful such as eye gnat control in the Coachella Valley, and is the only method of control. Other occasions of removal trapping are rare and usually compelled by other factors such as a sensitive environment (Key Island), public interruption during the fogging procedure (trapping in Thermal) or small residential areas where cost effectiveness is the major reason for using removal trapping.

Public perception, along with a reduction in number of mosquitoes in the traps, were the only indicators that removal trapping was a viable method for vector control in these cases. These first results were encouraging and indicate the need for future trials.

REFERENCES CITED

Day, J. F. and R.D. Sjogren. 1994. Vector control by removal trapping. Am. J. Trop. Med. Hyg. 30: 126-133.

Kline, D.L. and D.R. Bornard. 1996. A high-tech mosquito barrier. Agricultural Research/March 12-14.

Kline, D.L. and G.F. Lemire. 1995. Field evaluation of heat as an added attractant to traps baited with carbon dioxide and octanol for *Aedes taeniorhynchus*. J. Amer. Mosq. Control Assoc. 11:454-456.

Kline, D.L., G. R. Wood and C. D. Morris. 1990. Evaluation of 1-octen-3-ol as an attractant for *Coquilletidia perturbans*, *Mansonia* spp. and *Culex* spp. associated with phosphate mining operations. J. Amer. Mosq. Control Assoc. 6: 605-611.

APPLICATION OF ALTOSID® (METHOPRENE) FOR YELLOWJACKET CONTROL

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ABSTRACT

A two year study investigated the applicability of methoprene, in the form of Altosid Liquid Larvacide (ALL), for the control of scavenging yellowjackets. Bait stations were provisioned with Altosid-treated canned mackerel in picnic areas of two recreational parks within Marin and San Mateo counties. The bait stations were monitored and rebaited bi-weekly. The first year of the study concentrated on assessing the long term effects on yellowjackets. Results indicated that methoprene had a detrimental effect on yellowjackets, but it took approximately two months to bring about noticeable reduction of foraging workers. In addition, the unavailability of practical detection methods for methoprene residue made it difficult to confirm the observed results. In the second year, applications of higher dosages of methoprene at a concentration six times or higher than recommended field rates were made to shorten the time needed for control. Bait stations were placed adjacent to the established nests to evaluate the material's effectiveness for short term control. The study failed to demonstrate that the material can be relied upon to produce predictable results or effective control. A variety of factors were observed to have influenced the results including the attractiveness of the bait, competitive food sources and yellowjacket behavior.

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APPLICATION OF A NEW TAMPER RESISTANT BAIT STATION TO CONTROL ROOF RATS IN ORANGE COUNTY CALIFORNIA

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Vector control programs throughout California have been subject to historical changes brought about by modernization and increased regulatory standards. Effective September 1996, new regulations enacted by the United States Environmental Protection Agency now require that all commensal rodent baits be placed inside durable bait stations constructed in a manner to resist tampering and exclude entry by children under the age of six, domestic pets (dogs), and non-target wildlife. Prior to the implementation of the new regulations, rodenticides were placed either totally exposed or inside "open" stations that failed to prevent direct access to the bait. To comply with the new tamper resistance standards and commensurate rodenticide labeling, the Orange County Vector Control District proceeded to develop an economical tamper resistant station constructed from prefabricated sheet metal and extruded plastic components. This paper briefly summarizes the design features of the District's bait station and its application to controlling roof rats, Rattus rattus Linnaeus, in Orange County.

OCVCD TAMPER RESISTANT BAIT STATION

Operational Requirements. The roof rat control program operated by the Orange County Vector Control District relies heavily on chemical control and bait stations that can be firmly attached to fences and shrubbery at private residences. This aspect of the District's operations requires bait stations that are easily attached to vertical surfaces (e.g., walls and fences) and tree limbs. Commercially available bait stations are fabricated in various cubical and box configurations with access openings along the sides just behind the front panel. Attachment of these types of stations to tree limbs and vertical surfaces poses a number of technical problems that preclude ease of placement using either wire or a combination of hard anchor points. From a variety of designs submitted by technical and operational staff, a cylindrical concept was finally selected that conformed to the standards required for both economical production and quick deployment. A prototype of the station was submitted to the Environmental Protection Agency for technical evaluation and conformity with tamper resistant criteria. The OCVCD bait station was approved and now is formally listed by the EPA with special application for placement in vegetation.

Station Specifications. The station is cylindrical in shape (4.25" dia. X 14.0" long) with the access openings (ca 2.0") in the ends above center (Fig. 1A). The cylinder comprising the body of the station is molded (extruded to an OD of 4.20" and thickness of 0.20") from recycled plastic and colored dark green to blend with shrubbery. End cap assemblies are fabricated from two galvanized sheet metal (thickness 0.030 - 0.035") components spot welded together to produce a single component (Fig. 1B) that incorporates the access opening in the end cap (C) and baffle plate (P) to which the bait is attached to the underside (B). The baffle structurally prevents children and dogs from contacting the bait. A fully assembled station, including parts and labor minus the bait, costs approximately $4.50 to produce.

Field Evaluations. Prototype stations were baited with 16 ounces (two 8 oz. bait cakes) containing 0.005% chlorophacinone and deployed in the field to determine if the design was acceptable to accommodate foraging roof rats. All field tests demonstrated that rats would enter the station within several weeks of placement. The apparent prolonged acceptance period is attributable to the design of the station that requires wary rats to "become accustomed" to the "structure" before entering through the circular access openings. Previously, baits placed either in the open or in easily accessible non-tamper resistant stations were readily accessed by rats that "perceived" little danger associated with the unencumbered presentation of the bait. Other designs of commercially-available stations were not simultaneously evaluated with our station.

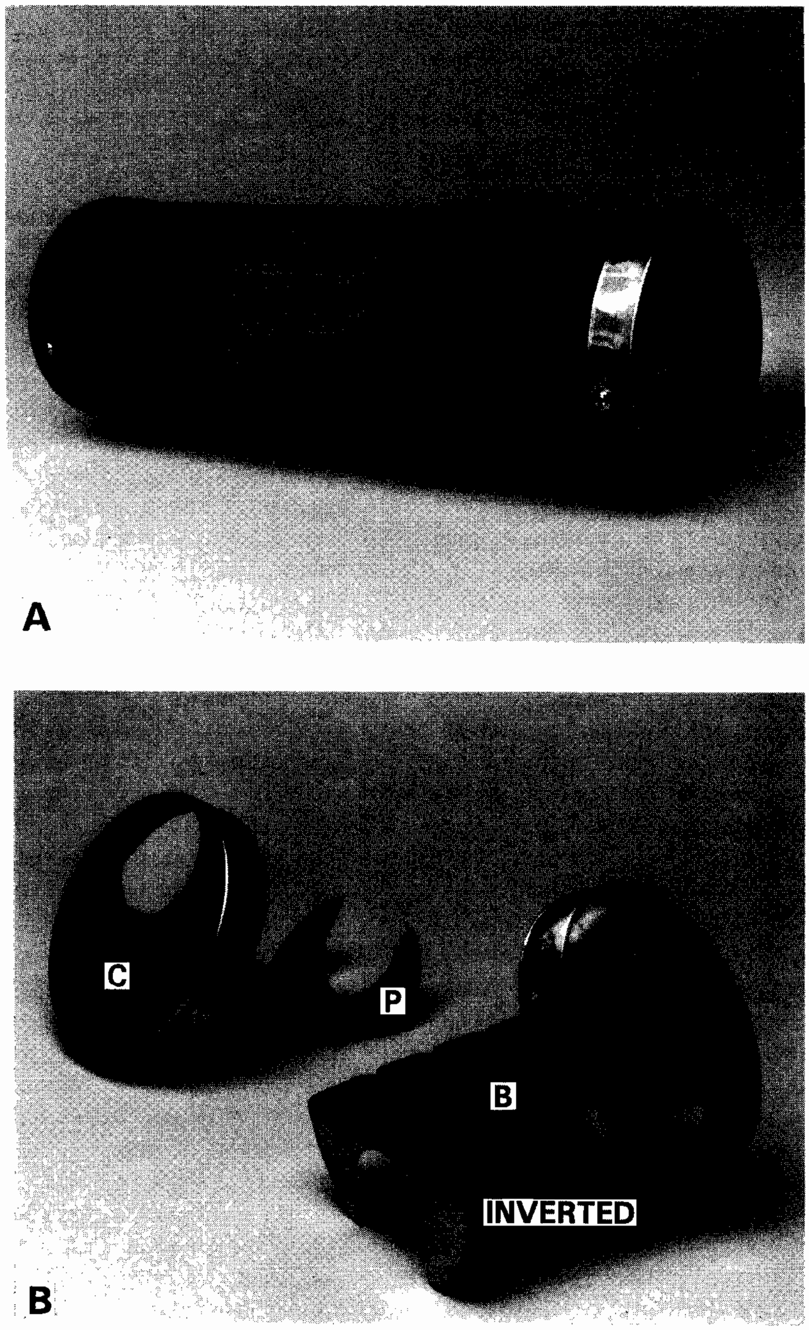

Figure 1. Photographs illustrating a fully assembled bait station (A) and assembled sheet metal components comprising the end cap and baffle assembly (B).

SYNOPSIS OF DISTRICT'S ROOF RAT CONTROL PROGRAM

Operations. The District receives on the average 12,000 service requests annually of which more than 90% are rat related. This level of service demand requires the services of 16 full time staff and one vector ecologist. In the process of providing rat control services, District technicians respond by deploying an excess of 25,000 pounds of bait presented in a minimum of 20,000 bait stations. The massive volume of baiting requires expedient placement and anchoring of stations plus the ease of recheck and management necessities.

Station Placement Guidelines. When placing bait in the field, District technicians are given the final decision whether or not to deploy a station if conditions (security/safety) allow them to choose that option. If a station is placed at a private residence, the site of placement is largely confined to the backyard where access is limited to the District technician and resident. For residents that have no children or pets, stations either can be attached (minimum 12 gage wire) securely to almost any vertical surface (e.g., walls, fences, etc.) or to the larger branches of shrubbery and trees. District policy requires bait stations to be securely anchored to walls, fences, and vegetation a minimum of 5-6 feet above ground level at residences with either pets (primarily dogs) or young children. This action provides an additional level of safety to assure an adequate out-of-reach zone for children and dogs. Additional wires and anchor points are often necessary to assure that stations can not be removed from their anchors without considerable effort.

SUMMARY

The design and component modularity of the Orange County Vector Control District's bait station delivers the utility required for rapid deployment, easy attachment to vegetation, and routine maintenance activities. The addition of the sheet metal end caps and baffle components effectively reinforces the extruded plastic housing and protects the plastic housing from being severely damaged by rats that continue to literally eat the station after the bait has been consumed. Though our station design facilitates placement in vegetation, the unit may be modified for ground deployment with the addition of restraining bands and brackets to accommodate either steel spikes or other types of comparable anchors.

ACKNOWLEDGEMENTS

The author extends his appreciation to the following District staff for their contribution in developing the final prototype of the bait station currently being used in routine District baiting operations: Ron Elliott, District Foreman, Lawrence Shaw, District Vector Control Specialist, and John Pett, District Vector Control Technician III. The efforts of the District's field operations staff deserve special recognition for implementing the new tamper resistance baiting program.

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MOSQUITO ABUNDANCE AND ARBOVIRAL ACTIVITY IN SAN BERNARDINO COUNTY DURING 1996

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ABSTRACT

Of the 5,988 mosquitoes collected in New Jersey light traps and CO_2-baited CDC traps in San Bernardino County during 1996, 59.3% were from the desert region (Needles) and 40.7% from valley sites. In the desert region, the dominant species were *Culex tarsalis* (94.6%) and *Culiseta inornata* (4.0%). In the valley region, *Culex quinquefasciatus* (41.0%), *Cx. tarsalis* (27.9%), *Culiseta incidens* (14.2%) and *Culex stigmatosoma* (13.3%) were found in significant numbers. All 68 pools of culicine mosquitoes submitted for testing were negative for both Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses.

The Colton and Needles flocks showed one and two SLE sero-conversions respectively in September and November. The posting of encephalitis warning signs, press releases and increased mosquito control activities were carried out in both areas.

As part of the state-wide encephalitis virus surveillance (EVS) program in California, The San Bernardino County Vector Control Program (SBCVCP) has carried out EVS and other mosquito control activities in both the valley and desert areas of San Bernardino County for several years. Geographically, the county consists of three distinct regions; the desert, mountains and valley regions. Demographically, the valley region houses over 80% of the nearly 1.6 million human population in the County with the remaining scattered over various parts of the desert and mountain regions. Historically, cases of both Saint Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) have been reported in the desert and valley regions from time to time.

After experiencing 26 human cases of SLE in southern California during 1984, the only human case of SLE in southern California during 1987 was reported from San Bernardino (Emmons et al. 1988). Of the two cases reported state-wide in 1988, one was from the same San Bernardino site (Emmons et al. 1989). Recently, two of the three SLE cases reported state-wide in 1993 and one case in 1994 were found to have been contracted in San Bernardino County (Emmons et al. 1994, Reilly et al. 1995). During the same period, both SLE and WEE virus activities were reported in the desert region, especially Needles, and adjoining areas along the Colorado River. Due to the periodic incidence of encephalitis disease, mosquito control and EVS activities have been routinely carried out in the desert and valley regions of this county. Data generated in routine EVS activities in 1996 are appraised here in relation to mosquito abundance and arboviral activity in San Bernardino County.

