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COORDINATION OF AGRICULTURE AND VECTOR CONTROL ACTIVITIES

Fred L. Starrh
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Kern County is faced with many serious problems, including mosquitoes. The mosquito abatement program is one which has been well recognized over the years. As early as 1948 the Kern Mosquito Abatement District established a "source reduction program". Early problems included the dumping of waste irrigation water out onto open areas of native land. Over the years the District has assisted in the development of return systems. The prime benefactor to the District's program, stronger than laws or other coercive programs, has been the cost of the water itself. Agriculturists are faced with the responsiblity of using that water to the greatest possible degree. Their continued improvements in water management will result in a great reduction of mosquito sources. Possibly other areas of the State do not have as critical a need for careful water management as does Kern County.

Both agriculture and mosquito abatement districts are faced with problems with the Environmental Protection Agency relative to the barring of use of some insecticides. Agriculture has been prohibited the use of some materials which I believe are effective and cause no real harm to anyone.

Agriculture looms large in the world. It has come to the forefront not only in Kern County but in the State, the Nation and the World. Last year in California there were 8,000,000 tons of canning tomatoes produced. California produced up to 1,000,000 acres of cotton, with production of bales almost equal to Texas, which uses 4 to 5 million acres to produce the same amount of actual cotton. One operator in California produces as much cotton as is grown in the entire nation of Isreal. California is involved in the production of over 200 farm commodities.

Whose fault is today's world hunger? Recently I was on a panel on this subject. One of the panel members was a minister who had been involved in various conferences around the world. He claimed we should be changing our system and sharing with everyone else. The concept of agriculture, and Farm Bureau, which is a general farm organization in the United States, with about 1250 members in Kern County, 65,000 members in California, and 2.5 million members in the United States, and of my own is that this is a falacy. Our system has established the USA as a leader in production of agricultural products. Other systems throughout the world, until they realize that an individual must be paid for what he does, will not contribute to the alleviation of world hunger. I do not believe we have the responsibility or capability of feeding the world. We should help where we can, and we should not feel guilty because we are unable to do the whole job.

Is the price of food too high? In relation to what? One farmer now feeds 56 persons whereas 10 years ago he fed 29, thus in 10 years there has been a doubling of production by agriculture. Industry has had a 1.8% increase in 20 years. In many ways agriculture has increased its production and has provided its products at a reasonable price.

Land grant colleges are one of the basic reasons our country has developed so well in agriculture. Experimental work has been going on for many years, the farmers have worked closely with farm advisers and with changing situations. This has benefited the entire country and in fact the whole world. Our society is based on change - sometimes many changes in a single operation. In many other countries significant change is not occurring, and hunger is severe. We try to find a better way to do things.

Our system provides us the freedom to do the things we can do, but it does not owe us anything. Government involvement in agriculture should be maintained at an absolute minimum, in order for supply and demand to operate within the agricultural sector of our nations economy.

LOCAL GOVERNMENT PESTICIDE SURVEILLANCE PROGRAMS

Thomas E. Corn

Fresno County - Agricultural Commissioner

1730 South Maple Avenue, Fresno, California 93702

The Agricultural Commissioner, under direction of the California Department of Food and Agriculture, conducts regulatory and service functions required by state and federal laws and by local measures and ordinances authorized by the County Board of Supervisors. The primary purpose of this office is to promote and protect the agricultural industry of the county and state. In Fresno County, two Divisions carry out the program workload of the Department.

1. Plant Protection and Apiary Division
   a. Pest Detection. Through continuing insect, nematode, and plant disease surveys.
   b. Air Pollution Injury to Vegetation. Through continuing surveys.
   c. Pest Abatement and Eradication. Control or elimination of a particular pest in a certain and usually restricted area.
   d. Collection and Identification of Common Pests. Dissemination of information pertaining to importance, distribution, host range, and life histories. Control measures for general pests of economic importance.
   e. Regulation of certain pesticides defined by law as "restricted" through issuance of use permits, frequent inspections, and dealer audits.
   f. Supervision and registration of State licensed pest control operators and agricultural pest control advisers. Review of dealer audits, and application surveillance.
   g. Apiary Inspection and Bee Disease Abatement. Inspection of hives for detection and eradication of American foulbrood disease and other serious diseases of bees. Enforcement of regulations pertaining to beekeepers and honeybees. Evaluation of colony strength through certification inspection.
   h. Plant Quarantine and Pest Exclusion. Inspection of plant material transported by mail, express, truck, or air to prevent introduction and spread of injurious insects, animal pests, weeds, and plant diseases. Controlled disposition of plant material found in violation.
   i. Seed Inspection. Vegetable and agricultural seeds inspected for conformity with State laws as to purity, germination, proper labeling, including that of poison - treated seeds, etc. Cooperation with University of California on regulatory aspects of Seed Certification program as conducted by California Crop Improvement Association and Agricultural Extension Service.
   j. Plant Nurseries inspected to detect and prevent spread of insects, weeds, diseases of plants; "cleanup" control measures, recommended enforcement of nursery stock grades, standards, and labeling laws.

2. Grower and Consumer Protection Division
   a. Vertebrate Pests. Measures for control or eradication of ground squirrels, gophers, field mice, jack rabbits, rats, etc., which cause serious losses to agricultural crops and may carry diseases transmissible to humans. Mixture and sale of treated baits to residents of County. Aid given by trappers to cattle producers, poultry raisers, sheep growers, and home owners to control predatory animals such as coyotes, feral dogs and cats, bobcats, and skunks. Inspection and surveillance of Nutria premises.
   b. Pest Bird Control. Suppressive measures to control starlings, horned larks, linnets, English sparrows, crowned sparrows, and blackbirds.
   c. Weed Control. Eradication of noxious weed infestations of limited distribution, and control of those having general distribution. Continuing surveys to detect new noxious weed pests. Field trials conducted to study new trends of control.
   d. Standardization Enforcement. Inspection for compliance with minimum State standards from producer to consumer concerning such requirements as packing, quality, maturity, container markings for all fruits, nuts, vegetables, eggs and honey. Prevention of deception in packing and marking of such products.
   e. U.S.D.A. Shell Egg Surveillance Inspection and Enforcement. Inspect and control the movement of "Restricted Eggs" (dirties, leakers, inedibles, loss, checks, and incubator rejects) as directed by the Eggs Products Inspection Act which was enacted by Congress as Public Law 91-597 on December 29, 1970.
   f. Crop Reporting. Compile reports of condition, acreage, production and value of agricultural products in the County as required by State law. Reports submitted to State Department of Food and Agriculture weekly, monthly and annually. Compile statistics for annual Fresno County Agricultural Crop Report.

One of the areas I have been asked to address is, "how to reduce the conflict on the use of pesticides that seems to be a growing area of concern". And, how we might improve communications.

First off, the "conflict" does not exist between the Mosquito Abatement Districts in Fresno County and the Agricultural Commissioner's staff -- at least to my knowledge. We have been requested to make a number of investigations on the possible misuse of chemicals and have found very little evidence to support any of these claims. I believe that we should improve our communications, along with Health Department personnel, to better understand our problems and concerns in the pesticide sector.

Pesticide surveillance goes back many years to 1935 when the Department and the Commissioner were involved in pest control operator certification and the Director of Agriculture was involved in surveillance of economic poisons. In 1949 Section 1080 was enacted on injurious materials. Section 1209 was enacted in 1969 which involved written recommendations and certain other mandates. Other statutes were enacted including A.B.246 in 1972, which resulted in the worker safety program. In 1973, A.B. 150 was enacted which carried the mandate that the Division of Industrial Safety shall enforce all occupational and safety health standards.

Pesticide surveillance programs vary between counties, as pointed out by Francis E. McGowan in the Legislative Analyst's report of December 27, 1972. The Agricultural Commissioners believe the pesticide programs will vary between counties depending on size, type of farming, population, potential hazards, available manpower as well as many other factors.

Pesticide surveillance includes many programs:

1. Restricted materials permits issuance.
2. Notice of Intents.
3. Observation of pesticide applications.
4. Inspection of Equipment.
5. Rinsing of empty pesticide containers.
6. Safe handling of pesticide containers.
7. Class I Dump Site operation responsibilities.
8. Registration of Pest Control Advisers.
9. Registration of Pest Control Operators.
10. Dealer Audits.
11. Pest Control Adviser Audits.
12. Inspection of recommendations made by advisers.
13. Protective clothing, safety equipment.
14. Label registration compliance.
15. Review of Use Reports.
16. Worker Safety intervals.
17. Posting of treated fields.
18. Employee training records.
19. Emergency medical care.
20. Medical supervision.
21. Supervision of rodenticide applications.
22. Calibration of equipment.
23. Review of application sites in reference to wildlife damage.
24. Control of pesticides for research and development.
25. Fumigation supervision.
26. Holding commodities with illegal residues.
27. Education.
28. Supervision of herbicide applications.
29. Report of Loss investigations.
30. Pesticide Illness investigations.
31. Apiary protection program.
32. Fire hazard prevention.
33. Pesticide accident investigation.
34. Pest Control Operator audit.
35. Residue sampling.
36. Storage of pesticides and containers.
37. Decontamination of spills.

The field of worker safety is of vital concern to the agribusiness community as well as the general public.

There are many unanswered questions relating to worker safety. Education is a vital part of the worker safety effort. Firm, uniform policy is being developed.

Fresno County utilizes the Restricted Materials Permit system with a Notice of Intent 24 hours before application of the pesticide as much as possible. This technique allows directed investigations of pesticide applications. Several materials with safety intervals on citrus, peaches, nectarines, grapes and apples are not on the restricted list. As a result, enforcement personnel investigate these pesticide applications on a random and complaint basis with a view to correcting serious violations where found.

Several enforcement techniques are used by Agricultural Commissioners and their staffs. Permits may be suspended, revoked or denied. Violations may be issued, registration of pest control operator or advisers may be issued if the offense is committed in the presence of the enforcement officer and there is an unlawful section.

The registration of chemicals by EPA is a major problem area facing all of us. Methods of developing appropriate data, reimbursement for data, and funding necessary for the process are just a few of the stumbling blocks.

We have many valuable chemical tools for use in mosquito control and agriculture. Everything possible must be done to protect these tools and keep them on the shelf.

WHAT'S BUGGING YOU?

Pamela Zinn

Pest Control Operators of California, Inc.
3444 West First Street, Los Angeles, California 90004

The Pest Control Operators of California, like the California Mosquito Control Association, is a non-profit Association made up of professional people concerned with protecting the health and property of man. Two things in my experience with the Pest Control Association "bug" me: 1) the obscenity of bureaucracy, and 2) the obscenity of profit.