MATERIALS AND METHODS

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

Adult Mosquito Population Dynamics. The abundance of various mosquito species was monitored weekly by the use of New Jersey light traps. In the valley, the traps were stationed at eight different locations; Yucaipa, Redlands, Highland,

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San Bernardino, Colton, Fontana, Ontario and Upland. Within the valley region there were at least two traps sites each in urban, suburban and rural environments. In the desert region (Needles area), one trap each was operated in urban, suburban and rural areas along the Colorado River.

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

Arboviral Activity in Female Mosquitoes. Arboviral activity in local mosquito populations was monitored by using CO_2-baited CDC traps to collect host-seeking adult female mosquitoes. Eight or more of such traps were operated twice a month in the valley area, once in the Colorado River (desert) region. Female mosquitoes collected overnight were anesthetized using triethylamine (TEA), counted, identified to species and sex then pooled by species with 10-50 adults per vial. All vials were stored on dry ice in the field or in ultra-low temperature deep freezer (60°F) in the laboratory before being shipped in dry ice-packed containers by overnight express mail to the Viral and Rickettsial Disease Laboratory (VRDL) in Berkeley.

Arboviral Activity in Sentinel Chickens. Both wild and domestic birds are known to play a significant role in the epidemiology of mosquito-borne encephalitides by acting as reservoir hosts for the encephalitis virus(es). Therefore three sentinel flocks, each consisting of ten white leghorn chickens, were used to monitor arbovirus activity in the area. Two of the flocks were maintained in the valley area in Colton and Redlands and one in the desert area in Needles. The Colton flock was stationed at the northeastern corner of Meridian Avenue and Olive Street in the city of Colton. This site is located in the general area of previous Saint Louis encephalitis human cases reported in 1987 and 1988. The Redlands flock was maintained at the Redlands Sewage Treatment along the Santa Ana River between Alabama and Nevada Streets. The desert flock was kept at the sewage treatment facility in the city of Needles. New Jersey light traps were regularly operated at all flock sites. Using the comb prick method, blood samples were taken from all sentinel chickens and placed on prelabeled filter paper strips on a bi-weekly basis during April through October, 1996. These samples were then mailed to the VRDL for detection of arboviral activity.

RESULTS AND DISCUSSION

Of the total 5,998 mosquitoes collected at all sites during the season, 59.3% were trapped in the desert area and 40.7% from the valley sites (Table 1). The most abundant mosquito in the desert region was *Culex tarsalis* Coquillet (94.6%), followed by *Culiseta inornata* Williston (4.0%), *Culex stigmatosoma* Dyar (0.5%), *Culex erythrothorax* Dyar (0.4%), *Aedes vexans* Meigen (0.3%), *Culex quinquefasciatus* Say (0.1%), *Anopheles franciscanus* McCracken (<0.1%) and *Culiseta incidens* Thompson (<0.1%). Unlike the desert region, the most abundant species in the valley region was *Cx. quinquefasciatus* (41.0%), followed by *Cx. tarsalis* (27.9%), *Cs. incidens* (14.2%), *Cx. stigmatosoma* (13.3%), *Cs. inornata* (1.9%), *An. franciscanus* (1.5%) and *Cx. erythrothorax* (0.2%). Earlier studies in this area indicated *Cx. tarsalis* as the most abundant species, comprising as much as 35% of the mosquitoes collected in 1989, (Mian and Prochaska, 1990).

A total of 68 pools of culicine mosquitoes (*Cx. tarsalis*, *Cx. quinquefasciatus* and *Cx. stigmatosoma*) were sent to VDRL for virus study. All pools tested negative for both SLE and WEE viruses.

The results on chicken serology showed one sero-conversion to SLE virus in the Colton flock and two in the Needles flock. The sero-conversion in the Colton flock was found in September (bleeding date September 4, 1996), whereas both sero-conversions in the Needles flock were reported late in the season (bleeding date November 12, 1996). There were no sero-conversions detected in the Redlands flock.

Upon receipt of confirmation on sero-conversions in both Colton and Needles flocks, the areas were posted with "Encephalitis Warning" signs followed by press releases to local newspapers advising residents to take necessary precautions during outdoor activities especially at dusk and dawn in the affected areas. In the wake of virus activity, mosquito source reduction and control activities were intensified in both areas. During the 1996 EVS season there were no human cases of mosquito-borne encephalitis in the SBCVCP territory.

During the 1996 mosquito season, the SBCVCP disease surveillance group responded to the request of Mohave County Environmental Health, Arizona to evaluate mosquito situation in communities along the Colorado River on the Arizona side across from Needles, CA. Based on mosquito surveys using CO_2-baited traps on June 20-21, 1996 at Willow Valley Marina and Club House, Indian reservation sewage ponds on Plantation Road, Topak Ranchero sites, Pintail Slough and North Dike, 12 *Cx tarsalis* pools tested negative for both SLE and WEE viruses. The expense incurred in these surveys were paid by Mohave County, Arizona.

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REFERENCES CITED

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

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

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

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

Reilly, K.F., M.S. Ascher, D.V. Dondero, B. Enge, F. Ennik, L.T. Hui, W.K. Reisen, D.A. Eliason, L.S. Mian, J. Lin, R.A. Murray, M.M. Milby, J.L: Hardy, S.B. Presser, R.E. Chiles, and W.C. Reeves. 1995. Surveillance for arthropod-borne virus activity and human disease in California during 1994. Proc. Calif. Mosq. Vector Control Assoc. 63: 18-22.

Table 1. Percent species composition of adult female mosquitoes caught in New Jersey light traps and dry -ice (CO^2) baited traps (and number of pools submitted) in San Bernardino County during 1996.

	Percent Composition at					
	DESERT AREA			VALLEY AREA		
Species	N.J. Traps	CO_2 Traps	Total	N.J. Traps	CO_2 Traps	Total
Aedes vexans	0.8	0.3	0.3	0.0	0.0	0.0
Anopheles franciscanus	<0.1	0.3	<0.1	7.6	0.3	1.5
Culex erythrothorax	0.2	0.9	0.4	0.5	0.1	0.2
Culex quinquefasciatus	<0.1	0.0	0.1	48.0	6.4	41.0
Culex stigmatosoma	0.7	0.0	0.5	12.9	46.4	13.3
Culex tarsalis	93.5	98.5	94.6	5.1	32.2	27.9
Culiesta incidens	<0.1	0.0	<0.1	14.2	14.2	14.2
Culiseta inornata	5.0	0.0	4.0	11.7	0.1	1.9
Total Number Caught	**2791**	**764 (17)**	**3555**	**394**	**2049 (51)**	**2443**
Region Total (%)			3,555 (59.3)			2443 (40.7)

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PLAGUE ACTIVITY IN SAN BERNARDINO COUNTY DURING 1996

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ABSTRACT

In 1996, the San Bernardino County Vector Control Program carried out 18 plague surveys at various sites in San Bernardino County. Of the 165 collected animals, 95.2% were ground squirrels, including 142 *Spermophilus beecheyi* and 15 *Spermophilus lateralis*. The remaining animals included seven *Tamias merriami* and one *Silvilagus audoboni*. A total of 546 fleas were collected from these animals. Of the total sera drawn from trapped animals, three tested positive for plague antibodies from two different locations: Glen Helen Regional Park campground and Rio & Baranca group camps (Silverwood Lake area). Upon receipt of laboratory results on plague positive samples, all public use sites showing plague activity were closed to allow for appropriate ectoparasites and rodent control. Public educational information on plague was disseminated through the local press.

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

Historically, plague is reported to have originated in Central Asia and spread to almost all continents of the world. This spread is evidenced from the first pandemic of AD 542, which involved Arabia, Europe and North Africa. The second pandemic, the "Black Death" of the Middle Ages (1300's) covered parts of both Asia and Europe. The third and last pandemic originated in Asia (Southwest China) and spread to South Africa and South America by 1899, then to North America (San Francisco) and Australia (Brisbane and Sidney) by 1900 (Twiggy 1978, Kettle, 1984).

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

Known foci of plague epizootics are distributed throughout the mountains and foothill areas of San Bernardino County. The mountain ranges along with natural recreational lakes provide a wide variety of camping, hiking, and water sport facilities to both local and out-of-county visitors. To safeguard public health and safety in these areas, the San Bernardino County Vector Control Program (SBCVCP) in collaboration with the California Department of Health Services, Vector-Borne Disease Section (CDHS-VBDS) and the United States Department of Agriculture-Forest Service (USDA-FS), carries out

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routine surveillance in plague enzootic areas during the season (April through November). The data generated in routine plague surveillance during 1996 are presented in this paper.

MATERIALS AND METHODS

In routine plague surveys, the general method described by Lang and Wills (1991) was used as follows: In a typical survey, 30-35 Tomahawk #103 live traps (Tomahawk Live Trap Co., Tomahawk, WI) baited with peanut butter and rolled oats were set at appropriate shaded locations close to rodent burrows in the survey area. In a campground situation, traps were also set near picnic tables that attract wild rodents, especially ground squirrels and chipmunks. For location purposes, each trap site was flagged with orange nylon ribbon hung from an adjacent bush or tree. The traps were set by 10:00 a.m. and picked up in the early afternoon the same day.

All traps with live animals were brought to a shady site for processing. Traps with animals were transferred into 45 x 90 cm clear polyethylene bags (3 mil). For each trap, a ball of cotton drenched in ethyl ether was introduced into the bag which was then kept tightly closed with a rubber band until the animal was anesthetized (usually in 5-15 min.). The unconscious animal was taken out of the bag and cage and transferred to a white enamel pan (30 x 20 x 5 cm deep), where the fleas were combed out using a stiff bristled brush. The fleas from each animal were collected in labeled 2 ml polypropylene screw cap tubes containing 2% saline solution. Next, through cardiac puncture, a 3 ml blood sample was drawn from each animal using a 23 gauge syringe. Before the animal regained consciousness and was released back into its habitat, all pertinent data such as species, sex and reproductive stage were recorded. All necessary survey site information was recorded before leaving the area

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

The CDHS-VBDS laboratory immediately informed us via telephone of any plague-positive samples. In the event of plague-positive sample confirmation, the standard plague epizootic protocol (Mian et al. 1996) would be followed. The protocol included posting the area with "Plague Warning" signs, public education, press releases (if warranted), immediate evacuation (if a campground), followed by flea control and rodent suppression (if necessary). Flea control was carried out by dusting rodent burrows with an insecticide (Diazinon® 2D). Post-treatment evaluation of flea index was performed prior to reopening the area for public use, especially a park or campground. Rodent control was done by using a rodenticide (Diphacinone bait blocks or aluminum phosphide tablets). Rodenticide treatment with aluminum phosphide (Fumitoxin®) tablets was done by placing two tablets into each active burrow which was then closed with a ball of crumpled newspaper and sealed with dirt. For follow-up if any, each burrow site was flagged with a bright colored nylon ribbon.

RESULTS AND DISCUSSION

During the 1996 season, the SBCVCP carried out routine plague surveys at various locations situated in plague enzootic mountain and foothill areas of San Bernardino County (Table 1). Of the total 165 animals collected, 157 (95.2%) were ground squirrels, namely 142 (47M, 95F) *Spermophilus beecheyi*, and 15 (8M, 7F) *Spermophilus lateralis*. The remaining animals included seven (2M, 5F) chipmunks, *Tamias merriami* and one (F) cottontail rabbit, *Silvilagus audoboni*. Except for two (one a cottontail rabbit from the Silverwood Lake area and the other a ground squirrel from the Phelan area) tested as carcasses, sera from all other animals were tested for plague antibodies.

Of the total 546 fleas collected from all trapped animals, 41.6% were *Diamanus montanus* (81M and 146F), 16.7% *Hoplopsyllus anomalus* (33M and 58F), 2.2% *Echidnophaga gallinacea* (4M, 8F), 0.9% *Oropsyllus idahoensis* (2M, 3F) and 0.5% *Nosopsyllus faciatus* (3F) with the remaining 38.1% (208) unidentified.

Based on rodent serology results, there were three seropositive samples, showing plague epizootics at two different sites: Glen Helen Regional Park campground and Rio & Baranca group camp sites near the Silverwood Lake area (Table 1). The number of plague-positive rodents (3) in 1996appeared to be 5x and 10X lower than those in 1994 and 1995, respectively. The number of surveys, total rodents trapped and fleas combed out, respectively, were 17, 165 and 511 in 1996, 24, 271 and 932 in 1995 and 23, 314 and 1219 [R1]in 1994 (Mian, et al. 1995, 1996). The Glen Helen Regional Park campground with positive serology and high flea index necessitated burrow dusting with Diazinon7 2D, according to the methods used in earlier surveys (Mian, et al. 1994, 1995). This weekends-only campground was treated during the week. The Rio & Baranca group camps near the Silverwood Lake area were closed and treated with aluminum phosphide at the rate of 2 tablets per burrow to control rodents and their ectoparasites. Burrow treatment at the Silverwood Lake sites was carried out by the certified staff of the California Department of Parks and Recreation. Post-treatment evaluation was carried out by the SBCVCP staff.