Bureaucracy within our form of government has come to be considered by consumers as obscene, as have profits by private industry. Our founding fathers came to the shores of this country seeking three basic freedoms: religion, philosophy and enterprise. As our country grew and matured, our surroundings and our goals changed. Certainly the desire to achieve, to become self-sufficient and to contribute to our environment should not be obscene. However, today the small business person is looked on with disdain. The same attitude may apply to those in local, city, state or federal government – they are accused of taking the easy way out, the sure pension, the soft job. How did this changed concept develop during the past few years – that we were in less than honorable professions?

I submit that our own laziness has been the cause. I believe the pest control industry has grown comfortable and complacent in just doing its job, and has forgotten its role as a good neighbor in the community. Let me illustrate. If you are sold a product which is good and useful and serves the purpose for which it was purchased, you should be satisfied. But – your next contact with the company which produced that product may be when you read the financial page and find that part of your purchase went towards making it a record-breaking profit year for the company.

The same principle applies to governmental agencies. We are excited by an announcement of new programs and new services, but we are left to flounder when no further communication is given, or when we call an agency for help and the switchboard operator plays Russian roulette with our good intentions and our valuable interest.

How do we defeat the obscenities of bureaucracy and profit? We need to go back into the community and follow through with our initial good work. We need to reestablish the liaison between government and private industry. We need to stop doing things to each other and start doing things with each other. We need to acknowledge that we are all professionals, we are all businessmen and individuals who have chosen to serve within our own rights, and that we do know what we are doing. Congress has advised us in many different ways that we will have to adhere to specific standards, so it is time our respective agencies and members start working together. We can let each other know what our problems are, we can sit down at common meeting tables and help each other obtain solutions. Both private and governmental agencies can profit by such a program.

THE CONFLICTS, CASE HISTORIES, SOLUTIONS AND RAMIFICATIONS OF A PERMIT SYSTEM

William E. Hazeltine

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In approaching the assigned subject, and having been told that I was allowed ½ hour to cover this topic, my first impression was, it's impossible. Perhaps this feeling of impossibility is merely an extension of the feelings one gets when they try to read and understand some of the thick Federal regulations which cover so many areas of our lives today. Nevertheless, the time has arrived and excuses will no longer prevail.

I want to begin with a historical review of the law which bears directly on the subject of permits and restrictions on operations of Mosquito Abatement Districts, as well as many other agencies in our Society. This is Public Law 92-500 better known as the 1972 Amendment to the Federal Water Pollution Control Act. To a lesser extent, the Federal Insecticide, Fungicide and Rodenticide Act as amended in 1972 provides for some additional permit requirements. I was admonished by friends not to wander off into the FIFRA to any great extent, even though I feel much better qualified and more at home talking about that law, and the Environmental Protection Agency's ineptness in its Administration.

To begin, you should understand some of the background and requirements of the present Water Pollution Control Act. The law seems to have grown out of the public's clamor of the early 1970's, to do something about our environment, or as some have portrayed it, to save us from ourselves. Apparently there was pretty broad support in the Congress for this legislation, as evidenced by the fact that on October 17, 1972, the President vetoed this bill but the Senate and House both overrode the veto and the bill became law the next day. This particular piece of legislation is divided up into 5 basic parts. These are described this way: (1) Research and related programs and including the general declaration of goals and policies which the Congress intended this act to accomplish; (2) Grants for Construction of Treatment Works; (3) Standards and Enforcement; (4) Permits and Licenses, the area which is of most concern to us today; (5) General Provisions of the bill, including authorization for money.

The law begins this way - "The objective of this Act is to restore and maintain the Chemical, Physical and Biological Integrity of the Nations Waters". Subsequent statements seem to make it clear that a weighing of risks and benefits is what was intended, particularly for materials such as pesticides, when it says "it is the National Policy that the discharge of toxic pollutants in toxic amounts be prohibited". Public participation in the development, revision and enforcement of regulations, standards and limitations are encouraged and it provides that the Administrator, in cooperation with the States, shall develop regulations for implementation. It also contains the instruction, which I paraphrase, requiring everyone to minimize the paperwork and try to get the best mileage out of available manpower and funds, ". . . so as to prevent needless duplication and unnecessary delays in all levels of government". I hope that as I go along, you will see a need to encourage all the regulating agencies to re-read this last provision so that we can all work together to accomplish the social good with the minimum amount of wasted effort.

Another provision of this law, is that in many instances, the State is designated as the enforcer, while the Federal Agencies write, or at least have to sanction, the regulations. From a practical point of view, this seems to insulate the Federal Agencies from direct confrontations and the expectation is that regulations adopted at the Federal level will be less reasonable because the Federal Agency is not the one on the firing line to receive the critical comments which seem to follow enforcement activities. There is nothing like making the regulator directly face the consequence of his regulation, to keep things on an equitable basis. In Section 404, the Corps is up front on issuing permits and taking the heat.

Getting into more detail, there are two sections in the Water Law which seem to affect mosquito abatement most directly. Section 402 of the Act is known as the National Pollution Discharge Elimination System, abbr. NPDES. The California State Water Board, through its Regional Water Quality Control Boards has been active in assuming this bureaucratic requirement. Ultimately anyone, including farmers, who discharge water, directly or through a drainage agency will be required to monitor and meet strict standards. The general pressure is to encourage water re-use through pump-back systems or similar methods. This way, there is no discharge to police. It should be obvious that the goals of the Water Board in implementing this provision could easily become a real hazard for mosquito control. Adding to this condition of seemingly unbalanced regulations has been some court actions which define navigable waters to include even dry washes, when water may flow through them and eventually enter public waters. A statement by the Central Valley Region Water Quality Control Board says "this implies that irrigators - - - could be subject to NPDES permits". If this section is carried to its logical end, at least where water is plentiful, good drainage for good mosquito control could be frustrated and thwarted. Limitations imposed, or at least proposed, in the State regulations for some pesticide discharges, seem to go beyond the realm of ridiculousness. They look nothing short of being impossible to meet and seemingly a complete neglect of any rational risk-benefit balancing.

Another aspect of this section of the Law has to do with the requirement that dairys, for instance, must be on a no-discharge type of liquid waste handling procedure. Usual implementation has been through ponding, with land disposal of waters from the lagoons. Unless handled with precision, dairy wastes can create large mosquito problems.

Section 404 seems to be the section of the Water Law which is of prime interest to us today because it seems to be a consistent theme for most of the reported mosquito control problems and conflicts. This section of the Law requires a permit from the Corps of Engineers for discharge of dredged or fill materials into navigable waters. Authority to issue permits seems to be shared by the Corps and the Environmental Protection Agency because the Administrator of EPA is authorized to prohibit a discharge of dredged material or spoils even though the Corps might issue a permit for such a discharge. The basis for the EPA Administrator over-riding or restricting the use of a defined area for discharge is that he finds, after a hearing, that the discharge of such material will have an "unacceptable adverse effect on municipal water supplies, shellfish beds and fishery areas, wildlife or recreation areas". EPA is required to consult with the Secretary of the Army but there is little doubt about who will make the final decision.

It should also be obvious from its absence that public health interests are not specifically listed as one of the criteria to be protected, which pretty well reflects the feelings of the hysteria prevalent in 1971 and 1972, particularly in some of the committees of the Congress. Those were the days when we were going to protect the environment "come hell or high water". And the environment was usually described as the defenseless animals, with man and his needs usually neglected, because man was supposed to be the cause of the problems in the first place.

Any district person here, reading Section 404 of the Law would probably not be troubled by this Section. Most mosquito abatement districts are not in the business of dredging navigable waters or discharging the spoil from dredging into navigable waters. Districts may construct levees, or drain ditches with small equipment for small areas. The aim is usually to control waters and manage mosquitoes through biological control or physical control systems, in order to eliminate the need and expense of having to use pesticides. Navigable waters, as a class of waters, also would seem to exclude us, because waters which are navigable by and large, are not the kind of waters which we can afford or even desire to try and manipulate for mosquito control, under usual circumstances.

There are some examples of dredged soil having water holding characteristics which breed mosquitoes and there the local control agencies have usually worked with the person doing the dredging to find solutions. From a rational viewpoint, keeping a navigable ship channel open usually is considered to be a decided social benefit and expenditures for mosquito control, when necessary in the spoil, is a necessary and acceptable part of the social cost of the dredging.

Up to now, I have talked about rational people and rational programs. The Corps of Engineers adopted what seemed to be a very reasonable definition of navigable waters, with the intent of providing reasonable protection in its originally proposed regulations. However, at this point, we see the intrusion of such environmental organizations as the Natural Resources Defense Council (NRDC) and the National Wildlife Federation who brought suit against the Secretary of the Army and Corps, as well as the EPA Administrator on August 16, 1974. Even the State of Florida joined the environmentalists in this action. These "Friends of Society" were able to get the Court to agree with their position and, in effect, order the Corps to "reconsider" its regulations, particularly a more bizarre definition of navigable water. The Corps accepted this environmental and Court intrusion and on May 6, 1975, (40FR: 19766) published new proposed regulations and invited comments. The Corps proposed 4 alternatives. The alternative, which was favored by the Corps, would have adopted a limited definition of waters of the United States, with a condition for State certification and authorization added in. This provision recognized that the EPA Administrator could still overrule, if he concluded that the proposed water disposal will have the unacceptable effects as listed in the Law.

The proposed rules show an absence of consideration for the effects that these regulations might have on public health, other than water quality for municipal water supplies. Our District commented to the Corps that we felt it would be wise to add a provision authorizing reasonable actions by a public health agency without a permit, when it was protecting the health of the public. We specifically referred to mosquito control as one of these very necessary actions, dealing with water management, as a way to reduce the need for pesticides.

The absence of an element dealing with health protection exclusions is hard to comprehend. Apparently the U.S. Public Health Service was unconcerned when these regulations passed their pre-publication review. In any event, the health protection exclusion did not get into the re-written regulations published on July 25th, 1975 (40FR:31320) where the Corps published its commentary and invited new public comment on these proposals. The 90 day comment period closed in late October of 1975. One of the nice parts in the latest proposal is that it carried along a provision allowing general permits for certain clearly described categories. In addition, these proposals deal with phased restrictions and discretionary authority to the District Engineer to regulate certain projects on a case by case basis. This case by case wording is a nice way to saying that risk-benefit balancing can be applied.

It seems obvious in reading these regulations that the farming and agricultural community made their voice heard, because many of the normal agricultural operations are considered to not fall within the term fill material, and farm conservation practices would not be regulated unless they occur in navigable waters. Health protection did not get comparable attention, most likely due to our general negligence.

A few years ago, most of us would not have believed it necessary to lobby for the needs of the public health; it should now be clear that nothing can be taken for granted when we have to compete with some of the environmental organizations active today. Some of the people on the Advisory Boards of the organizations who sued the Corps, include Nelson Rockefeller and Jimmy Roosevelt, Dave Sine, the environmental lawyer, Stewart Udall, one time Secretary of the Interior, Drs. Rene DuBois and Josiah Lederberg, not to mention Mrs. Louise Auchincloss, Jackie Onassis' mother. These are some of the uncommon people who hamper our efforts to protect the public health. They are fighting our program by trying to force the Corps to restrict our programs. This may not seem charitable and it may even seem personal, but this is the battle scene that we face with the environmental interests and it's better that you know the people who are against you, than to go around with your head in the sand or elsewhere.