During the foregoing plague epizootics, public educational information was provided through local newspapers, radio and television. Plague warning signs were posted immediately and left in place for the remainder of the season in the affected areas. There were no human plague cases in San Bernardino County.

Lastly, a carcass sent to the State Microbial Disease Laboratory from a ground squirrel die-off earlier in the season (May, 1996 - presumably due to plague) in the Phelan area about 10 miles northeast of Wrightwood, tested positive only for *Pasteurella multocida*, the causative agent for avian cholera. *P. multocida* is an opportunistic and facultative pathogen that has a wide mammalian and avian host range. Earlier studies support the occurrence of this bacterium in a wide variety of hosts, from carnivores to lagomorphs to rodents and their fleas (Quan et al. 1986).

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REFERENCES CITED

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

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

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

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

Mian, L.S., C.N. Nwadike, J.C. Hitchcock and M.B. Madon. 1995. Plague activity in San Bernardino County during 1994. Proc. CA Mosq. Vector Control Assoc. 63: 35-38.

Mian, L.S., C.N. Nwadike, J.C. Hitchcock, M.B. Madon and C.M. Myers. 1996. Plague activity in San Bernardino County during 1995. Proc. CA Mosq. Vector Control Assoc. 64: 80-84.

Quan, T.J., K.R. Tsuchiya and L.G. Carter. 1986. Recovery and identification of *Pasteurella multocida* from mammals and fleas collected during plague investigations. J. Wildl. Dis. 22: 7-12.

Salmon, T.P. and W.P. Gorenzel. 1981. Plague, What You Should Know About It. U.S. Dept Agric. And Univ. CA Coop. Exten. Leaflet No. 21233. 14 pp.

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

Table 1 Summary of plague surveys carried out in San Bernardino County during 1996

Survey Date	Location (Altitude in feet)	Number of Sera Tested (M/F)	Tested Positive	Number of Ectoparasites (M/F)	Flea Index
3/2	Silverwood Lake Rec. Area (3700)	1 (0/1) c/	0	0	0
4/11	Hesperia-7800-8000 W. Arrowhead Lake Rd. (4200)	4 (1/3)	d/	3 (1/2)	0.7
5/2	Hanna Flat CG a/ (7000)	15 (4/11)	e/	26 (10/16)	1.7
5/3	Phelan-5048 Sunnyslope (4200)	1 (1/0) c/	f/	0	0
5/3	Serrano CG (6800)	7 (3/4)	0	77 (25/52)	11.0
5/21	Barton Flats CG (7000)	19 (2/17)	e/	46 (17/29)	2.4
6/3	San Gorgonio CG (7000)	14 (2/12)	-	16 (3/13)	1.1
6/13	Silverwood Lake CG (3800)	16 (3/13)	-	26 (12/14)	1.6
7/3	Glen Helen Reg. Pk. CG (2700)	15 (6/9)	2 (1:32)	38 (14/24)	2.5
7/8	Holcomb Valley CG (7400)	13 (8/5)	0	29 (13/16)	2.2
7/15	Tanglewood CG b/ (7700)	0	-	0	0
7/31	Glen Helen Reg. Pk. CG (2700)	21 (11/10)	e/	98 (46/52)	4.7
8/14	Glen Helen Reg. Pk. CG (2700)	4 (2/2)	g/	0	0
8/27	Applewhite CG (3300)	5 (1/4)	0	20 (not sexed)	4.0
9/9	National Children's Forest (6800)	3 (1/2)		0	0
9/16	Northshore CG (5300)	13 (5/8)	0	45 (not sexed)	3.4
9/23	Rio & Baranca CG (4200)	14 (8/6)	1 (1:128)	122 (not sexed)	8.7
11/6	Rio & Baranca CG (4200)	N/A	h/	0	-
Total		**165 (58/107)**		**546**	

a/ Campground.
b/ Group Camp.
c/ Tested as a carcass.
d/ Not tested.
e/ Pre-treatment survey.
f/ Tested positive for *Pasteurella multocida.*
g/ Post-treatment survey.
h/ Post-treatment evaluation based on burrow swabbing of two reopened burrows out of a total of 42 burrows treated with aluminum phosphide tablets at the rate of two tablets per burrow.
N/A-not applicable.

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VECTOR MONITORING OF GREEN LEAF WASTE AT A SANITARY LANDFILL IN SAN BERNARDINO COUNTY

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ABSTRACT

The San Bernardino County Vector Control Program (SBCVCP) carried out studies on the vector monitoring of the green leaf waste at the Mid-Valley Sanitary Landfill, Rialto, during 1995. Data generated in these studies showed that except for one weekly observation (October 26, 1995) during the 53-week study period, fly grill counts remained below 6, the threshold considered under the performance standards pursuant to Section 17683, Title 14, California Code of Regulations (CCR). The fly grill count of 15.6 on October26, 1996 was a very rare occurrence which was due to a combination of factors such as right temperature, humidity and wind speed, most favorable for fly activity on that day. Except for one yellow jacket wasp, and a couple of bees on a few occasions, no cockroaches or other insect vectors were observed during these weekly surveys. Also, no breeding of mosquitoes was found at the three catch basins or in used tires temporarily stored at the landfill. In monthly rodent surveys, no domestic rats or any members of the genus *Rattus*, were trapped at the active site. Among field rodents, ground squirrels, kangaroo rats, deer mice and harvest mouse were trapped at the landfill. However, trapping near the green leaf waste at the active site, yielded 1 to 4 deer mice on three occasions and one ground squirrel during the entire study period. During the weekly observations, bird activity at the landfill site was frequently noticed. Crows, seagulls and pigeons were more apparent than passeriformes—sparrows and finches, at the active site. The overall data on vectors generated in these studies suggest that the green leaf waste meets the performance standards for an alternate daily cover to be used on the active face of the daily lift. It needs to be emphasized that the use-timing of the green leaf waste, i.e. from the late afternoon through early next morning (7:30 a.m.), further minimizes the likelihood that activity of diurnal flies will be significant.

Sanitary landfills or solid waste disposal facilities operate under permits by local enforcement agencies and comply with all applicable laws and regulations as defined by the California Code of Regulations (CCR) and the California Integrated Waste Management Board (CIWMB). Pursuant to Section 17683, Title 14 of CCR under the performance standards, the trash at sanitary landfills is compacted and covered daily with a 6 inch thick layer of soil on the top, sides and active face of the trash lift. Besides other uses, the daily soil layer is used to deter the attraction and propagation of flies, rodents and other vectors of diseases at these facilities. To offset the cost of soil and soil hauling to the site, many landfills in southern California have been faced with evaluating alternate materials such as green leaf waste, synthetic geo-textile cover, etc., as substitutes to the soil cover especially at the active face of the lift. Besides other requirements, the substitute cover must meet the performance standards pursuant to Section 17683 of CCR.

In evaluating the use of pulverized green leaf waste as an alternate daily cover at the Mid-Valley Sanitary Landfill in Rialto, The San Bernardino County Vector Control Program (SBCVCP) in an agreement with the San Bernardino County Solid Waste Management Department, carried out the monitoring of various vectors at the green leaf waste. In accordance with the performance standards criteria, this paper presents data on the vector monitoring of green leaf waste at the Mid-Valley Sanitary Landfill during a 12-month period, January 9, 1995-January 8, 1996.

MATERIALS AND METHODS

A study was conducted on the vector monitoring of the green leaf waste at the Mid-Valley Sanitary

Landfill in Rialto, California. The study site and vector monitoring procedures used in the study were as follows:

Study Site. The study sites were situated in the Mid-Valley Sanitary Landfill located at the northeastern corner of Highland Avenue and Sierra Avenue, Rialto, CA. Of the total 302-acre county-owned landfill area, 142 acres were used for actual landfilling; the latter was divided into two parcels namely, an 82-acre triangular shaped northern parcel and a 60-acre rectangular southwestern parcel. Another 5-acre area was used for gas flow station and recycling , whereas the remainder 160 acres were not permitted for landfilling. Three catch basins approximately 1-acre each, were situated to the east of the southwestern parcel of the landfill.

Landfill Operations. The landfill operation was based on the area-fill method where daily refuse was placed in 10-15 feet thick lifts each consisting of 2 feet compacted layer of refuse with maximum perimeter slopes of 3:1 (base to height). Pursuant to Title 14, CCR, a 6-inch thick cover of soil was placed daily on the top and sides of each advancing lift. Refuse and cover soil were compacted and graded to drain run-off water from rain. An average of 450 cubic yards of soil cover was used daily at the landfill. A more detailed description and operation of the landfill can be found in the San Bernardino County Mid-Valley Solid Waste Disposal Facility, Report of Disposal Site Information (EMCON 1994)

Survey Methods. The monitoring methods of various vectors used in these studies were in accordance with the performance standards in Section 17683, Title 14, CCR. The vectors monitored in these studies included muscoid flies, domestic rats, field rodents, mosquitoes and other vectors such as cockroaches wasps, etc.

A. Flies. The flies under surveillance at the green leaf waste, Mid-Valley Sanitary Landfill, included members of the families Anthomyiidae, Muscidae, Calliphoridae, Sarcophagidae and Drosophilidae.

Prior to fly grill observations, weather data such as temperature, relative humidity, wind velocity and sky conditions were recorded. Temperature and relative humidity were measured using a VWR® digital Humidity/Temperature Meter (model #35519-043, Control Company, Friendswood, Texas) and wind velocity was read from an anemometer (Turbo Meter, Davis Instruments, Hayward, California).

Weekly fly grill surveys were carried out during the afternoon hours when the green leaf waste was available at the active site. The grill used in these surveys was constructed at SBCVCP with the construction similar to the typical scudder fly grill (Scudder 1949); it consisted of 24 slats each 36 x 0.75 x 0.25 in. placed 0.75 in. apart on a Z-shaped frame. Each observation consisted of placing the grill at one spot over the green leaf waste for 4 minutes and then during the next approximately 30 seconds all flies landing on the grill were counted. Flies alighting repeatedly on the grill were counted only once. Using different sampling spots at the green leaf waste, ten fly grill counts were made. Of the 10 counts, five with the highest counts were averaged to obtain the mean value for that survey.

During the weekly surveys for flies, visual observations on other vectors such as cockroaches, wasps, etc. were recorded along with information on other animals especially birds and mammals found at the active site. Moreover, mosquito breeding, if any, was monitored at the three catch basins as well as used tires when present. Sampling was done by taking at least 5 dips at several accessible spots at the basins, using the standard mosquito dipper.

For monthly fly composition studies, six 22.4 x 1.6 in. sticky tapes (Aeroxon FlyCatcher™ Roxide International, Inc., New Rochelle, New York) were hung near the active site. These tapes were picked up after one week. Due to windy conditions and dust interfering with the efficacy of sticky tapes, monthly sweeps (n=4) of on-wing flies at the green leaf waste were also carried out using standard insect collection net. Flies collected on sticky tapes or those collected in sweep nets and killed in an insect killing jar, were brought to the laboratory. All specimens were identified to family, genus and species using both descriptive and pictorial keys by Ecke (1963), Pratt et al. (1976) and Axtell (1986).

B. Rodents. To monitor rodent activity at the landfill, two trap lines each consisting of twenty traps were operated overnight each month during 1995. Each trap line in turn had equal numbers of both National and Sherman type live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin). Traps baited with rolled oats were set up approximately 20 ft. apart. Of the two trap lines, one was operated close to the active surface and the other was run on the periphery in a suitable undisturbed location at the landfill. During the trapping activity, visual arrays. A summary of the technical specifications for each piece of spray equipment used in the District's AHB program is provided in Table 1. On several occasions local municipalities allowed the use of their truck-mounted "cherry pickers" to access tree top swarms that were situated well beyond the reach of the orchard sprayer.

M-Pede Usage in Swarm and Colony Control. A preferred method for treating honey bee swarms or colonies is the application of an aqueous solution of insecticidal soap and/or surfactant (Sames et al. 1991, Visscher et al. 1995). At the time of this writing, the only product labeled for this purpose in California was M-Pede insecticide (Note: use facilitated by a 24-C local need). The results of our treatments using different quantities of M-Pede applied to different sizes of swarms and colonies are summarized in Fig. 4. Overall, the quantity of M-Pede applied was directly proportional to the number of bees being treated. Swarm size was qualitatively rated by relative size in relation to common spherical objects. Small swarms were rated as being "softball" size or smaller and contained an average of 5,000 bees. Medium "football" or "volleyball" sized swarms were comprised of 5,000 to 20,000 bees. Large swarms greater than or equal to the size of a "basketball" usually contained more than 20,000 bees. The average volumes of M-Pede applied per treatment for small, medium and large swarms was 4.0 (N=68), 6.6 (N=51), and 9.0 (N=80) fluid ounces, respectively.

Swarm Control: Honey bee swarms by their nature are not aggressive, but caution should be exercised when removing or spraying a swarm with an approved insecticide. The necessity for caution is advised because some "exposed" colonies with no visible evidence of comb can be mistaken for a swarm.

Since human activity around a swarm can present problems in establishing a safe boundary before removal, the District's pretreatment protocol included procedures for clearing the immediate area and advising all spectators to either leave the area or proceed indoors for their personal safety. Once the area was secured, treatment of a swarm was preceded by placing a plastic tarp beneath the swarm (not always practical) to catch the dead bees as they fell from the swarm mass. The actual treatment began with the application of a fine mist that enveloped the swarm mass [. The swam was continuously misted until the outer layer of bees sloughed to the ground to expose more bees . This process was continued until only a small number of bees remained attached to the original swarm site. These subsequently were removed by hand or dislodged by shaking the substrate supporting the swarm. On an operational note, it is recommended from our observations that power sprayer nozzles be kept at least 10 feet from the swarm; too much pressure from the force the spray stream will tend to literally blow the swarm apart, spreading angry bees everywhere.

Table 1. Application equipment used in the Orange County Vector Control District to remove honey bee swarms and established colonies.

Backpack Sprayer (portable)
- Used in 94 percent of swarm calls
- Effective on swarms within 15 feet of the ground
- 2.5 gallon capacity with 1.5[RP1] wand and adjustable nozzle
- One gallon per minute application rate
- Average application in the range of 4.0-9.0 fluid-ounces of M-Pede

Orchard Sprayer (vehicle mounted)
- Used in 67 percent of all service requests (1995 data)
- Effective on swarms between 15 and 30 feet above ground
- 50 gallon tank capacity with interchangeable nozzle tips
- 5 gallons per minute application rate
- Average application in range of 25.0 to 60.0 fluid-ounces of M-Pede

Hand Duster (portable)
- Plunger style for ground applications of dusts
- Used in a majority of confined space applications
- Effective on colonies in tree rot cavities and utility vaults
- Dusts applied at label rates (varies by product)

Colony Control: The treatment protocol used for eliminating an established honey bee colony was similar to that used for controlling a swarm with increased caution required to avoid being stung during the process of removing the honeycomb. Preceding any control action against a colony in a residential setting, all surrounding residents were moderating influence of maritime conditions Observed honey bee activity in this region was considered not to be significant as indicated by the fewest number of service requests received from coastal communities. The comparatively minimal service to this area during peak swarming in May and June may have been due to the daily formation of coastal fogs that often extended more than 10 miles inland. This is supported by the fact that further inland, calls were received both during the morning and afternoon hours, but were not received in the coastal region until the afternoon fog burn-off; the presence of the fog layer in the morning likely suppressed swarming. activity.

AHB Surveillance. A county wide array of 19 standard blue 5-gallon (bucket-type with snap-on lids) bee traps was placed by the Agricultural Commissioner's office during the spring of 1992 in anticipation of the imminent arrival of the AHB from the southern deserts. At the time of placement, bee traps were inspected biweekly during the honey bee swarming season and once every 30 days between October and March. With the District's new role in AHB management within the County, surveillance activities were transferred from the Agricultural Commissioner's Office to the District in 1994. Since then, the District AHB staff have been responsible for maintaining and monitoring all bee traps deployed within the County. The current deployment array places bucket traps at sites likely to intercept AHB's dispersing into the County from the Santa Ana River corridor, Ortega Highway along route 78 and San Clemente up the coast from Camp Pendelton via San Mateo Creek and the balance of San Diego County. Supplemental surveillance is being provided by identifying samples of swarms and colonies obtained from routine control operations. Samples of bees taken either from bee traps, swarms or colonies currently are being identified by the Agricultural Commissioner's office using the FABIS (Fast Africanized Honey Bee Identification System) test, with further DNA testing at the State Entomology Lab if a sample is "suspect" using FABIS wing length parameters [e.g., wing length mean (N=10) of less than 9.01 mm).

APPLICATION EQUIPMENT AND CONTROL STRATEGIES

Application Equipment. Each AHB control technician is provided with an open-bed pickup truck that supports a vehicle mounted orchard sprayer plus portable auxiliary spray equipment consisting of a backpack sprayer and manual hand duster (Fig. 3.). A rear-mounted locking tool compartment accommodates wash water and chemical storage while a cab-high steel rack running the length of the cargo bed carries ladders and supplemental lighting

Fig. 3. District "bee vehicle" fully complemented for field operations.

County for supporting substantial feral bee populations.

The coastal (C) region was characterized by warm winters and cool summers caused by the

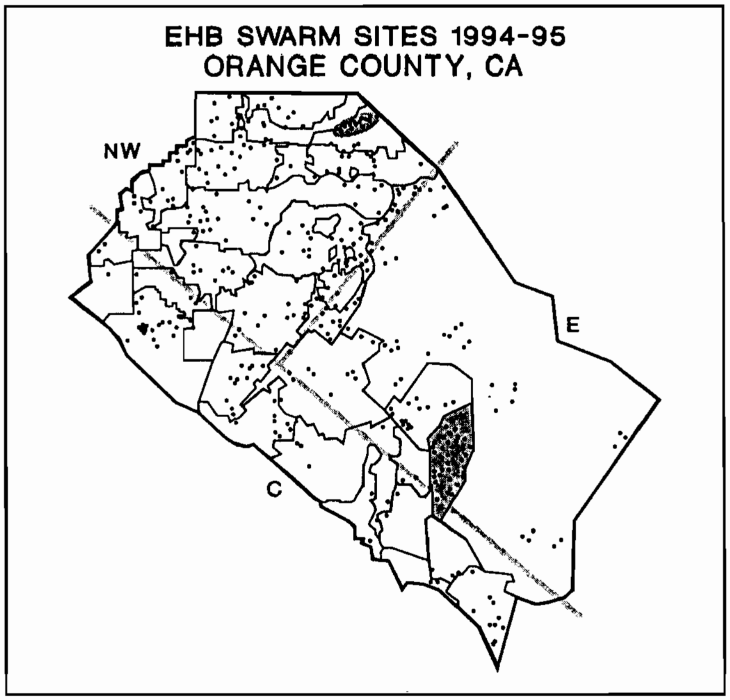

Fig. 1. Distribution of honey bee service requests in the northwestern (NW), eastern (E), and coastal (C) regions of Orange County, 1994-1995.

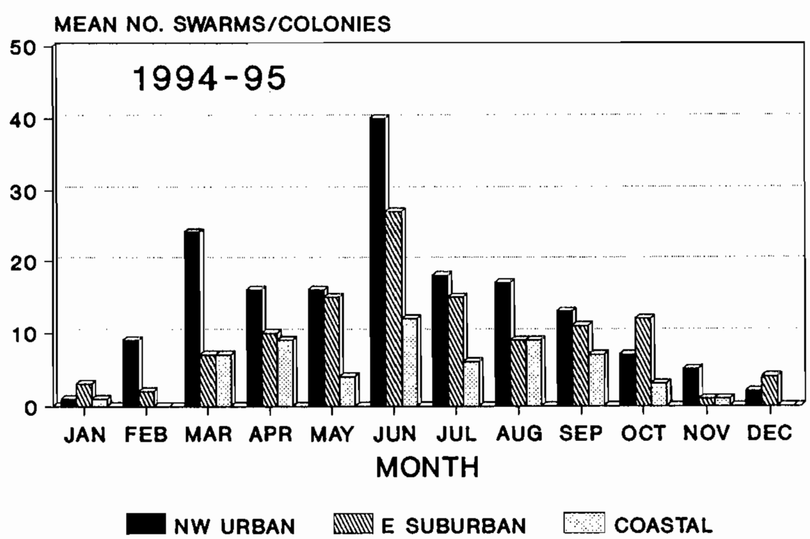

Fig. 2. Monthly distribution of service requests in the northwestern, eastern, and coastal regions of Orange County, 1994-1995.

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ORANGE COUNTY VECTOR CONTROL DISTRICT'S AFRICANIZED HONEY BEE PROGRAM: MANAGING EUROPEAN HONEY BEES IN ADVANCE OF THE ARRIVAL OF THE AFRICANIZED HONEY BEE INTO ORANGE COUNTY CALIFORNIA.

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Africanized Honey Bees (*Apis mellifera scutellata* forms - AHB) are the most recent challenge to agricultural and public health interests in California. With the approach and subsequent arrival of the AHB into the southern desert regions of the state, the anticipated infiltration of the bees into Orange County prompted the Orange County Vector Control District's Board of Trustees to respond by directing the District to develop and implement an AHB control program. Accordingly, an AHB program was established in April of 1994 with the hiring of 4 technical staff and a "bee team" supervisor supported by equipment dedicated to controlling honey bees. The initial purpose of the program was to develop familiarity with managing honey bees and gain valuable experience with the application of effective control measures. With the support of the Orange County Agricultural Commissioner's Office, a special local need (SLN) was issued to the District that provided for controlling swarms and colonies of existing European Honey Bees (EHB, *Apis mellifera ligustica*) to gain experience in bee control before the arrival of the AHB. This paper briefly summarizes the District's AHB control program with particular emphasis on logistics, response to honey bee service requests, surveillance activities, control techniques, equipment applications and public education.

PROGRAM OVERVIEW

The initial objective of the District's program was to establish public contact, develop a protocol for responding to honey bee service requests, and deploy a surveillance network to detect the arrival of the AHB into the County. The District's intent to "engage" the AHB was conveyed to the media, private sector, and other agencies that ultimately would be involved with AHB's. Honey bee calls normally received and processed by the Orange County Agricultural Commissioners Office were directed to the District where they were evaluated and control teams dispatched when justified by the situation. As of January 1996, over 1,029 service requests regarding "bees" were received by the District. Of these, approximately 507 involved honey bees, with the remaining service requests being associated with either information or response to other Hymenoptera including wasps, yellow jackets, and bumble bees.

Operational Regions. Environmental heterogeneity justified segregating the District into three operational regions based upon local ecological affinities (Fig. 1). The northwestern region, (NW) characterized by heavily populated urban and residential developments was interspersed with park lands, riparian corridors, and man-made drainage conveyances. Included within this area were several large commercial apiaries that provided a continuous source of "surplus swarms". Overall, this region produced the greatest number of service requests linked to resident reports of bees in highly populated areas (Fig. 2).

The east (E) region was less developed and included large tracts of upland brush lands (e.g., chaparral), canyons, and riparian corridors emanating from the west slope of the Santa Ana Mountains. By comparison to those in the northwestern region, commercial apiaries in this region were situated in remote areas well removed from established urban and residential developments. Not surprisingly, fewer service requests were received from residents in this area even though local environmental conditions are perhaps the best in the

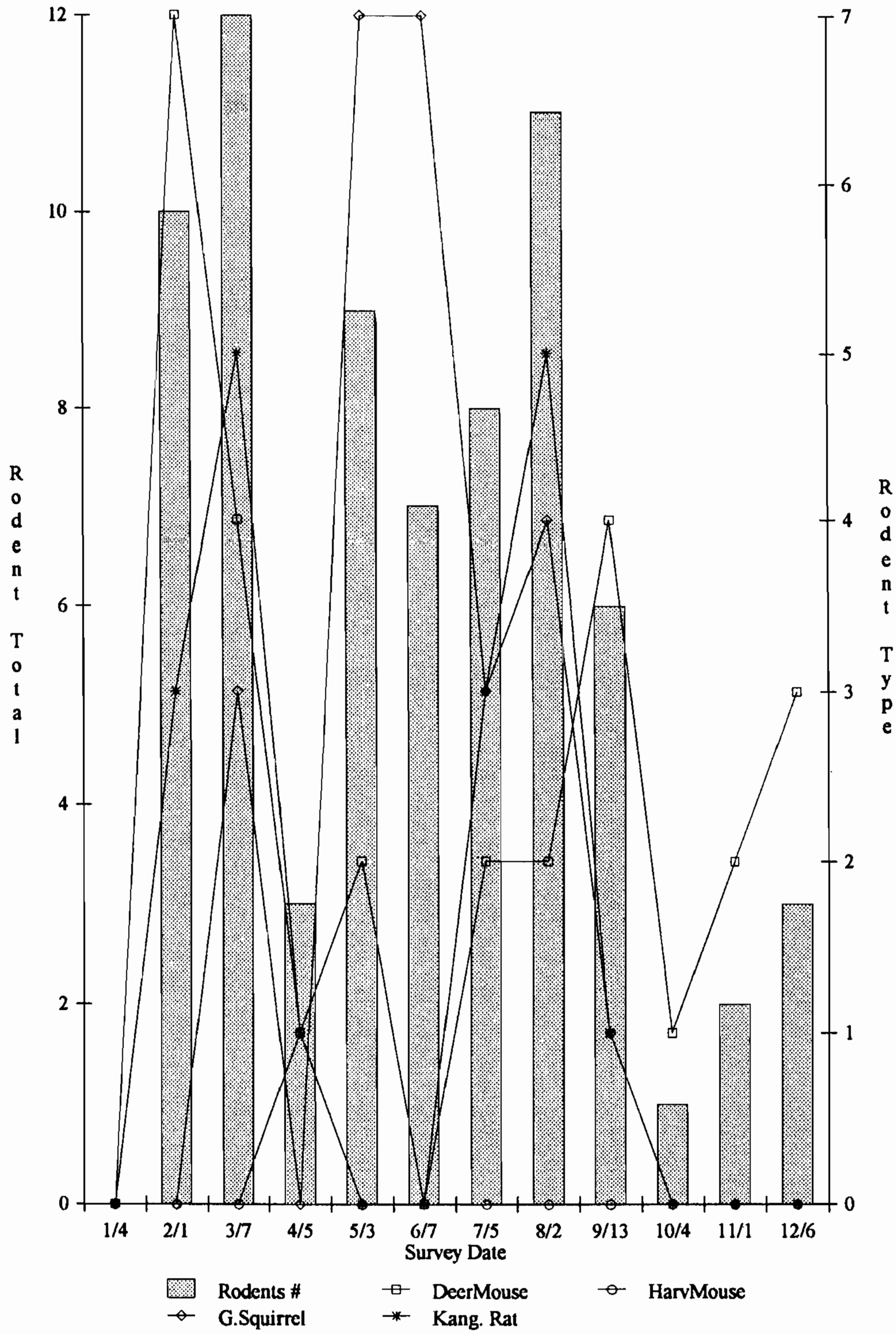

Figure 3. Monthly rodent survey data at Mid-Valley Landfill, 1995.