As things now stand, navigable waters apparently include just about all waters, as defined by the Corps, under the Court Order. In addition, the Environmental Protection Agency will be issuing regulations on navigable waters and the discharge of dredged or fill materials. Their proposed regulation is in the Federal Register for September 5, 1975 (40FR:41292). Comments were invited and the closing date for them was December 4, 1975. EPA's proposals seem to be consistently negligent of health benefits.

One final comment before moving into the case histories on how the requirements have affected mosquito control and mosquito abatement agencies. The regulations to implement the water law have apparently included both dredging and filling, as well as ditching, under the assumption that the water which might run off of the dredged material would have to flow back into some other water or that ditching might change water flow characteristics and, therefore, theoretically at least, change water quality, or some other equally tenuous argument. You know I was really hung up about the way ditching and draining regulations had been added to what the law required, until I received a copy of a letter dated November 6, 1975 addressed to the Honorable Larry McDonald, who is a Georgia Congressman, and signed by Russell E. Train, as Administrator of the Environmental Protection Agency. Train's letter was in response to some statements which I had made and which Congressman McDonald had published in the Congressional Record, regarding the place and responsibility of EPA in the 1975 encephalitis epidemic. Because this letter is signed by the Administrator and seems straighforward in its wording, it should be important to show what the intent of the regulations really are, and I would like to quote a portion of it here:

> "- - - while it is true that EPA, in conjunction with the Corps of Engineers, has promulgated rules for the regulation of some ditching and draining operations, I do not believe that these will impact on the current encephalitis outbreak. In the past, ditching for mosquito control was of questionable value, and in many cases, marshes were drained when no real need was indicated. **Only when dredged material is to be discharged into a water body will there be a need for a permit under the regulations.** Thus the majority of ditching and draining operations of swamps and marshes will not require a permit. The extent of the regulation is to insure that the need for ditching is carefully examined and the cost and benefits thoroughly considered." (emphasis added)

An additional two sentences within the pertinent paragraph of Mr. Train's letter, shows the extent of the Agency's misunderstanding of our needs in mosquito control. The paragraph continues:

> "- - - under the new rules and regulations, procedures allow for emergency ditching and draining if circumstances warrant, such as when an encephalitis outbreak is expected. Furthermore, the regulations required that in emergency permit conditions, a permit must be expedited so as to provide quick, effective action."

Now we know the positive intent of EPA, and have a basis for conducting source reduction within Mr. Train's stated intent. I was also struck by the lack of understanding expressed in trying to use drainage to stop an encephalitis epidemic. Ditching and drainage is for prevention, and quicker action like pesticides are necessary for control of epidemics.

Let's proceed to look at some of the examples of what has taken place in our world.

Example No. 1. A copy of a telegram dated April 3, 1975 to the Director of the Department of Sanitation, City of New Orleans, Louisiana, says in effect, to stop ditching for mosquito control or the District Engineer will seek an immediate injunction. Even the work already done might subject the City to legal action for violation of Section 10 of the Rivers and Harbors Act of 1899 or Section 404 of the FWPCA of 1972. Recall that Section 404 deals with the discharge of dredged materials into navigable waters. Apparently this order stems from a complaint from a lawyer who observed the drag line excavating ditches in the tidal wet land. The telegram continues "you are directed to perform no further work until a permit has been issued or it has been determined that none is required". It also states, "regulations governing our regulatory permit programs preclude my acceptance of an application for an after-the-fact permit until all legal issues have been resolved".

Those of you familiar with the New Orleans Control Program realize that without the use of a reasonable source reduction program, mosquito control is going to be severely restricted.

Example No. 2. Comes from the Solano County MAD and deals with the Suisun marsh. In addition to the Corps of Engineers and EPA, permits have been required by the San Francisco Bay Conservation and Development Commission (BCDC), the San Francisco Bay Regional Water Quality Control Board and the State Lands Commission. I hope I haven't missed any other State Agency. If I have, I'm sure Embree Mezger will correct me.

The problems encountered were for permits for maintenance of **pre-existing** drainage ditches on 5 private properties, comprising one thousand and five acres in the marsh. Securing the necessary permits required about 11 months and during this time, the District was forced to spend public monies for extended ground and aerial application of insecticides, to suppress the mosquitoes being produced on these 5 properties.

The Grizzly King Duck Club Tidal Marsh project took approximately 12 months to get all the required permits, and the approximate cost for pesticide control was about $2,100 of public monies. A portion of this marsh belongs to the State of California and is under the jurisdiction of the Department of Fish and Game. As noted by Embree, the Solano District has used source reduction as its primary control tool for mosquitoes on these marshes since June of 1930, using the techniques of drains for effective mosquito control.

Another case history outlined by the Solano District discusses the problems of securing State and Federal permits to clean or re-align a drainage ditch in a tidal marsh on privately owned property. This account, which was part of the material provided at a recent Health Department Seminar on the subject of Permits, had taken over 1 year and had still not been resolved by the August 14 date of the report.

Example No. 3. Comes from the Alameda County MAD in a letter dated August 27, 1975. In the letter, Fred Roberts discusses a specific example this way:

> "Last year we attempted to have Oakland's Scavenger Company correct drainage problems and repair levees for mosquito control purposes on a 450 acre reclaimed marsh. After an initial request, to the Bay Conservation and Development Commission, we had to obtain permission from the Corps of Engineers, the State Lands Commission, the Regional Quality Control Board and the County of Alameda. The tangle created by the various requirements at each Agency, delayed the project until it became impossible to accomplish the necessary work before climatic conditions intervened."

The letter continues:

> "the staff of these various agencies were responsive and tried to facilitate a solution. The problem lies in the permit procedure policies of the Agencies. There are currently no mechanisms to rapidly process permits for maintenance and repair work needed for mosquito control purposes. Your effort to that end is greatly appreciated."

Example No. 4. In a letter dated August 21, 1975 from Dr. Allen Telford of the Marin-Sonoma MAD, the procedures used to reduce the mosquito breeding problems in salt marshes were considered. The program consisted of a source reduction program and that program has eliminated plane spraying and pesticide use. Dr. Telford's letter states:

> "as far as we can determine, our source reduction projects have returned these marshes to a state of near natural tidal flow bringing mosquito population to levels equivalent to those which we observed on a relatively undisturbed marsh in our District. We conclude that our program is fiscally and ecologically sound."

And the letter continues:

> "should we be forced to abandon this program because of restrictive permit procedures and other requirements, we would be compelled to return to pesticide applications."

The basic concern here seems to be one that changing conditions could necessitate new physical changes in the future, as a reasonable way to protect the marsh resource and avoid the need for pesticide controls in that area. It continues:

> "It is imperative therefore, that we continue to manage marsh mosquito sources effectively and without delay in order that we may properly maintain this publicly mandated and publicly financed mosquito suppression."

The letter then goes on to suggest the establishment of inter-agency cooperative agreements, directed toward this end.

Lest there be any misunderstanding, it should be clearly stated that the examples stated here all come from people dedicated to source reduction as the basic tool for good mosquito control. These men are adept at risk-benefit balancing and, I know from my own experience, that each of them thinks well beyond the simple requirement of a single or special mandate. Each of them does consider the impact that various alternatives will have on the larger environment as well as the responsibilities they have to protect the public health through mosquito control.

Example No. 5. The last example I want to mention concerns a dairy operator in Butte County and the problems he had with the Regional Water Quality Control Board's attempt to implement the requirements of the Federal law. In this case, the landowner was sent two registered letters, one being a follow-up of the other, telling the man he was out of compliance and that he had to eliminate any discharge of waste water from his dairy and cattle operation or face legal action. Prior to receiving these letters, the operator had spent about $10,000 for a lagoon and had made arrangements to use a small field for irrigation with waters from this collection site. As far as we could see, he had complied with his pasture irrigation. We were trying to get him to correct some problems on another piece of land and when these letters hit, he said, "no more changes for anybody until somebody figures out what I can do legally". We understand that later an oral apology was received. Apparently the two Certified letters were sent in error and the operation seemed to be in compliance.

The part of the Water Board letter which really bothered me was a suggestion by the Regional Water Board that help to comply with the restrictions could be obtained from the Farm Advisor, from the Agricultural Commissioner, the Soil Conservation Service or the Mosquito Abatement District. As I recall, it listed 5 sources, but the Water Board was **not** one of those listed as a source to help the man find ways to comply with the law. The real test of an expert is one that can tell you how to solve a problem and, it seems inappropriate for any agency to order correction to a prescribed standard, without also telling the recipient how he can comply with this standard or order.

Moving from examples back to legal authority, I tried to prepare a list of all of the agencies we deal with which require some kind of a permit or have regulatory authority over Mosquito Abatement Districts. On the Federal level, besides the Federal Water Quality Control Act of 1972, there is the Air Quality Act (the 1970 Amendments), the Federal Insecticide, Fungicide and Rodenticide Act as amended in 1972, the National Environmental Policy Act and the Federal Aviation Administration, not to mention OSHA, DOT Noise laws and now even the Fair Employment Practices Commission. At the State level, regulation comes from the State Health Department, the Department of Agriculture, the Resources Agency, the Water Quality Control Board, the State Division of Forestry, the Division of Industrial Safety, possibly the San Francisco Bay Conservation and Development Commission, the State Reclamation Board, the Coastal Zone Conservation Commission, the State Lands Commission, California Department of Fish and Game, California Department of Parks and Recreation and perhaps others. The one advantage we have, at the State level, is our statute, and it would seem reasonable to expect the Health & Safety Code would be a more potent law than some of the other laws. The justification is that human health should be more important than the health of a fish or a bird, or a tree.

Let me see if I can put this topic into some kind of a perspective, which will give our respondents the opportunity to react a little and come back to you with their side of the story. The Corps of Engineers has worked with mosquito control and other health interests, and I believe without the interference of some of the environmental groups, that the Corps would have adopted regulations which would have allowed us to live in productive harmony with nature, recognizing that mosquitoes are a pollutant, even though they really are wildlife, in every sense of the word. The proposed regulations which the Corps published are ample evidence of their desire to be reasonable and represent all interests fairly. Perhaps it is our fault for not actively lobbying the needs of public health that the regulations, as published subsequently, were not clearly favorable to our needs when they neglected to consider the direct exclusion of public health.

I understand from Marvin Kramer that great strides have been made with the Corps and that mosquito control permits will proceed as expeditiously as possible. This is evidence that the Corps wishes to work with us in reducing the amount of time and paper work necessary to allow us to accomplish our mandate of protecting the public health. It is nice to see some agencies follow the Statutes.