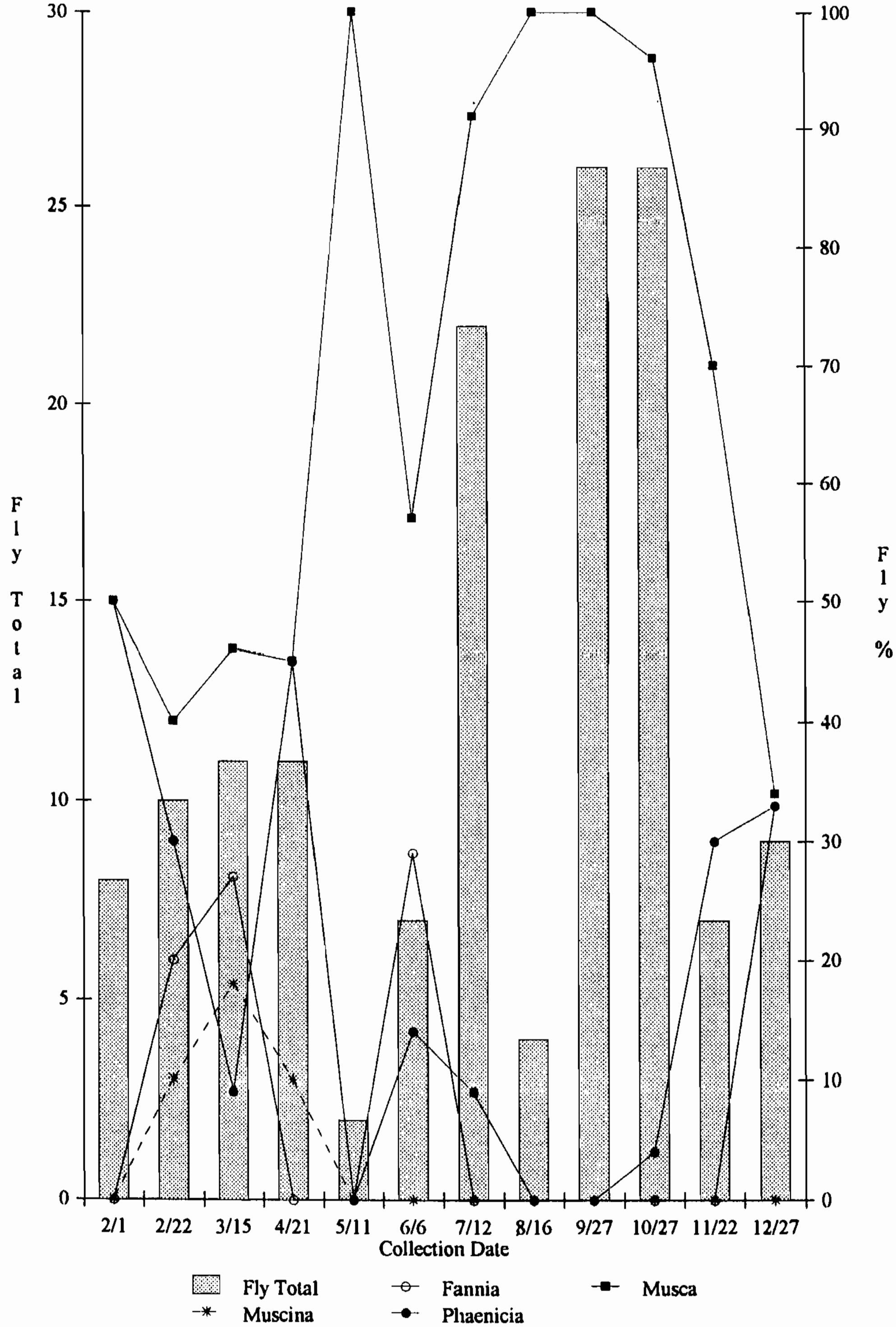

Figure 2. Monthly fly composition at the green waste, Mid-Valley Landfill, 1995.

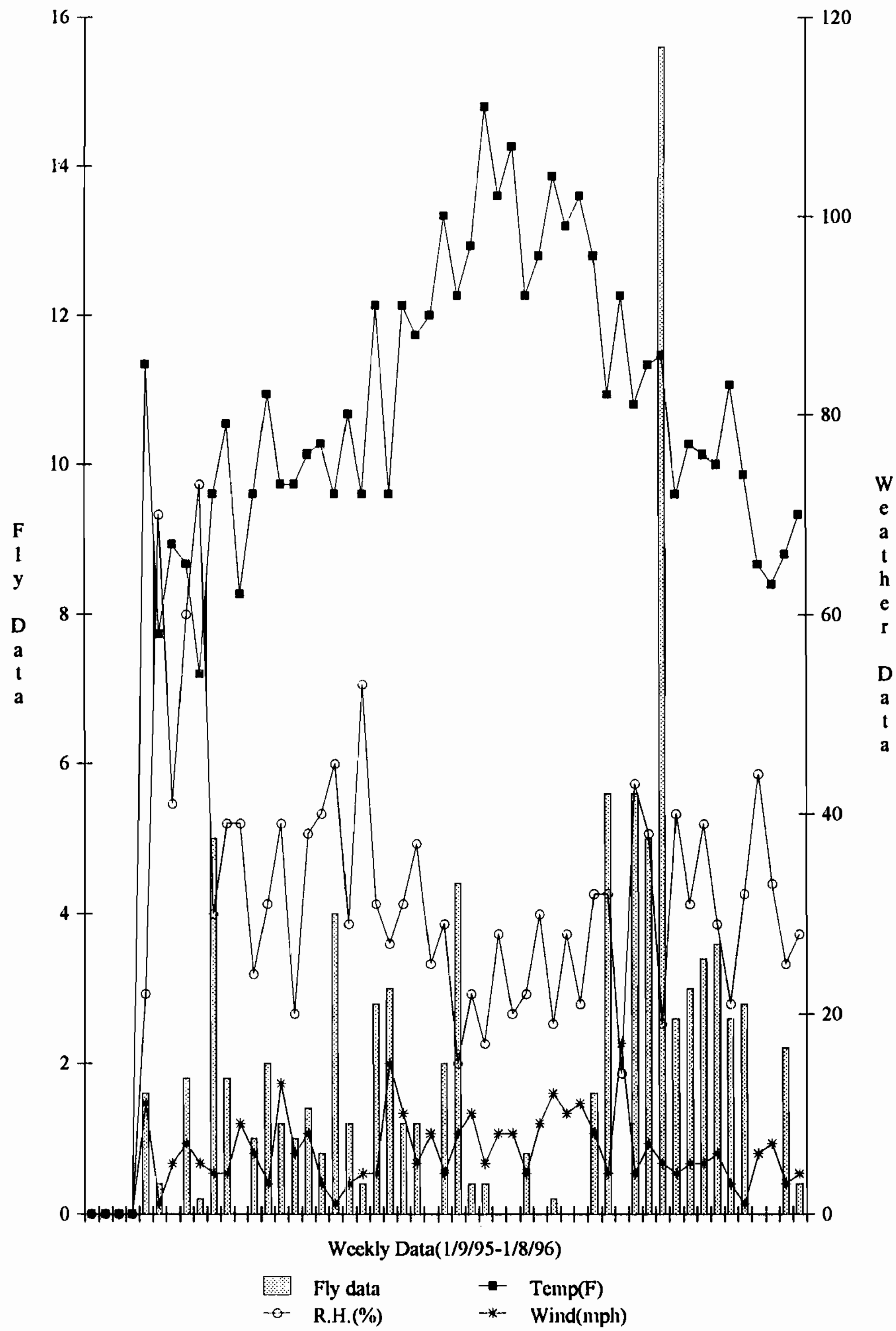

Figure 1. Weekly fly population and weather data at the green waste, Mid-Valley Landfill, 1995.

Guide to the Mammals, North America North of Mexico. 3rd Edition. Houghton Mifflin Company, Boston, MA. 289 pp.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1-42

Ecke, D.H. (ed.). 1963. A Field Guide to the Domestic Flies of California. California Mosquito and Vector Control Association-Entomology Committee. Visalia, CA. 31 pp.

Mian, L.S. 1994. Population dynamics of muscoid flies at three commercial poultry ranches and associated fly complaints in San Bernardino County. Proc. Calif. Mosq. Vector Control Assoc. 61: 63-69.

Pratt, H.D., K.S. Littig and H.G. Scott. 1976. Flies of Public Health Importance and Their control. HEW Public No. (CDC) 76-8218. U.S. Department of Health, Education and Welfare, Public Health Service, Centers for Disease Control, Atlanta, GA. 46 pp.

EMCON 1994. San Bernardino County Mid-Valley Solid Waste Facility-Report of Disposal Site Information (Revised, 1994). Colton, CA. 59 pp.

Scudder, H.J. 1949. Some principles of fly control for the sanitation. Amer. J. Trop. Med. 29: 609-623

Zeiner, D.C., W.F. Laudenslayer, Jr., K.E. Mayer and M. White (Eds.). 1990. California's Wildlife. Volume III: Mammals. California Department of Fish and Game. Sacramento, CA. 407 pp.

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was low (<5 flies/grill) under prevailing conditions of temperature, humidity and wind. The only exception was week #43 (October 26, 1995) when the fly grill count rose (15.6) above the threshold value of 6. Evidently this was due to a combination of weather conditions (Temp. 86°F; R.H. 19%; wind 4.5 mph). This could further be explained by comparing these figures with the data of week #40 (October 4, 1995) when prevailing conditions, i.e. temp. 91.6°F; R.H. 14% and wind 16.8 mph, resulted in zero flies alighting on the grill. Clearly high wind was the dominant factor followed by high temperature and low humidity affecting fly activity at the green leaf waste. Week #34 is another example where the combined effect of the three abiotic factors was evident especially when compared with data of the two earlier (#31 and #32) and two later weeks (#36 and #37).

Apart from weekly observations on fly activity, monthly species composition data were plotted against total fly numbers collected on sticky tapes or insect net sweeps (Fig. 2). The faunal composition consisted of four main species namely little house fly, *Fannia canicularis L.*, common house fly, *Musca domestica L.*, false stable fly, *Muscina stabulans* (Fallen) and green blow fly, *Phaenicia sericata* (Meigan). During the first quarter, especially February and March, 1995, all four fly species were found in varying numbers with *M. domestica* being the highest followed by *F. canicularis*, *P. sericata* and *M. stabulans*. During the rest of the year, *M. domestica* remained to be the dominant fly with occasional peaks shown by *P. sericata* and *F. canicularis*. The seasonal distribution of these flies especially of *F. canicularis* and *M. domestica* is similar to fly distribution reported in earlier studies in the area (Mian 1994).

Besides the above-mentioned flies, other less important and smaller species that were found in the monthly samples included the black garbage fly, *Ophyra leucostoma* (Wiedeman), black scavenger fly (Sepsidae), the pomace fly, *Drosophila* sp., and small dung fly (Sphaeroceridae). Seasonally, the occurrence of these flies was sporadic during the first half of the year except for *O. leucostoma* that also showed up during the last quarter.

Monitoring of other insect vectors such as mosquitoes, cockroaches wasps, etc., was also carried out during weekly samplings. The three catch basins and used tires stored occasionally at the landfill showed no mosquito breeding. Also, no cockroaches or other vectors, except for a yellow jacket wasp and few honeybees, were noticed at the green waste site.