The Environmental Protection Agency seems to have taken a different track and stance. The Agency seems to be consistently dedicated to trying for a no-risk society which is about as inappropriate and unrealistic a position as anyone could expect. Nothing is without risk and the failure of EPA to apply a reasonable risk-benefit balance, e.g., in looking at the needs for pesticides as well as the risks from pesticides, or the needs for source reduction as well as the risks to the environment from such operations, can only be characterized as jaundiced and, from my experience, this characterization is charitable.

My instructions were not to dwell on the specific area of pesticide regulation, even though these do constitute areas where permits are required and the EPA regulations of these chemicals does prohibit reasonable operation. I will bow to that request. However, for any of you who wish to privately pursue the subject of pesticide regulation and strategies for corrections, I would be happy to discuss the issues with you.

As a closing item, let me point out that the issue of permits is not nearly as bad as it might seem. Permits, as well as laws and regulations, are in the realm of politics, and a politician has been described as a person who senses where people are going and then gets out front to lead them. Almost all of the signs that I see suggest that the pendulum of reactions has already reached its maximum and is now swinging back toward the center. One sociological study in Wisconsin, which is an area of excess environmental concern, suggested that the total percent of people concerned about the environment in 1968 was something of between 15 and 16 percent of the population. In 1970, this exceeded 40% of the population and in 1972 was back down to about 15 or 16% of the population. Interestingly, the study showed that those with the best education retained their environmental concerns longer, and that city people retained a concern longer than rural people. In addition, it pointed out that when the less affluent people realized that achieving the environmental goals was going to cost them jobs, that apparently they lost their enthusiasm more rapidly than those who were more affluent at the time.

I suggest to you that if we express the needs of our constituency for health protection, and if we do this effectively to our Congressman and Senators, that we can expect a better response this year. I believe the Congress and the voters are fed up with EPA's over-regulations and the environmentalist's over-kill and that changes through amendment of the laws or forced changes in the regulations will occur very soon.

ENABLING LEGISLATION, PERSPECTIVE OF PROBLEM, REASON FOR PERMITS AND POSSIBLE SOLUTION

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The State of California has two basic policies relative to permits: 1) processing of permits should be expedited as rapidly as possible, and 2) the environment must be protected. There is no need to assume that expediting a process will mean that you cannot also protect the environment.

The Resources Agency encourages the use of long term and general permits for routine maintenance and for minor activities. We try to avoid situations in which an individual who wishes to carry out a minor job must go through the same process that is required for a major project, either from a time or a cost standpoint. A permit handbook for State permits is being considered. The thrust of that handbook would be to define explicitly what permits are needed, in what areas, for what purposes, and how to make them available as quickly as possible.

Within the Resources Agency my job is "Projects Coordinator". A major function of this job is to expedite the processing of comments on proposed projects. We have set some stringent deadlines for comments from the various State Departments. If a Department has not replied by the deadline then the permit goes without any comments by such Department.

ENABLING LEGISLATION, PERSPECTIVE OF PROBLEM, REASON FOR PERMITS AND POSSIBLE SOLUTION

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In July, 1975, the U. S. Corps of Engineers continued its jurisdiction over the navigable waters of the United States as they were in existence at that time, and at the same time included all wet lands contiguous or adjacent to those navigable waters. On July 1, 1976, the Corps will exert jurisdiction over all primary tributaries to the published navigable waters. It will also include all lakes of 5 acres or greater. Under the third phase, which will go into effect July 1, 1977, the Corps will go to the headwaters of all streams, to a point where the normal flow is 5 cubic feet per second, and will also include adjacent and contiguous wet lands to all the streams included in both phases 2 and 3.

One of the provisions in the regulations to lessen the administrative work load and to make the permit program more viable is the provision for a general permit. This matter was discussed during the past year with mosquito control personnel. It is the desire of the Corps to get permits processed as quickly as possible. General permits will be issued to include certain types of activities which are minor in scope and which do not have a bad cumulative or singular effect on the environment. Mosquito control activities have been considered as good candidates for general permits. Contacts have been made with the Mosquito Control Section of the State Department of Health to start the development of the general permit for mosquito control. A general permit would cover all the activities and conditions described in the permit for a certain geographical area – the first one for the mosquito abatment districts will cover only the San Francisco geographic boundaries of the Corps. If this proves acceptable, the Los Angeles and the Sacramento boundaries will be developed. It was first thought that a state-wide permit would be best, but it has been determined that activities are sufficiently different in various parts of the State that 3 general permits will be preferable.

The final regulations concerning permits for activities in navigable waters were published in the Federal Registry Volume 40, Number144, on 25 July, 1975, Section 33, CFR 209.120.

1 29 of these regulations allows the Corps of Engineers to issue general permits for categories of activities that are similar in nature and would be expected to have only a minor or minimal adverse impact when performed separately and cumulatively.

Notice is hereby given that the San Francisco District proposes to issue a general permit to the California Department of Health as the sponsoring agency for mosquito control activities in Alameda, Contra Costa, Del Norte, Humboldt, Lake, Marin, Mendocino, Monterey, Napa, San Benito, San Francisco, San Mateo, Santa Clara, Santa Cruz, Solano and Sonoma Counties. The purpose of this permit is to authorize the ongoing routine mosquito abatement practices performed or directed by health agencies. These activities would include the installation and maintenance of water control structures, repair of flood gates or wiers, excavation and maintenance of lateral ditches on irrigated lands, and the excavation and dredging of large 6′x4′ and small 18″x18″ drainage and water circulation ditches on tidal and reclaimed marshes. Also included would be the maintenance of levies, the clearing of brush on stream banks, the filling of isolated pot holes in stream beds, and such minor ditching of stream beds as required to interconnect isolated pools of water.

After review and a concensus is reached by concerned agencies, all mosquito abatement activities falling within the scope of these criteria would not require separate permits. Individual activities that do not conform to such criteria would require separate permits. The general permit, if issued, would not authorize activities which individually or collectively serve to alter the existing environment. The maximum quantities of work per site authorized by the proposed permit would be the clearing of 5,000 linear feet of water circulation ditch or the dredging of 1,000 cubic yards of material for the purpose of maintaining either drainage and collection ditches or levies. All material excavated during the clearing of either drainage or collection ditches must be disposed of in such a manner as to permit the free exchange of water with the surrounding area.

The activities governed by this permit which also fall within the jurisdiction of either the California Coastal Zone Commission or the San Francisco Bay Conservation and Development Commission would require the approval of those agencies. The National Environmental Policy Act of 1969 requires a consideration of the environmental impact of the work for which a permit is needed. For example, proposed maintenance work would bring about a reduction of the mosquito producing areas, thereby reducing or eliminating a public health problem. Control in this manner would also permit a reduction or elimination of the use of pesticides. Mosquito reduction may cause an increase in the human use of wetland areas creating an additional impact on the wetlands habitat. Benthic organisms would be destroyed by dredging and disposal of the dredged material would temporarily eliminate vegetation adjacent to the ditches. Presently unknown archeological, prehistorical or historical data and scientific knowledge may be lost or destroyed by work to be accomplished under the requested permit.

A permit issued by the Corps does not give any property rights either in real estate or materials, nor does it give any exclusive privileges, nor does it authorize any injury to private property or invasion of private property rights, nor does it authorize any infringement of federal, state or local regulations, nor does it eliminate the necessity of obtaining state assent to the work authorized. The decision on whether to issue a permit will be based on an evaluation of the probable impact of the proposed activity on the public interest. That decision will reflect the national concern for both protection and utilization of important resources. The benefit which reasonably may be expected from the proposal must be balanced against the reasonably foreseeable detriments. Many factors which may be relevant to the proposal will be considered, including conservation, economics, aesthetics, general environmental concern, historic values, fish and wildlife values, flood prevention, land use classification, navigation, recreation, water supply, water quality, and in general the needs and welfare of the people. No permit will be granted unless its issuance is found to be in the public interest.

A general permit may be revoked if it is determined that the cumulative effects of the activities will have an adverse effect on the public interest. Following revocation, any future activities in areas covered by the general permit shall be processed as individual permits under existing regulations. Any person who has an interest which may be adversely affected by the issuance of a Corps permit for the work described may request a public hearing. The request must be submitted in writing to the District Engineer and must clearly set forth the interest which may be adversely affected by the activity. Interested parties may submit in writing any comments they may have on the proposed work.

It is probable that general permits will be valid for a period of about 5 years.

MERCED COUNTY MOSQUITO ABATEMENT DISTRICT

VS

FRANKLIN COUNTY WATER DISTRICT

A. James Fagundes

Merced County Mosquito Abatement District
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ABSTRACT

The Franklin County Water District is described with regard to its encompassment, framework and operational treatment process. Mosquito breeding problems resulting from the treatment operation is researched from District files with emphasis on the cause. Failure of cooperative source reduction measures made it imperative for the Mosquito Abatement District to institute legal abatement action against the Water District. A chronological sequence of events illustrating personal contacts, official correspondence, abatement letters, Board of Trustee actions regarding filing and amending of legal abatement notices, and collaborative efforts performed by local and state regulatory agencies to assist the Abatement District in eliminating the nuisance are discussed. As a result of the Merced County Mosquito Abatement District action, the Franklin County Water District converted the problem discharge area into additional oxidation ponds and thus eliminated the mosquito nuisance situation.

ROLE OF THE COOPERATIVE AGREEMENT BETWEEN THE CALIFORNIA DEPARTMENT OF HEALTH AND LOCAL VECTOR CONTROL AGENCIES IN COORDINATING PESTICIDE USE

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In the early 1960's, popular and scientific concerns over the adverse effects of pesticides, coupled with increasing governmental controls, became apparent. By the middle of the decade, a fear developed that the beneficial uses of pesticides in public health programs might be lost in an avalanche of antipesticide action. This prompted a number of people with vision to seek a means of protecting the rights of local vector control agencies to use pesticides judiciously. Prominent among the leaders were Frank M. Stead, forward-thinking environmental engineer with the Department of Public Health and chief of the Environmental Health Services Division; Richard F. Peters, chief of the Bureau of Vector Control; Thomas D. Mulhern, mosquito control technical consultant; and Oscar V. Lopp, late manager of the Merced County Mosquito Abatement District.

Early in 1967, the State Board of Health adopted a policy statement entitled "Recommended Standards Relating to the Use of Pesticides in Vector Control." Department staff prepared a descriptive document entitled "Acceptable Pesticides and Their Use by California Abatement Districts and Other Official Mosquito Control Agencies." This included an "Official List of Pesticides" which set forth use specifications for parathion, methyl parathion, fenthion, malathion, and Abate®. Concurrently, the California Department of Agriculture amended its regulations to allow cooperating local agencies to apply highly toxic insecticides, defined as "injurious materials" (parathion and methyl parathion on the "official list") without consent of the landowner or notification of persons on the property. By this action the Department of Agriculture recognized not only the need for pesticide applications in mosquito control, but also that the low dosage rates presented no significant risk to people who might be on treated property.

In its first year, 49 agencies signed the cooperative agreement. The 1967 document required the local agencies to agree:

- To use the pesticides listed in the "official list" without exceeding the dosage rates and application rates specified, and in the manner described, for the control of mosquitoes.
- To maintain for review by appropriate governmental agencies for at least two years, a record of all pesticides applied, showing the kind, the dosage rate, the area treated, and the location.
- To avoid the use of pesticides in a manner that would produce illegal residues on agricultural produce or on meat or milk, as specified by the California State Department of Agriculture.

In return, the agreement provided:

- For exemption of cooperating agencies from the requirements for consent of landowners and notification of persons on property treated with injurious materials.
- For Department of Public Health support of local programs utilizing pesticides in conformity with the agreement.
- For the Department to add other pesticides to the "official list" when and as warranted.

Annual revision to fit changing needs has been coordinated with the California Department of (now) Food & Agriculture and the California Department of Fish & Game, as well as the local agencies involved. In 1969, two important steps were taken. One allowed many mosquito control products to carry this label addendum: "To be used by mosquito control agencies as provided under the contractual agreement with the California Department of Public Health relating to pesticide usage." The other made use of a procedure under which the Department of Food & Agriculture issued variances allowing prescribed uses not in accordance with a product's label. This mechanism, utilized each year since 1969, has been most useful in dealing with rapidly-developing pesticide resistance in many mosquito populations.

Also in 1969, legislation was enacted requiring pesticides used to be reported. A simplified system of reporting was negotiated, involving monthly pesticide use reports to the Department of (now) Health which are then tabulated and sent to the Department of Food & Agriculture. The information developed through this system has provided invaluable documentation on the use of pesticides in vector control.

In 1973, the intent of the Department to provide for certification of pesticide applicators was included in the cooperative agreement. This program came into being a year later.

By 1974, the number of signatory agencies had increased to 73. Specifications for mosquito control pesticides included parathion, methyl parathion, malathion, fenthion, and Abate, as in the 1967 document, but by this time the list had been expanded to add chlorpyrifos, naled, dichlorvos, propoxur, Lethane® 384, pyrethrins, synthetic pyrethroids, petroleum oils, fuel oils, and insect growth regulators. In addition, special exemptions had been granted for some uses of propoxur, fenthion, and chlorpyrifos.

Exemptions granted cooperating agencies had been expanded to incude the following:

Food & Agricultural Code –

Sec. 11408(e). Uses under the cooperative agreement are exempted from the definition of "agricultural use".

Administrative Code (Title 3) –

Sec. 2451.5, 2465. Requires reporting of specified pesticides. A memorandum of understanding exempts cooperating agencies which report to the Department of Health.

Sec. 2463. Exempts cooperating agencies from permit requirements relating to specified pesticides.

Sec. 2489. Exempts cooperating agencies from provisions related to re-entry of treated fields.

Sec. 3092(d). Exempts cooperating agencies from requirements for an experimental use permit.

Sec. 3094(b). Allows pesticide application upon property without consent of the landowner.

Sec. 3094(c). Allows pesticide application without notifying persons on the property.

At about that time (1974), major changes in pesticide regulation were initiated as the impact of the Federal Environmental Pesticide Control Act of 1972 began to be felt. This act amended the earlier Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which had been basically a labeling act. The new law, known as "FIFRA, as amended", greatly expanded the powers of the U.S. Environmental Protection Agency over pesticide use. Some of the key provisions empowered EPA to preempt intrastate regulation of pesticides, required that applicators of certain dangerous pesticides be certified, and forbade use of a pesticide in a manner inconsistent with its labeling. Civil and criminal penalties might be imposed. The Act was signed on October 21, 1972, with its provisions to be implemented during a four-year period. (A later amendment delayed full implementation one year.) Some authority could be delegated to the states in registering pesticides to meet special local needs, certification of applicators, and certain other areas, but in the first two or three years it was unclear just what California's role would be.

Inevitably, the uncertainties caused a great deal of confusion throughout the state, which extended to the cooperative agreement. Beginning in 1975, specifications for pesticide use were not included because of the difficulties revolving around the meaning of not using pesticides in a manner inconsistent with the label. It was also unclear whether the Department of Food & Agriculture would be able to continue to issue label variances; however, as of this writing, the procedure is still allowed.

Probably the most far-reaching impact upon the cooperative agreement, precipitated by FIFRA, as amended, was to necessitate making the cooperative relationship available to agencies controlling vectors other than mosquitoes. The cooperative agreement currently has seven provisions, all consistent with federal and state laws and regulations. A cooperating agency agrees:

- To use pesticides in a manner not inconsistent with the label, or as authorized by official variance, to control pest and vector organisms.
- To ascertain, at least once each year, that application equipment is correctly calibrated, and to maintain calibration records.
- To maintain for review by appropriate governmental agencies a record of each location treated, showing the kind, formulation, and amount of pesticide; the method and equipment used; the size of the area treated; the type of habitat treated; the date of application; and the name of the applicator.
- To submit to the Department each month a Pesticide Use Report, showing the target organism treated, the product, and the formulation and amount of each pesticide used.
- To report to the Department, in a manner specified, any conspicuous or suspected adverse effects upon humans, domestic animals and other nontarget organisms, or property.
- To dispose of used pesticide containers and waste materials only at locations and under conditions as specified by the appropriate regulatory agency.
- To certification of its control technicians by the California Department of Health in order to verify their competence in performing all aspects of the control of pest and vector organisms.

Several major changes have occurred and are foreseen in the format and role of the cooperative agreement. Specifically:

- The agencies now signing the cooperative agreement include not only those which are limited to controlling mosquitoes but also those which control other pests and vectors.
- Specifications are now being prepared for pesticides used to control all vectors. Aid is being obtained from the registration units of both EPA and the California Department of Food & Agriculture.
- The full utility of such specifications is not yet clear, but it is possible that a basis might be provided for a regulatory agency enforcement policy which would allow the specifications to be considered part of a product's labeling.
- As part of the cooperative relationship, the possibility is being explored that the Department's Vector Control Section might become the registrant for products registered by the Department of Food & Agriculture to meet special local needs, or even products registered by EPA.
- The document, and the cooperative relationship, may serve as the means for applying the provisions of the FIFRA, as amended, for emergency and crisis exemptions.
- Other utilizations will be developed as the need arises, perhaps even extending the concept of the cooperative agreement to cover physical and biological control approaches as well as pesticides.

In summary, the cooperative agreement has proved to be effective in resolving the dilemma of protecting people **from** pesticides, yet **with** pesticides. It has provided an environment within which local vector control agencies are able to conduct positive programs, with the Department of Health representing all cooperating agencies before federal, state, and local regulatory groups. This has lessened the burden imposed by the proliferation of regulations at all levels of government. The flexibility of the cooperative agreement has allowed it to continually fulfill its purpose as conceived ten years ago, and it will be useful in solving problems that arise in the future.

ENCROACHMENT BY ADMINISTRATIVE ACTION OF REGULATORY AGENCIES UPON THE PREROGATIVE OF LOCAL MOSQUITO CONTROL AGENCIES

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Good morning to all of you sturdy souls, who have heard the call of duty and responded at this somewhat early hour. In this our "Bicentennial Year", I am reminded of the reason for this celebration of independence and the happening that brought it about. We first think of freedom -- religious, personal, but most of all freedom from unreasonable regulations. It would seem that our country is fast approaching the same level of unreasonable regulations that caused the formation of our nation 200 years ago.

We find that our mosquito abatement districts (and we as individuals) are subject to ever mounting demands for reports, summaries, questionnaires, and a full rainbow of forms to be filled out in triplicate from inspectors, reviewers, consultants, and auditors, all of this demands great time and energy. We ask ourselves what does this accomplish? Is it all necessary or are we strangling ourselves in Bureaucracy?

I am not advocating throwing barrels of tea into the ocean, or even taking up arms, but we must analyze the situation, realize the consequences and plan our actions to continue in our efforts to prevent and control mosquitoes.

By way of background information, let me try to bring you all up to date on mosquito control districts and what is happening to "make life and work miserable" for the districts. More then 60 years ago the Legislature of the State of California adopted mosquito control statutes to permit the prevention of one form of degradation of the environment, and to provide for reclamation or improvement in areas of the state where the environment had already been severely damaged by faulty land use and water management, which produced hordes of mosquitoes. The then new statutes provided a procedure by which the public in any sizeable area of the state could organize a unit of local government which they would support by taxing themselves, and which would be responsive to the will of the people of the area in preventing environmental damage or correcting damaged environments. The statute is called the Mosquito Abatement District Act of 1915. It is unique because it has stood the test of time: now, 60 years later, with only minor amendments to update its details, it continues to provide a satisfactory basis for organized local mosquito control, even though the technology of mosquito control has advanced radically, population has increased enormously, and land uses and water management have substantially changed the face of California. It is equally significant that the number of local mosquito control agencies has multiplied to keep pace with the development of the respective areas of the state and with the many new mosquito sources created and the newly populated areas to be protected. Meanwhile, the environment protection programs of these agencies have been carried out without serious interference with primary land and water uses, through the application of efficient mosquito control services aided by cooperation of the land and water users. Only rarely has it been necessary to resort to legal action, even though the Statutes provide ample legal powers which could be used to abate as public nuisances the man-made sources of mosquitoes. The history of the past 60 years of mosquito control in California under the Mosquito Abatement District Act is a dramatic representation of responsive, effective, economical local government in the best democratic tradition, serving the needs of the public with minimum interference with the freedom of the individuals who constitute the "public". The districts formed under this law have provided mosquito control services second to none in the world; and the law is regarded as the stongest and best in existence. It has also been used as a pattern by various other states.

One may ask how this law came into existence, and why it contains various provisions. Prior to the establishment of mosquito abatement districts, malaria was rampant in California. Frequent flights of mosquitoes from the natural breeding places about San Francisco Bay infested the surrounding residential communities, and in the interior valleys spring and early summer floods brought hordes of mosquitoes. As irrigation developed there were added extensive man-made sources which continued to produce mosquitoes later in the season. These conditions led to studies of the mosquito and mosquito-borne disease problems by the University of California, the State Department of Public Health, the Commonwealth Club of California, and various committees of the State Legislature. Features of the law to which its success is attributed are as follows:

1. The policy-making Board of Trustees called for is composed of citizens of the District, to represent all of its people, including those to be protected and those upon whose lands the mosquito sources exist.