Rodent monitoring yielded no domestic rats belonging to the genus *Rattus*, e.g., roof rat, *Rattus rattus L.* and Norway rat, *Rattus norvegicus* (Berkenhout) (Muridae), during the entire study period. Other rodents that were trapped are shown in Fig. 3. Ground squirrel, *Spermophilus beecheyi* (Sciuridae), a known vector of sylvatic or rural plague, was found more during the second and third quarters. The Pacific kangaroo rat, *Dipodomys agilis* (Heteromyidae), showed a somewhat similar seasonal distribution. Deer mouse, *Peromyscus maniculatus* (Cricetidae), a known vector of Hantavirus, was more abundant during the first and third quarters, whereas the western harvest mouse, *Reithrodontomys megalotis* (Cricetidae), was trapped only once during the month of April.

Most of the rodents were trapped at the southwestern parcel of the landfill and away from the active site. At the active site, there were 1, 1 and 5 deer mice during February, May and September respectively; there was only one ground squirrel trapped by the active site during September.

Apart from rodents, two lagomorphs, namely cottontail rabbit and jack rabbit, were observed away from the active site. Among birds, crows, seagulls and pigeons were more frequently observed by the active site than smaller passeriformes such as sparrows and finches.

In light of the foregoing discussion on the vector monitoring of the green leaf waste as an alternate daily cover on the active surface, it is quite evident that the green leaf waste does not pose any significant health risk with respect to the attraction and propagation of disease vectors such as filth flies, rats and other rodents. Moreover the daily time period (late afternoon through next morning, 7:30 a.m.) during which the green leaf waste will stay on the active surface, further minimizes the chances of fly activity to be noticeable since flies are diurnal insects.

REFERENCES CITED

Axtell, R.C. 1986. Fly control in confined livestock and poultry production. Technical Monograph. CIBA-GEIGY Corp., Greensboro, NC. 59 pp.

Burt, W.H., and R.P. Grossenheider. 1976. A Field

observations of field rodents or other animals were also recorded. Identification of collected rodents was carried by using available identification keys (Burt and Grossenheider, 1976; Zeiner et al. 1990).

(Table 1) These variations were due to changes in weather conditions such as temperature, relative humidity (R.H.), wind speed (mph), etc. The data on fly populations presented along with corresponding

Table 1 Mean weekly fly grill counts taken at the green leaf waste, Mid-Valley Landfill, Rialto, CA during 1995-96

Week number[1]	Date	Mean number of flies[2]	Week number	Date	Mean number of flies
5	02/01/95	1.6 ab[3]	30	07/26/95	0.4 a
6	02/08/95	0.4 a	31	08/02/95	0
7	02/15/95	0	32	08/09/95	0
8	02/22/95	1.8 ab	33	08/16/95	0.8 a
9	03/01/95	0.2 a	34	08/23/95	0
10	03/07/95	5.0 ab	35	08/29/95	0.2 a
11	03/15/95	1.8 ab	36	09/06/95	0
12	03/22/95	0	37	09/13/95	0
13	03/29/95	1.0 a	38	09/20/95	1.6 ab
14	04/05/95	2.0 ab	39	09/27/95	5.6 bc
15	04/13/95	1.2 ab	40	10/04/95	0
16	04/21/95	1.0 a	41	10/11/95	5.0 ab
17	04/26/95	1.4 ab	42	10/18/95	5.0 ab
18	05/03/95	0.8 a	43	10/26/95	15.6 c
19	05/11/95	4.5 ab	44	11/01/95	2.6 ab
20	05/17/95	1.2 ab	45	11/08/95	3.0 ab
21	05/24/95	0.4 a	46	11/16/95	3.4 ab
22	05/31/95	2.8 ab	47	11/22/95	3.6 ab
23	06/07/95	3.0 ab	48	11/29/95	2.6 ab
24	06/14/95	1.2 ab	49	12/06/95	2.8 ab
25	06/21/95	1.2 ab	50	12/12/95	0
26	06/28/95	1.2 ab	51	12/20/95	0
27	07/05/95	2.0 ab	52	12/27/95	2.2 ab
28	07/12/95	4.4 ab	53	01/04/96	0.4 a
29	07/19/95	0.4 a			

[1] Due to rain during weeks 1 through 4, no data was taken.

[2] Mean of 5 fly grill counts.

[3] Means followed by the same letter(s) are not significantly different from one another at P ≃ 0.05 (Multiple range test, Duncan 1955). Due to some zero values in the data, the analysis of variance was carried out on transformed data using the square root ($\sqrt{X+1}$) transformation.

Last but not least, proper safety precautions were duly taken by the SBCVCP staff during all vector monitoring activities at the landfill. The staff always wore a hard hat and orange vest while at the landfill. Moreover, during monthly rodent surveys, the survey personnel followed all necessary precautions by wearing Tyvek® suit (Lyons Safety, Inc. Carson, CA) and proper respirator (battery powered air pressure type with HEPA 10 filter) to guard against the Hantavirus or other air-borne pathogens carried by wild rodents.

RESULTS AND DISCUSSION

Statistical analysis of fly data showed significant variations from week to week during the study period

temperature, relative humidity, and wind speed data (Fig. 1), show that except for week #43 observations, the mean fly grill counts during 1995 remained below 6, a threshold value under the performance standards. Looking at the combined effects of temperature, humidity and wind velocity, any deviation from optimum conditions (temperature 75-85°F; R.H. 40-60%; wind<5 mph) could significantly affect the activity of adult flies. Notwithstanding slight variations due to the aforementioned abiotic factors, mean fly numbers stayed at ≤ 5 flies/grill during the first 25 weeks of the study period. During weeks #25 through #40, very high temperature (90-110°F) and low R.H. (<30%) coupled with wind speed >5 mph, had a significant effect on the movement of flies. Fly activity at weeks #40 to #50 advised of our activities and cautioned to stay indoors until the treatment was completed. Adjacent residents not home at the time of our control actions were informed by proper notification left at their front door. Treating a colony commenced with misting the outer surface of the colony plus comb to preempt any bees from flying off before the main body of spray was applied. Once soaked with M-Pede, the outer layer of bees quickly succumbed with subsequent spays applied as needed to untreated bees that were encountered as the comb was removed in either layers or sections. In treating colonies, an effort was made to remove as much of the comb as possible to discourage the site from being reused by future colonizers.

Remnant Trapping. Treatments of swarms and colonies invariably leave a small number of bees alive that either were not contacted by the insecticide or were in the field foraging at the time of the treatment. These "stragglers" can present a problem where a swarm or colony has been removed from sites juxtaposed to residents, schools, and common areas. As a safeguard against residual bees, various types of remnant traps (Visscher and Khan, unpublished data) baited with aggregation pheromones (e.g., Queen Mandibular Pheromone or QMP) have been used with great success in capturing most straggler bees. Subsequent to the treatments of swarms and colonies in sensitive situations, remnant traps were placed within three feet of the treated site and left overnight. The following morning remnant traps were retrieved, placed in plastic ziplock bags and the trapped bees destroyed by agitating the M-Pede supplied to the reservoir of the trap (Tapper et al. 1997).

Confined Space Treatments. Colonies found inside cavities associated with trees, fences, and other non-structural situations were treated with insecticidal dusts that produced a rapid knockdown. Of the three dusts (e.g., Drione, Ficam "D", and Tempo) used in confined space treatments, Ficam "D" was the most effective followed by Tempo and Drione, respectively.

The basic technique used for introducing dusts into the colony space involved placing the nozzle of the duster within the access opening and delivering several "puffs" from either a bellows or plunger style duster. Knockdown usually occurred within minutes of treatments with occasional retreatments required if a significant portion of the bees failed to drop. Plugging the entrance after the introduction of the dust also assured a complete treatment by not allowing any of the sequestered bees to escape prematurely or avoid being contacted by the dust.

PUBLIC EDUCATION

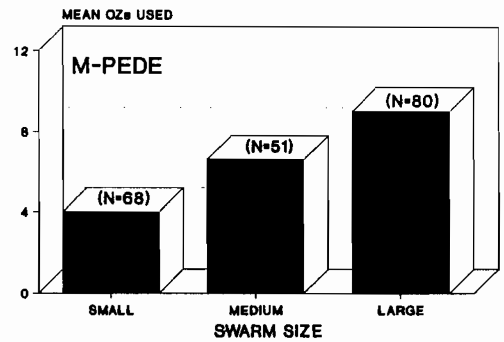

Fig. 4. Mean fluid ounces of M-Pede insecticide used for small, medium and large sized honey bee swarms and colonies.

One of the key elements of the District's AHB control program is public awareness in coping with the eventual arrival and establishment of the AHB within Orange County. The District's public education coordinator has compiled a multimedia program to inform the public of the risks associated with the presence of the AHB. At the core of the program are "common sense" preventative measures intended to illustrate that simple precautions can significantly reduce the chances of being accidentally stung in the backyard, at school, and while recreating at County parks. Special programs designed for schools inform students (primarily grades 3 through 6) of: 1) the importance of honey bees in nature, 2) history of the AHB in the Americas, 3) differences between AHB's and EHB's, 4) common sense precautions to avoid being stung, and 5) ways to make the home and backyard environment less attractive to AHB's.

SUMMARY

The Orange County Vector Control District's Africanized Honey Bee Program and attendant experiences with EHB's has provided an excellent model in advance of the arrival of the AHB. The existing array of surveillance traps plus identification of treated swarms and colonies appears to be an adequate method of detecting AHB infiltration into Orange County. Operational familiarity with treating and removing swarms or colonies has provided our "bee team" with valuable experience and time to refine various technical aspects associated with the art of bee control. Our public education program also has been field-tested with great success in the classroom and other venues where informative presentations have been well received.

ACKNOWLEDGMENTS

The authors wish to extend their appreciation for the contributions of the following District Staff for their efforts in the development of our AHB program: Stephen G. Bennett, Danny R. McCarty, Daniel P. Merrick, Agripino Rodriguez, and Valerie Williams.

REFERENCES CITED

Visscher, P.K., R.S. Vetter, and A.M. Khan. 1995. The use of insecticidal soap in abatement of honey bee swarms. Bee Science 3: 179-184.

Visscher, P.K., and A.M. Khan. Unpublished report. Management of bees remaining after swarm removal. Report submitted to Mosq. Vect. Control Assoc. of Calif.

Sames, W.J., W.T. Wilson, J.W. Smith, and H.D. Peterson. 1991. Effective destruction of honey bee colonies. Southwestern Entomologist 16: 19-24.

Tapper, J.J., R.P. Meyer, C.L. Ross, D. Loughner, and L.H. Shaw. 1997. Remnant traps and their use in the Orange County Vector Control District's Africanized Honey Bee Program. Mosq. Vect. Control Assoc. of Calif. 65: (editors add pagination)

1997 Proc. Mosq. and Vector Control Assoc. of CA 65:94-100

EVALUATION OF MOSQUITO AND ARBOVIRUS ACTIVITY IN ORANGE COUNTY, 1996

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In 1996 the Orange County Vector Control District (OCVCD) continued its mosquito and encephalitis virus surveillance throughout the year. Mosquitoes were collected at 11 permanent sites throughout the County. Eighteen CDC/CO_2 traps were utilized as well as 5 gravid female traps and a single Australian Crow Trap, modified to retain mosquitoes that enter through slots on the sides. The summary of the number of each mosquito species pooled from each trap type is shown in Table 1. A total of 1,601 mosquitoes (79 pools) was was sent to the Viral and Rickettsial Disease Lab in Berkeley, California for virus testing; 62 pools of *Culex quinquefasciatus,* 15 pools of *Culex tarsalis* and 2 pools of *Culex stigmatosoma.* None of the pools tested positive for St. Louis encephalitis (SLE) or western equine encephalitis (WEE) viruses. No human cases were reported from Orange County. Wild bird sera were tested for SLE and WEE by OCVCD using HAI techniques. Nine Australian Crow traps (McClure, 1984) were used to trap a total of 13,011 birds and 4,061 blood samples were taken during 1996 yielding 46 SLE positive birds (35 sparrows and 11 finches) and 4 WEE positives (3 sparrows and 1 finch). The summary of results is given in Table 2.