2. This Board is empowered to employ specialist personnel and labor, and to obtain equipment and supplies as necessary.
3. Specific powers necessary for mosquito control operations are vested in the Board of Trustees, including:
   a. The right to enter upon public and private lands for inspection and to perform mosquito control operations.
   b. Specific authority to use chemicals to control mosquitoes.
   c. Specific authority to perform drainage and water management functions necessary for mosquito control.
   d. Authority to abate as public nuisances man-made sources of mosquitoes, on private lands.
   e. Responsibility to compensate landholders for any damages caused by the taking of land for mosquito control purposes.
   f. Authority to do and perform any other acts necessary to protect the public from mosquitoes.

During 60 years of operation under this law, the mosquito abatement districts have operated through cooperation rather than by legal prosecution, and through effective and economical service programs. It is significant that in 1975, when unprecedented encephalitis epidemics were common in many other parts of the USA, California escaped – even though this state is recognized by the experts as endemic for encephalitis.

The mosquito control program has provided the desired protection of the environment by controlling the pest and vector mosquitoes which threaten the health and comfort of Californians. Internal discipline of the organizations has prevented misuse of the powers provided by the Legislature and it is clearly evident that these agencies should not be subjected to repressive interference by regulatory agencies which have been created by Congress and/or the state government for purposes parallel with those of the mosquito abatement districts and the health departments which engage in mosquito control.

An outstanding characteristic of local mosquito control agencies in California is the willingness of these agencies to cooperate with federal, state, and other local govenmental units having interests in land and water management and in safety of the public as well as an equal willingness to cooperate with the landholders upon whose lands mosquito problems develop. Active and practical cooperation has been fostered and encouraged by the State Health Department and the California Mosquito Control Association, each of whom contributes substantially to the coordination of mosquito control among local agencies and with other governmental units for mutual benefit. The practical results of joint consideration of mutual problems often has been modification of control approaches for integrated or multiple-purpose benefits, as where fish and wildlife objectives have been combined with those of mosquito control.

Technical developments in California have been used as a basis for some practical and workable specifications included among the rules set out by EPA and other regulatory agencies. Such items as the selection of approved chemicals and dosage rates, types of equipment, and acceptable safety procedures closely resemble usage adopted in California before EPA came into existance! The list of developments is far too long to be included here in detail, but a few examples may be illustrative:

1. Training of personnel for effective and safe use of chemicals, based upon valid research and evaluation, has been carried on by all local agencies throughout the period of their existence.
2. Evaluation of the efficacy and safety of control measures has been performed continuously by the State Health Department, with standards issued or modified as technology advances in accordance with valid research. An example is the elimination of DDT from extensive field operations when research demonstrated the phenomenon of concentration through the food chain to possibly dangerous levels in some wildlife species.
3. As early as 1949, mosquito control agencies in California subscribed to "Standards and Recommendations" for balanced scientific programs of biological, physical, and chemical control.
4. In 1951, long before the creation of EPA, the California Mosquito Control Association, with guidance by the State Health Department and participation by many mosquito control agencies, developed and published standards and manuals for safe and effective use of chemicals, with separate specifications for the materials of relatively low toxicity and for the highly toxic insecticides such as parathion.
5. More recently, when it became apparent that workers would have to be certified as competent to perform the tasks demanded of them, CMCA with approval of its member agencies requested the State Health Department to develop a training manual and a training program, and to conduct certification examinations for all personnel. After three examinations, this is now an accomplished fact and substantially all current personnel have completed intensive undated training and have successfully passed comprehensive examinations to demonstrate their competence.
6. Throughout the past five years or so of rapidly changing technology, the University of California (in response to petitions of the California Mosquito Control Association) has been increasing its mosquito control research, to keep ahead of the demands for advancing technology.

These are just a few outstanding examples of the advances characteristic of the programs in California which have established a basis for the belief that Congress would not want regulatory agencies of federal government to impede the progress of public health by overriding the powers granted local agencies by the State Legislature.

Encroachment upon these powers by administrative units of various governmental agencies has taken various forms the propriety of which could possibly be questioned on contstitutional grounds, related to invasion of State's rights and upon the premise that a responsible unit of government within a state should be presumed to be qualified to carry out its responsibilities, without having to obtain a permit or approval from the administrative unit of some other government agency not as well qualified to judge the needs of the specific technical field. (This would in no way relieve the mosquito abatement district of responsibility of performing its operations in a manner compatible with the local environment, nor would it be relieved of criticism or legal action should its operations result in damage to the property of a landholder or to the environment).

1. The limitations upon the use of chemical pesticides imposed by EPA is the most familiar encroachment upon the prerogatives of local mosquito control agencies, which by State law are given the right to "take all necessary or proper steps for the extermination of mosquitoes and to enter upon without hindrance any lands – to treat with appropriate chemical or biological control agents the breeding places of mosquitoes –".
   1) There seems to be a great deal of doubt about the wisdom of the EPA ban on heptchlor and chlordane. Federal Judge Herbert Perlman ruled on December 12 that the factual evidence against these pesticides was not sufficient for suspension. In November a subcommittee of the National Cancer Advisory Committee considered cancer criteria and decided that these criteria were not appropriate for regulatory decisions. But it appears that in this decision, EPA has bypassed the experts.
2. The permit systems recently imposed by administrations of the U. S. Corps of Engineers and by regional agencies appear to violate the powers granted directly by the State Legislature "if necessary or proper, to build, construct, repair, and maintain necessary dikes, levees, cuts, canals, or ditches upon any land. . ." In practice, operation of such permit systems has meant delays of up to a year in urgently needed cleaning of ditches previously maintained for many years by mosquito control agencies, with resultant emergence of mosquito broods in the interim period.
   2) The Army Corps of Engineers has recently expanded permit regulations. In section 404 of their regulations, their authority extends to all coastal and inland navigable waters and contiguous or adjacent coastal and fresh water wetlands, as well as tributaries of navigable waters to their headwaters. Freshwater wetlands and coastal wetlands are those areas which are periodically inundated and that are normally characterized by the prevalence of vegetation and requires salt or brackish water on saturated soil conditions for growth and reproduction. You'd better believe this affects mosquito control operations!
3. Although the Districts are empowered by the Legislature to "employ such personnel and contract for such services as may be necessary. . ." (for mosquito control) the requirement by EPA that the personnel so employed prove by certification approved by EPA that they are qualified to discharge the duties expected of them appears to be a violation of the prerogative granted the District by the Legislature, and presumably could require that the District employ only individuals approved or certified by EPA.
4. Until a few years ago, the local districts and the local populations enjoyed the prerogative of determining areas to be included within individual Districts. boundary limitations, annexations, consolidations, etc. However, it appears now that the functioning of LAFCO's has largely deprived the local Districts of their prerogatives in this respect.
   3) Let me point out that mosquito control districts are not alone in their feeling of frustration due to overlapping and conflicting regulatory agencies. Quoting Mary Henderson, a fellow councilman from Redwood City, from a report given at a seminar on Regional Growth & Development in November 1975 – "We desperately need a reasoned, conscious, deliberated federal policy on growth to replace the present unstated, nondeliberative, inadvertant, often internally inconsistant, 'de facto' policies which presently exist in federal laws, programs, rules and regulations departmental edicts and in some instances, even in whims of individual bureaucrats."

   She then quotes the President's Growth Report – "What is known is that Federal programs often have conflicting objectives; that strategies of one agency may be blurred or reversed by the actions of another and that the effect on growth of programs designed explicitly to change growth patterns seems to be far less than the impact of other programs whose purposes ostensibly have nothing to do with growth!"

   Frankly, all else pales in comparison to specific instances of the impact of federal government single purpose policies, or inadvertent policies or unconscious policies. In Redwood City we are preparing, in conjunction with neighboring local governments, to build a subregional sewage treatment plant in order to raise the quality of discharge into the San Francisco Bay as required by Federal government policy. The outfall line has already been built, with assistance of federal funds. The location of the treatment plant to go with the outfall line has been in all our plans for a number of years. But suddenly, long after plan approvals by assorted government agencies, now Fish and Wildlife objects to the site of the plant and the Corps of Engineers will not issue a permit for the plant until that single objection is removed. All this comes at least 7 years after state and federal agencies have approved and even funded the project. Which Federal policies will prevail, clean water or protection of certain wildlife? The irony is that the Federal government does not know which outranks what. While there are Federal policies in both areas, there are no Federal policies to interrelate the two concerns.

It is to be hoped that these regulatory agencies referred to were formed for a purpose fundamentally similar to that of the mosquito abatement districts, i.e., that of protecting the environment for man, or in some cases reclaiming environments already impaired by man. Therefore, it appears inconsistent that they should function by administrative decision in a manner that will impair the work of the mosquito abatement districts. The question then becomes one of "How can the responsibilities of the several agencies be met by mutually acceptable, cooperative action, rather than by conflict or confrontation".

Several possibilities are immediately apparent (there may be others), each of which may have some limitations. These deserve consideration by this group and by the regulatory agencies and possibly by Congress and the respective State Legislatures. These have been defined as follows:

1. Where public health agencies are engaged in mosquito control, there should be categorical exemption of program functions which are specifically authorized by statutes adoped by State Legislatures from compliance with restrictive regulations of other agencies. (This would in no way limit the prerogative of the Congress or the State Legislatures to limit the functions of these agencies, nor would it relieve the local agencies of their current responsibility for safe operation and liability for damage, nor would it lessen their current broad responsibility to cooperate with other agencies in protecting the environment without unjustified limitation on freedom of action by the citizenry).
2. Public health agencies engaged in mosquito control should be granted annual permits in advance, based upon their plans for the coming year, by governmental agencies empowered by Congress or by the State Legislatures to regulate uses of chemicals, water management practices such as ditching, diking, filling, impoundment, etc., and the use of biological control agents, on public or private lands and waters.
3. Because of the urgent need for immediate action when mosquito problems develop, in lieu of permits as presently required, regulatory agencies should accept informative reports of operations performed by public health agencies engaged in the control of mosquitoes and mosquito-borne diseases.

I would hope that you as district trustees representing various areas will take back to your boards the concerns expressed here today. I feel it is our duty as appointed representatives of the people to use whatever power or "clout" as it has been often described, to assist our staffs in this effort to lift the heavy thumb that is being applied to the responsible control of mosquitoes. Using the words of Austin Morrill in the latest Mosquito News in reference to New Jersey having problems in Mosquito Control – "the problem is not in controlling mosquitoes – but people".

SURVEILLANCE FOR ARTHROPOD–BORNE VIRUSES AND DISEASE BY THE CALIFORNIA STATE DEPARTMENT OF HEALTH, 1975

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Surveillance for mosquito-borne encephalitis in California during the 1975 season was remarkable in revealing an unusually low level of activity for western encephalitis (WEE) and St. Louis encephalitis (SLE) viruses. For the second year in a row, and only the second year since record-keeping began in the 1940's, there were no indigenous human cases of WEE or SLE (Table 1). As usual, extensive efforts were made by the State Viral and Rickettsial Disease Laboratory and five county public health laboratories to detect any cases which might have occurred (Table 2). The experience this season was in marked contrast to the large epidemics which occurred in the eastern, southern and central United States during 1975.

Two cases of SLE occurred in persons who acquired infection out-of-state: a 75 year old woman, resident of Chicago, acquired SLE apparently while visiting in Illinois State Park, Zion, Illinois, an epidemic area during 1975. She became ill August 25, 4 days after arriving for a visit in Los Angeles. The complement-fixing (CF) antibody titer was stationary at 1:64, and the indirect fluorescent antibody (IFA) titer was stationary at 1:512. Serum samples were insufficient to do neutralization tests or other confirmatory tests, but it was felt this represented a presumptive positive case, since history of exposure, clinical findings, and the high antibody titers were compatible with SLE. She recovered completely and returned home. The second case was a 23 year old woman from Modesto, Stanislaus County, who became ill on August 29, 2 days after returning home from Houston, Texas, another SLE endemic area in 1975. The CF antibody titer for SLE rose from <1:4 to 1:128, and IFA antibody titer rose from 1:128 to 1:1024. Paired cerebrospinal fluid samples showed rising IFA antibody titer for SLE of <1:4 to 1:256. She recovered and was discharged from the hospital, but later had to be readmitted because of persisting neurologic problems.

No equine cases of WEE were laboratory-confirmed, although 40 clinically suspect cases were reported to the Department (Table 3). No WEE virus isolates were made from equine brain samples tested in suckling mice, but 3 cases of rabies in equines were confirmed by the FRA test and by virus isolation.

A total of 1,000 mosquito pools were tested in suckling mice, from the usual sampling sites throughout the state (Tables 4-6). One isolate of Turlock virus was made from a pool of 50 *Culex tarsalis* collected October 10 in Mohave County, Arizona. Surprisingly, no virus isolates were made from any of the mosquito pools collected in California However, more intensive study of research sites by Dr. W. C. Reeves' group did reveal low levels of activity for California encephalitis, Hart Park, Turlock and other viruses in the Sacramento Valley, and WEE antibody conversion in chicken flocks in Kern County; and Dr. Telford Work's research in the Imperial Valley revealed the presence of WEE and SLE viruses in certain foci there. In summary, this was an unusually quiet surveillance year, in contrast to the large epidemics of WEE and SLE in the midwestern, southern

Table 1.—Human cases of arthropod-borne encephalitis – California, 1950-1975.

YEAR	TOTAL	WEE	SLE	DEATHS		
1950	157	88	69	2	–	(1 WEE, 1 SLE)
1951	55	22	33		–	
1952	420	375	45	10	–	(9 WEE, 1 SLE)
1953	36	14	22	1	–	(SLE)
1954	121	22	99	2	–	(SLE)
1955	9	6	3		–	
1956	21	14	7		–	
1957	26	3	23	1	–	(SLE)
1958	53	37	16		–	
1959	42	2	40	1	–	(SLE)
1960	13	1	12		–	
1961	10	2	8	1	–	(WEE)
1962	21	5	16	1	–	(SLE)
1963	15	3	12		–	
1964	12	10	2		–	
1965	10	9	1		–	
1966	17	9	8		–	
1967	15	7	8		–	
1968	15	11	4		–	
1969	5	–	5		–	
1970	2	–	2		–	
1971	5*	3	2		–	
1972	8**	3	5		–	
1973	5	–	5		–	
1974	–***	–	–***		–	
1975	–****	–	–****		–	

*In addition, 1 case of VEE confirmed, in traveler to Mexico

**In addition, 2 cases of VEE confirmed, in travelers to Mexico

***1 case of SLE contracted in Texas

****1 case of SLE contracted in Texas; 1 case in Illinois

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[2]Vector Control Section.

[3]Veterinary Public Health Unit, Infectious Disease Section.

Table 2.–Humans tested serologically for arbovirus encephalitis by the Viral and Rickettsial Disease Laboratory, California State Department of Health, and by county health department laboratories, by county of residence and month of illness onset. 1975.

COUNTY	TOTAL	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	UNK.
California, Total	583	2	–	1	10	41	80	106	85	100	78	39	9	32
Alameda County	3	–	–	–	1	–	–	2	–	–	–	–	–	–
Berkeley	8	–	–	–	–	–	1	1	–	–	4	–	–	2
Butte	3	–	–	–	–	–	–	1	1	1	–	–	–	–
Contra Costa	21	–	–	–	–	2	5	2	4	4	2	1	–	1
El Dorado	16	–	–	–	–	–	–	4	1	7	2	1	–	1
Fresno*	104	–	–	–	–	8	17	20	18	19	8	5	8	1
Glenn	1	–	–	–	–	–	–	–	–	–	–	–	–	1
Humboldt-Del Norte	10	–	–	–	–	2	–	1	3	2	–	1	–	1
Imperial	2	–	–	–	–	1	–	–	1	–	–	–	–	–
Inyo	1	–	–	–	–	–	–	–	–	–	–	1	–	–
Kern	27	–	–	–	–	1	4	6	4	6	2	2	–	2
Kings	1	–	–	–	–	–	1	–	–	–	–	–	–	–
Lassen	1	–	–	–	–	–	–	1	–	–	–	–	–	–
Los Angeles County*	10	–	–	–	1	1	–	–	1	1	2	2	–	2
Long Beach	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Los Angeles City	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pasadena	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Madera	1	–	–	–	–	–	1	–	–	–	–	–	–	–
Marin	8	–	–	–	–	1	–	–	–	3	2	1	–	1
Mendocino	1	–	–	–	–	1	–	–	–	–	–	–	–	–
Merced	3	–	–	–	–	–	2	1	–	–	–	–	–	–
Modoc	1	–	–	–	–	–	–	–	–	1	–	–	–	–
Monterey	10	–	–	–	–	–	1	1	–	3	2	1	–	2
Napa	2	–	–	–	–	–	–	1	1	–	–	–	–	–
Nevada	3	–	–	–	–	–	1	1	–	–	–	1	–	–
Orange*	8	–	–	–	–	1	–	2	4	–	1	–	–	–
Placer	11	–	–	–	–	–	3	1	2	–	2	1	–	2
Riverside	7	–	–	–	1	–	–	–	2	2	1	1	–	–
Sacramento*	42	–	–	–	1	4	8	7	5	7	10	1	–	–
San Bernardino	5	–	–	–	–	–	1	2	1	–	–	–	–	1
San Diego*	69	2	–	–	2	1	14	20	6	10	9	5	–	–
San Francisco	61	–	–	1	1	5	5	14	5	9	13	5	–	3
San Joaquin	14	–	–	–	–	1	2	4	3	–	1	1	–	2
San Luis Obispo	4	–	–	–	–	–	–	1	–	1	1	1	–	–
San Mateo	38	–	–	–	1	3	5	2	8	11	4	2	–	2
Santa Barbara	13	–	–	–	–	2	1	2	2	2	2	2	–	–
Santa Clara	3	–	–	–	–	1	–	1	–	–	–	1	–	–
San Jose	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Santa Cruz	3	–	–	–	–	–	1	–	1	–	1	–	–	–
Shasta	7	–	–	–	–	–	3	–	–	1	1	–	–	2
Sierra	2	–	–	–	–	–	–	1	–	1	–	–	–	–
Siskiyou	3	–	–	–	1	–	–	1	–	–	–	–	–	1
Solano	6	–	–	–	–	1	1	–	1	2	1	–	–	1
Sonoma	9	–	–	–	–	1	–	1	3	1	1	1	–	1
Stanislaus	12	–	–	–	–	–	1	1	2	2	3	1	1	1
Sutter-Yuba	2	–	–	–	–	–	–	–	2	–	–	–	–	–
Tehama	7	–	–	–	–	–	1	1	3	1	–	–	–	1
Tuolumne	3	–	–	–	–	–	–	–	–	1	2	–	–	–
Ventura	10	–	–	–	1	2	1	1	–	2	1	1	–	1
Yolo	5	–	–	–	–	2	–	1	1	–	–	–	–	1
Undesignated	1	–	–	–	–	–	–	1	–	–	–	–	–	–

*Tested by county health department laboratory.

Table 3.–Suspected clinical cases of arbovirus encephalitis in equines by county and month, for California 1975.

County	Month of Onset Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Undetermined[a] or Not Tested	Totals[b]
Totals	0	0	2	5	3	8	5	6	3	1	0	1	6	40
Amador	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Butte	-	-	-	1	-	1	-	-	-	-	-	-	-	2
Del Norte	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Fresno	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Inyo	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Kern	-	-	-	1	-	-	2[c]	-	2	1	-	-	3	9
Lake	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Los Angeles	-	-	-	1	-	1	-	-	1	-	-	-	-	3
Mendocino	-	-	-	-	-	-	1	-	-	-	-	-	1	2
Merced	-	-	1	-	1	-	-	-	-	-	-	-	-	2
Plumas	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Riverside	-	-	-	1	-	-	-	1	-	-	-	-	1	3
San Bernardino	-	-	-	-	-	-	1	1	-	-	-	-	-	2
San Diego	-	-	-	-	-	-	-	-	-	-	-	1	-	1
San Joaquin	-	-	-	-	-	-	1	-	-	-	-	-	-	1
Santa Clara	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Santa Cruz	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Siskiyou	-	-	-	-	-	2	-	1	-	-	-	-	-	3
Sonoma	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Sutter	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Tulare	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Yuba	-	-	-	-	-	-	-	-	-	-	-	-	1	1

[a]Inadequate serum specimens available for testing.

[b]Complement fixation tests were performed on all serum specimens submitted and virus isolation techniques were attempted on all brain specimens by the Viral and Rickettsial Disease Laboratory, California State Department of Health.

[c]One possible case of WEE in a six-year-old gelding from Kern County; onset July 1 and euthanized on July 5; vaccination history unknown; complement fixation antibody on a single blood sample taken July 5 was 1:64. No brain specimen submitted for virus isolation attempts.

and eastern United States, and to virus activity in previous years in California (Table 5).

Surveillance for 1976 will consist of approximately the same level of effort as for 1975. The sparse rainfall and Sierra snow levels of the 1975-1976 Winter and Spring may help in mosquito control and in maintaining the low level of virus transmission and rarity of human and equine disease.

There were 18 Colorado tick fever cases confirmed during 1975, the third highest year on record. Continuing field studies, including possible tick control methods to interfere with the virus transmission and virus persistence, are planned by the Vector Control Section and the Viral Rickettsial Disease Laboratory.

ACKNOWLEDGMENT

The assistance and cooperation of many staff members of the Viral and Rickettsial Disease Laboratory, the Vector Control Section, and others in the California State Department of Health, as well as of local mosquito abatement districts, the California State Department of Food and Agriculture, and other local, county and state or federal agencies in carrying out the surveillance program are gratefully acknowledged.