A total of 2,643 blood samples from house sparrows was taken, of which 1.32% tested positive for SLE and 0.11% tested positive for WEE. Of the 1,418 house finch samples 0.78% were positive for SLE and 0.07% were positive for WEE. House sparrows positive for SLE (Figure 1) were found from May through December, with the percent positive increasing from 0.6% (2 of 308 birds) in May to a high of 3.6% (14 of 391) in July and back down to 1% (2 of 203) in September. This was a substantial increase from 1995 when a total of 6 birds (all sparrows) seroconverted during the entire year. House finches positive for SLE (Figure 2) were found from June through December, with the percent positive increasing from 0.9 % (1 of 112) in June to 1.7% (3 of 173) in August. Three of 4 WEE seroconversions in 1996 occurred in house sparrows, 1 each in January (Garden Grove), July, (Anaheim), and August (Garden Grove). The single house finch WEE seroconversion occurred on July 29 in Irvine. The majority of wild bird SLE seroconversions occurred from June 13 - 28 (total = 2.0% positive, 3 different sites); July 3 - 31 (2.4%, 7 sites); August 7 - 29 (2.0%, 5 sites); and September 3 - 26 (1.0%, 4 sites). In contrast to wild bird seroconversions, the first sentinel chicken in the Los Angeles Basin seroconverted for SLE on July 19, followed by seroconversions on August 19-20, September 5 (Riverside Co., Corona), September 16, and 30. The most active sites in the county included a residence in Huntington Beach with 14 SLE seroconversions between May and October; Garden Grove with 9 SLE, 2 WEE seroconversions between January and November; and Modjeska Park in Anaheim with 6 SLE, 1 WEE seroconversions between June and December. Figure 3 shows the SLE positive sparrows from Modjeska Park plotted against host-seeking female mosquitoes. The highest percent positive (5.7%) at this site occurred in June (week 26) during a slight increase in *Cx. quinquefasciatus* activity from 0-5 per trap night in week 25. At the Garden Grove site (Figure 4) *Cx. quinquefasciatus* activity fluctuated between 1 and 8 per trap night for most of the year and in most cases peaks preceded bird seroconversions by 1 to 3 weeks. An average number of 8 *Cx. quinquefasciatus* per trap night occurred during week 23 with the highest percent positives for SLE (8.7% and 6.7%) occurring

[1] Deceased

during weeks 25 and 26. *Culex tarsalis* activity was virtually non existent at these two localities during 1996. Blood fed *Cx. tarsalis* and *Cx. quinquefasciatus* aspirated from a modified crow trap were prevalent at the residential site in Huntington Beach and, once again, peaks preceded bird seroconversions by 1 to 3 weeks (Figure 5). This particular locality consistantly produces large numbers of *Culex* and usually has the highest seroprevalence of SLE in the County. With the exception of the Huntington Beach site, *Culex tarsalis* has always been more abundant in rural areas such as the San Joaquin Freshwater Marsh in Irvine (Figure 6). Host-seeking *Cx. tarsalis* numbers in the San Joaquin Freshwater Marsh were highest in July and August (140-250 per trap night), but were almost absent the rest of the year. In contrast, during 1995, *Cx. tarsalis* numbers in the Marsh averaged 40-50 per trap night in July and August and displayed the usual pattern of occurrence from March through October. The most common mosquito, *Culex erythrothorax*, only averaged between 1000-1300 per trap night during March and April (10,000 per trap night in June and July, 1995) and *Culex quinquefasciatus* was uncommon in the marsh averaging less than 10 per trap night between March and November. Treatment of the San Joaquin Marsh with SCOURGE began on week 14 and continued through week 40. Gravid females of *Cx. quinquefasciatus* were obtained from Reiter ovipositional traps at 5 sites in the County. Figure 7 illustrates gravid mosquito activity at Irvine Valley College in Irvine (suburban) and Central Park in Huntington Beach (rural). Both sites are approximately 13 miles apart and, although the numbers of mosquitoes collected were different at each site, activity periods were similar. In 1995 Irvine Valley College was the most productive site for gravid *Cx. quinquefasciatus* (225 per trap night in July), but averaged less than 10 per trap night during all of 1996. In general, mosquito counts from all trap sites (CDC and Reiter traps) in Orange County were considerably lower and SLE and WEE activity in the wild bird populations was noticeably higher when compared to 1995.

REFERENCES CITED

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

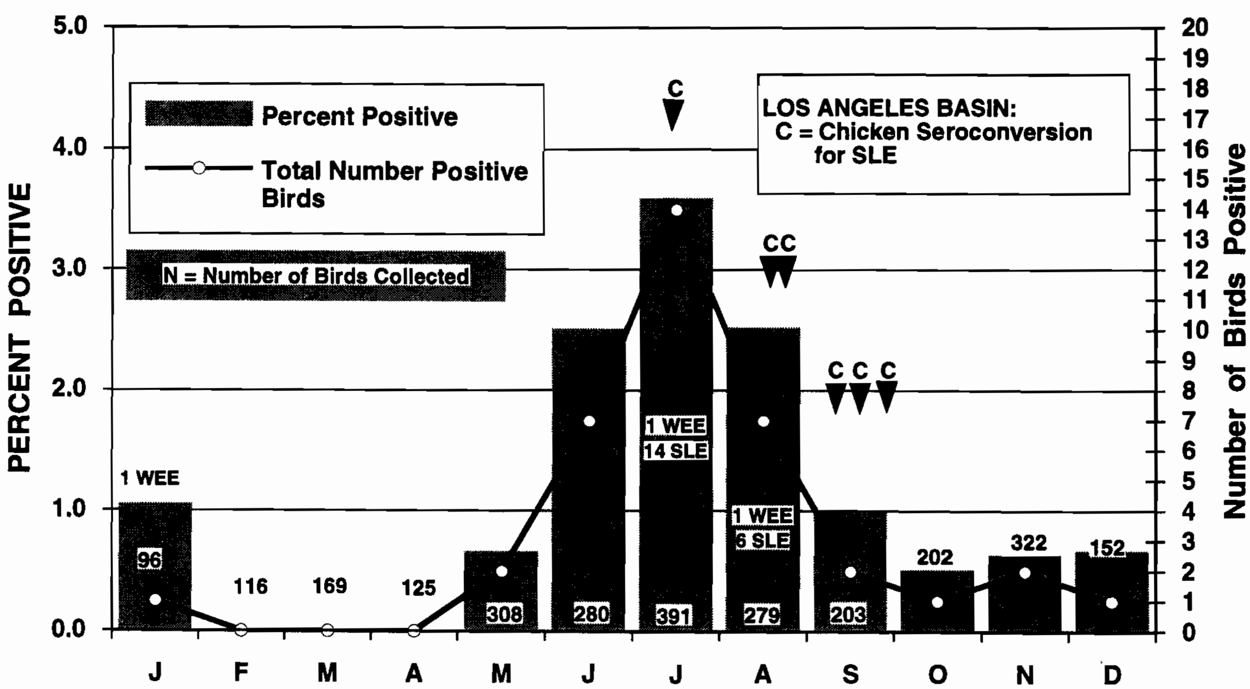

Figure 1. SLE and WEE virus activity in the Los Angeles Basin and house sparrow seroconversions in Orange County during 1996.

Table 1. Number of mosquitoes and mosquito pools submitted for SLE and WEE virus testing by species and trap type from Orange County during 1996.

Species	Number of mosquitoes	Oviposition Traps (pools)	Modified Crow Trap (pools)	CDC Traps (pools)	Total Number of pools
Culex quinquefasciatus	**1,302**	**22**	**38**	**2**	**62**
Culex tarsalis	**278**	**0**	**8**	**7**	**15**
Culex stigmatosoma	**21**	**1**	**0**	**1**	**2**
Totals	**1,601**	**23**	**46**	**10**	**79**

Table 2. Small bird seroconversions for SLE and WEE antibodies in Orange County during 1996.

Species	SLE	WEE	No. Blood Samples	% SLE	% WEE
House Finch	11	1	1,418	0.78	0.07
House Sparrow	35	3	2,643	1.32	0.11
Totals	**46**	**4**	**4,061**	**1.13**	**0.10**

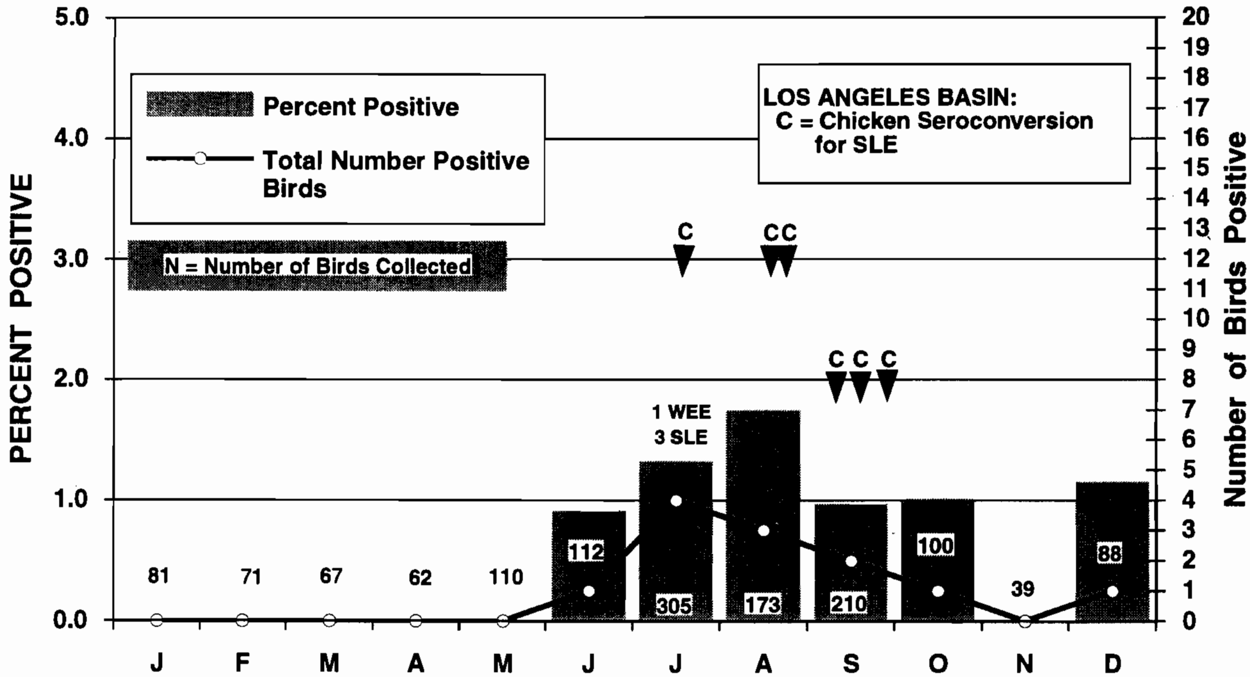

Figure 2. Arbovirus activity in the Los Angeles Basin and house finch seroconversions in Orange County during 1996. Bars represent SLE

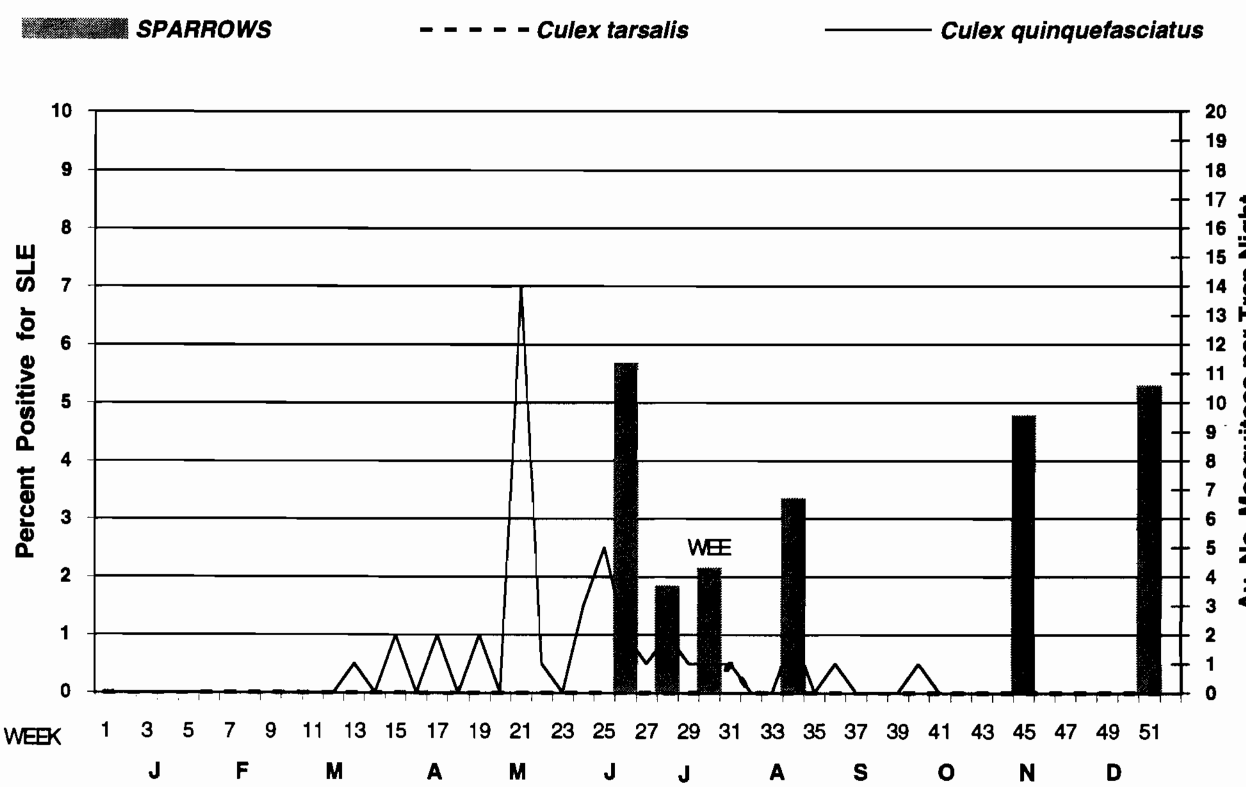

Figure 3. Host-seeking mosquito (CDC traps) activity, SLE and WEE positive house sparrows at Modjeska Park in Anaheim during 1996.

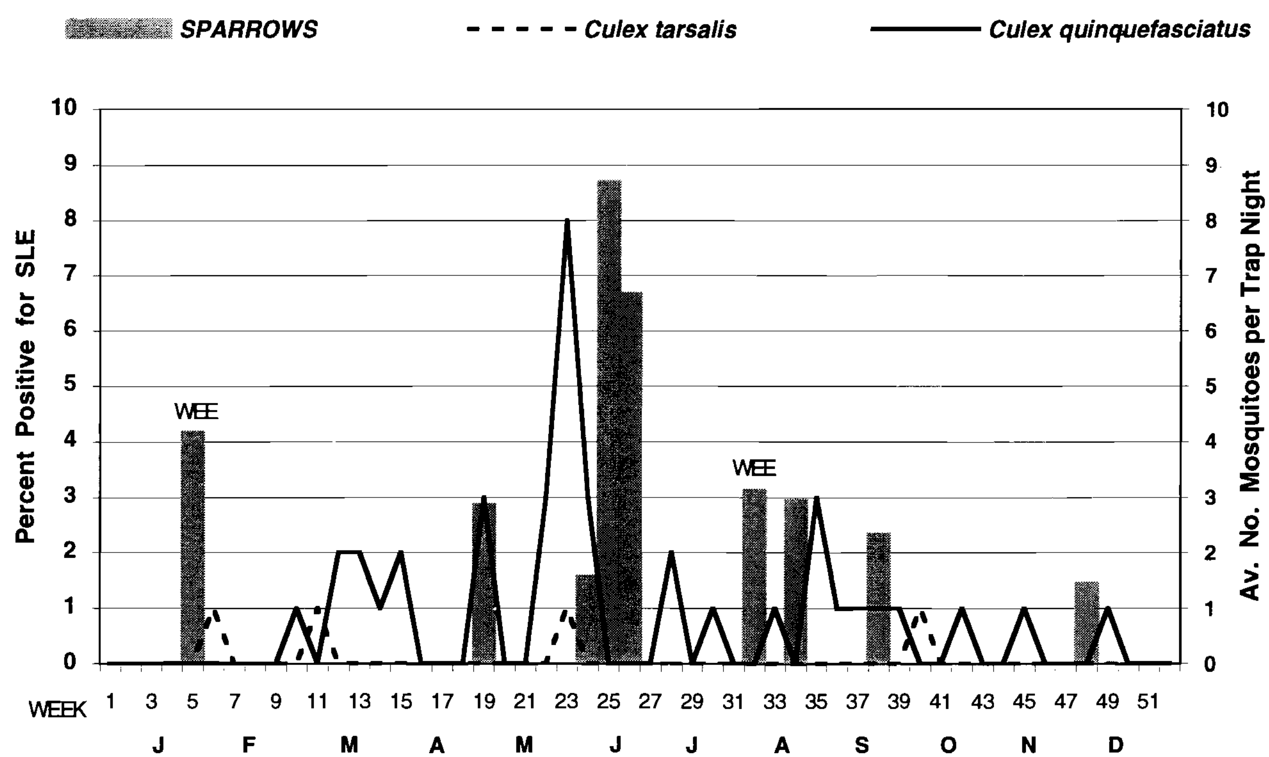

Figure 4. Host-seeking mosquito (CDC traps) activity, SLE and WEE positive house sparrows at OCVCD in Garden Grove during 1996.

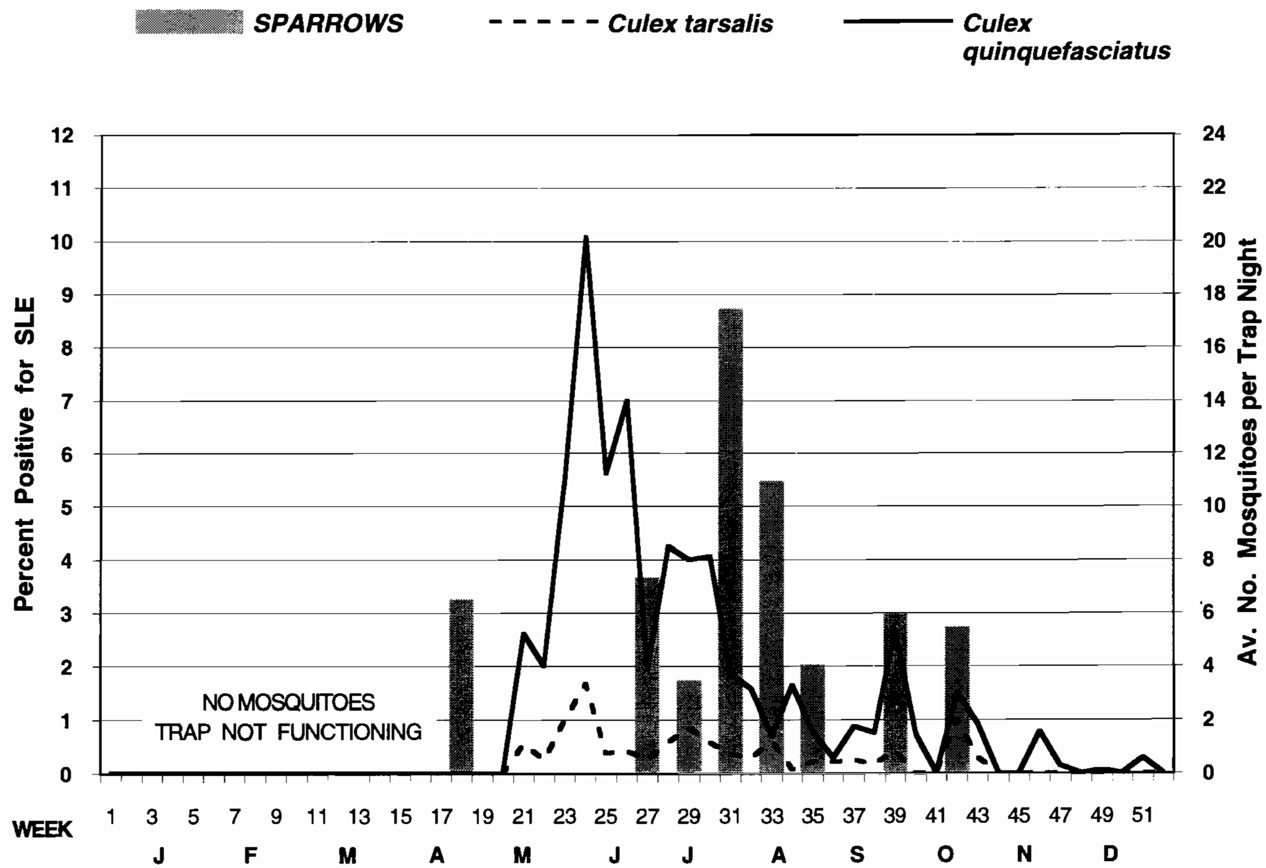

Figure 5. Host-seeking mosquito activity (modified Australian Crow Trap) and SLE positive house sparrows at a residence in Huntington Beach during 1996.

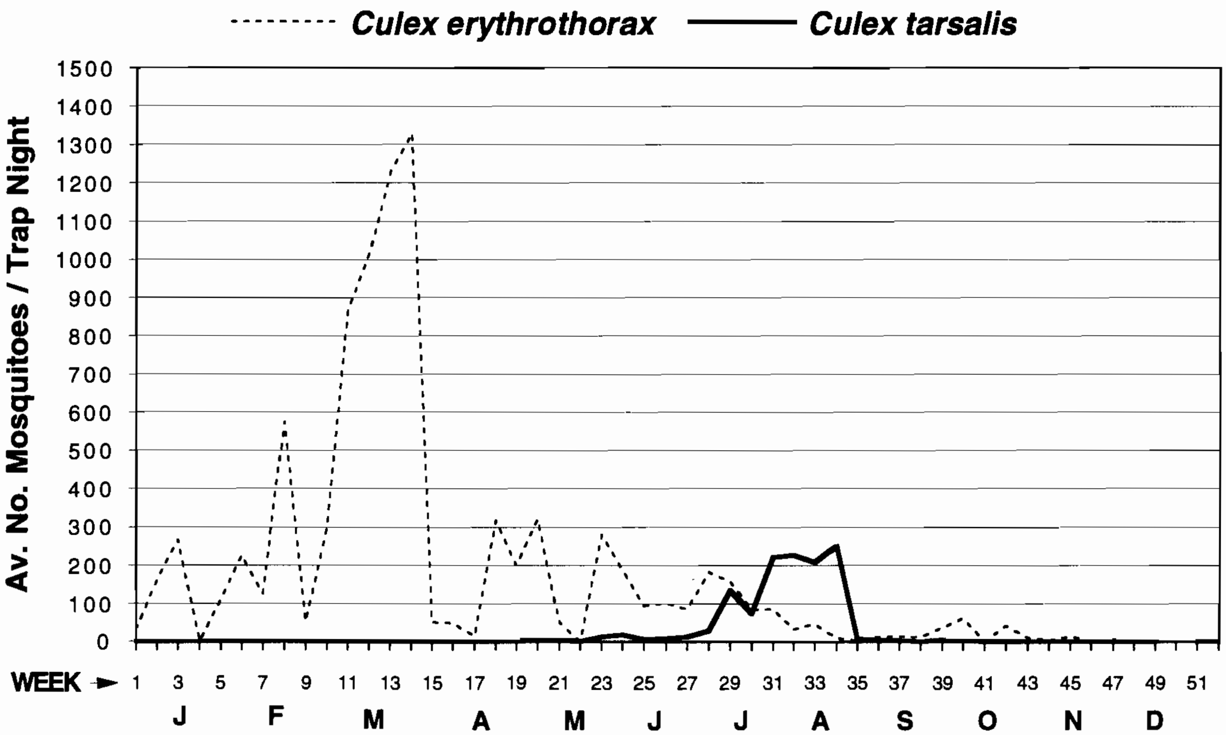

Figure 6 . Host-seeking mosquito (CDC traps) activity in the San Joaquin Freshwater Marsh in Irvine during 1996.

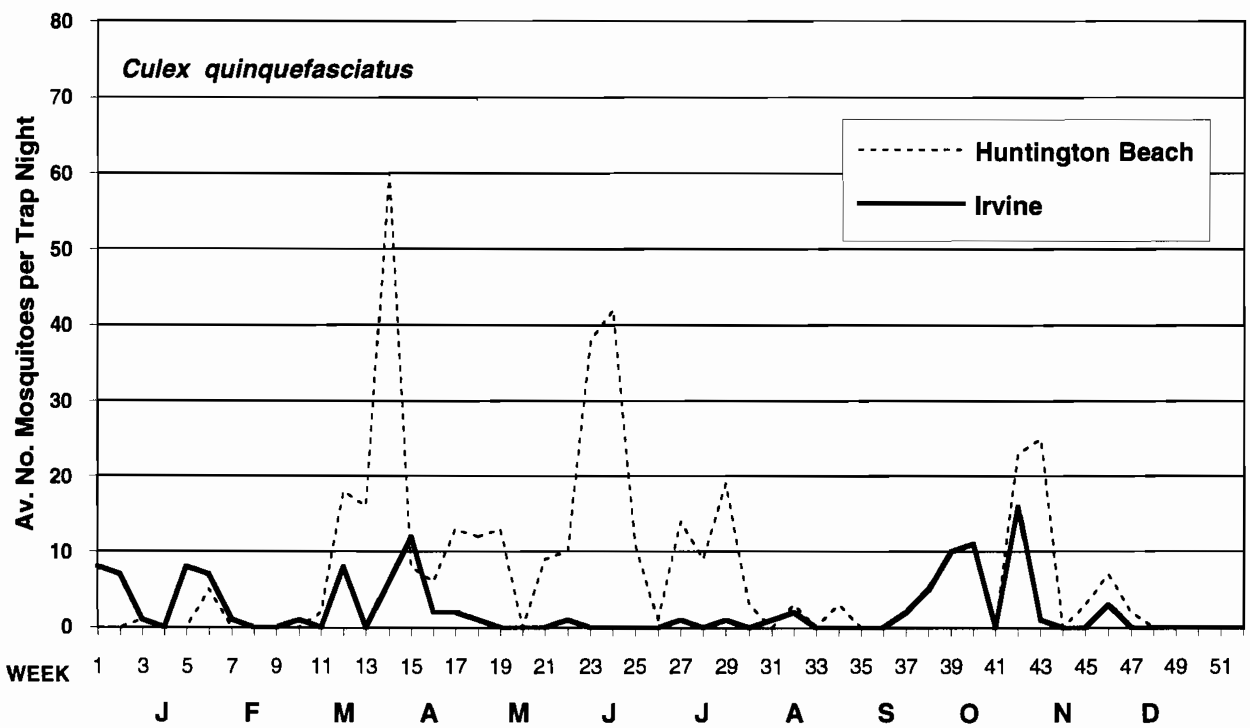

Figure 7. Gravid female mosquito activity at two sites in Orange County, CA. during 1996.

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

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