Table 4.—Number of mosquitoes (pools) tested, by species and month, by the Viral and Rickettsial Disease Laboratory, California State Department of Health, 1975.

Species	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	TOTALS
Culex												
tarsalis	215 (10)	142 (6)	48 (5)	505 (18)	1,364 (50)	3,797 (96)	5,361 (136)	6,595 (164)	3,373 (80)	2,341 (60)		23,741 (625)
erythrothorax	73 (3)	139 (3)	9 (3)	996 (25)	1,311 (33)	1,748 (40)	2,816 (68)	708 (20)	1,119 (27)	951 (21)		9,870 (243)
peus			3 (1)		1 (1)	2 (2)	33 (2)	562 (18)	12 (1)			613 (25)
pipiens					1 (1)	4 (1)	2 (2)	51 (4)	9 (2)			67 (10)
Aedes												
dorsalis					12 (2)		9 (1)					21 (3)
sierrensis						823 (18)	495 (9)	1,043 (21)	220 (4)			2,581 (52)
Anopheles												
franciscanus							8 (4)					8 (4)
freeborni							9 (3)					9 (3)
Culiseta												
incidens			2 (1)		3 (1)		200 (4)	91 (2)	10 (2)	20 (3)		326 (13)
inornata	2(1)		13 (4)	1 (1)	10 (2)		11 (4)				18 (3)	55 (15)
particeps					15 (1)		40 (3)					55 (4)
Psorophora												
confinnis								8 (3)				8 (3)
TOTALS	290 (14)	281 (9)	75 (14)	1,502 (44)	2,717 (91)	6,374 (157)	8,984 (236)	9,058 (232)	4,743 (116)	3,312 (84)	18 (3)	37,354 (1,000).

Table 5.—Arbovirus surveillance activities and results by the California State Department of Health.

	1971	1972	1973	1974	1975
Suspect Human cases of encephalitis/meningitis tested serologically	620	729	1,037	643	583
SLE +	3	5	5	1*	2*
WEE +	3	3	0	0	0
VEE +	0	2*	0	0	0
Suspect equine cases of encephalitis tested serologically	145	68	56	61	40
WEE +	16	1	2	2	0
Number of mosquito pools tested	1,784	6,336	4,838	1,690	1,000
WEE +	16	42	97	4	0
SLE +	6	64	75	2	0
Other +	43	74	109	38	0
Total +	65	180	281	44	0

*Out-of-state contraction.

Table 6.–Number of mosquitoes (pools) tested, by county and month, by the Viral and Rickettsial Disease Laboratory, California State Department of Health, 1975.

County	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	TOTALS
Colusa						798 (16)		550 (11)	550 (11)			1,898 (38)
El Dorado						262 (6)	435 (8)	1,134 (23)	220 (4)			2,051 (41)
Fresno							20 (1)	36 (1)				56 (2)
Glenn									490 (10)			490 (10)
Imperial	253 (10)	158 (3)		235 (8)	17 (2)	331 (9)	189 (9)	55 (5)	50 (3)	146 (4)		1,434 (53)
Kern					42 (2)	112 (2)	269 (8)		364 (8)	91 (3)		878 (23)
Los Angeles					205 (6)				11 (1)			216 (7)
Madera					20 (2)		46 (1)	42 (2)				108 (5)
Marin						561 (12)	260 (5)					821 (17)
Merced							785 (17)	128 (4)				913 (21)
Orange								71 (2)		14 (1)		85 (3)
Placer								500 (10)				500 (10)
Plumas								1,546 (31)				1,546 (31)
Riverside					734 (19)	971 (27)	850 (25)	331 (10)	952 (21)	1,539 (31)		5,377 (133)
Sacramento						22 (1)	310 (7)					332 (8)
San Bernardino		1 (1)	40 (10)	153 (10)	968 (40)	423 (19)	2,045 (66)	657 (34)	42 (3)	276 (11)	11 (2)	4,616 (196)
San Diego				501 (11)	722 (17)	1,331 (31)		517 (13)	438 (13)	503 (12)		4,012 (97)
San Joaquin							75 (2)					75 (2)
Shasta							999 (22)					999 (22)
Siskiyou								1,947 (42)				1,947 (42)
Sutter						211 (5)		475 (12)	500 (10)			1,186 (27)
Tehama							1,158 (24)	984 (20)				2,142 (44)
Tulare						10 (1)	63 (3)					73 (4)
Ventura								1 (1)	642 (18)	45 (5)		688 (24)
Yolo						500 (10)	530 (11)					1,030 (21)
Yuba							444 (10)					444 (10)
Mohave, Arizona			35 (4)		9 (3)	842 (18)	162 (6)	18 (8)	78 (6)	431 (10)	7 (1)	1,582 (56)
Yuma, Arizona	37 (4)	122 (5)		613 (15)			344 (11)	66 (3)	406 (8)	267 (7)		1,855 (53)
TOTALS	290 (14)	281 (9)	75 (14)	1,502 (44)	2,717 (91)	6,374 (157)	8,984 (236)	9,058 (232)	4,743 (116)	3,312 (84)	18 (3)	37,354 (1,000)

ARBOVIRUS RESEARCH IN KERN COUNTY, CALIFORNIA, THE EVOLUTION OF INTERESTS AND DISCOVERIES OVER MORE THAN 40 YEARS[1]

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When I was invited to present a research oriented paper to this conference I decided the Bicentennial year of 1976 was an appropriate time to present an historical review of arboviral research in Kern County. In past conferences we attempted to limit discussion to our latest research developments and future problems. Kern County has been a focal area of field research on encephalitis for over 40 years. The period has witnessed an evolution of research interests and many of the discoveries are now integrated into your control programs and the general scientific knowledge of such diseases.

This presentation is dedicated to the numerous agencies that provided the cooperation essential to the research effort and particularly to the Kern Mosquito Abatement District and its late manager Arthur F. Geib. The leadership and support of this agency were a key to whatever success was achieved.

The form of this presentation will be different from the usual scientific paper in that I have divided the over 40 years that begins in 1930 into 5 decades. First, I will give you a general characterization of research in each decade. In the next step I will review the principal research questions that were pursued and then I will present a series of summary statements that represent a judgement of the principal findings. I hope you will find this different approach instructive and that the statements of findings will be useful as a guide to much of what is currently known and accepted about western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) and their control in the western United States. The analysis is largely limited to field studies in Kern County and to related laboratory research. Well over 200 persons have contributed to the research. Omission of individuals names and of an extensive bibliography is done only for economy of time and space. I will refer several times to the statement a farmer made to me some 15 years after we started research in Kern County, it was "Doc if you don't know all the answers now you never will." Ladies and gentlemen he was right!

1930 - 1939 The Pioneering Decade

I will call 1930-1939 the "Pioneering Decade of Encephalitis Research". The studies were initiated in Kern County in the early 1930's by staff of the George Williams Hooper Foundation of the University of California and the research has had an unbroken continuity since then. The area was selected because the rates for the diseases known as poliomyelitis and encephalitis were as high or higher in Kern County than in any other county in the U. S. A. at that time. Poliomyelitis was known to be caused by a virus, in 1930 WEE virus was first isolated from a horse in California and in 1933 SLE virus was isolated from a fatal human case in Missouri.

The basic questions posed with reference to these 2 diseases in Kern County were:

1. What are the causes?
2. Are their epidemiologies different?

The epidemiological and virological studies clearly showed that:

1. Three distinct viruses were involved: western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE), and poliomyelitis.
2. All 3 viruses could produce both inapparent and clinical disease in man.
3. Encephalitis viruses most likely were transmitted by mosquitoes.
4. Poliomyelitis virus most likely was transmitted man to man.

The above findings dictated an evolution of the research into what I will call "The Discovery Decade" of 1940-1949.

1940 - 1949 The Discovery Decade

The principal research questions in this decade were related to the development of practical approaches to control of encephalitis, and were:

1. What is or are the vectors of WEE and SLE viruses?
2. What is the biology of the vector?
3. Can the vector be controlled?
4. What are the sources of vector infection?
5. What is the extent of disease and inapparent infection in man and horse?

The first breaks that provided answers to these questions occurred when epidemics of encephalitis were investigated by the University of California team in the Yakima Valley, Washington in 1941 and 1942. *Culex tarsalis*, a mosquito previously held to be innocuous, was found infected with WEE and SLE viruses and birds with inapparent infections were sources of vector infection.

Again during this decade, Kern County was an ideal site for extension of the findings because:

1. The diseases were highly endemic.
2. All cases went to the Kern County Health Department and Kern General Hospital.
3. The physicians and veterinarians in the area were in-

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terested and well informed.

4. The Dr. Morris' (now the Kern) Mosquito Abatement District covered an urban area of 82 square miles and was willing to expand its control program.

The discoveries regarding mosquito vectors were conclusive and indicated that in the summer *C. tarsalis*, only 1 of some 22 mosquito species in the area:

1. Was the primary vector of WEE and SLE viruses.
2. Preferred to feed on birds but also fed on man and horse.
3. Became infected by feeding on birds.
4. Largely originated from agricultural, industrial and domestic use of water.
5. Could be controlled by an integrated program of water management DDT application and *Gambusia* planting.

This mosquito preferred to feed on birds and when it fed on viremic birds it readily became infected and subsequently could transmit the infection.

The findings regarding the clinical disease in humans and horses were equally clear:

1. Attack rates per 100,000 population rose as high as 16 for WEE and 11 for SLE.
2. Clinical disease was most likely to occur in infants and children.
3. Over 20% of persons over 20 years of age had experienced inapparent infections.
4. Almost 100% of horses were immune because of vaccination or natural infections through mosquito bites.
5. Risk of disease was lowest in years of low *C. tarsalis* populations.

The preceding findings led to several specific efforts to evaluate *C. tarsalis* control in order to achieve encephalitis control. It was hypothesized that residual spraying of domestic shelters, where *C. tarsalis* rested, would reduce the vector population and interrupt viral transmission from chicken to chicken. The first "civilian lot" of DDT was made available in 1945 and it was disappointing to learn this cheap and easy approach to control was ineffective. However, it did lead us to find that wild birds were more effective hosts than chickens and that vector populations would have to be drastically reduced to have any reduction in viral activity. We also did the first flight range studies with fluorescent markers and found that *C. tarsalis* readily moved a mile or more. We discovered that we did not know how these viruses survived the winter period when transmission ceased.

The above research had required the development of collaboration between a wide variety of agencies including but not limited to:

1. University of California – George Williams Hooper Foundation – School of Public Health
2. Kern Mosquito Abatement District (Originally Dr. Morris MAD)
3. Kern County Health Department
4. Kern General Hospital
5. Practicing M.D.'s and D.V.M.'s
6. California State Department of Health
7. California State Department of Fish and Game

During this decade, the Kern MAD expanded from 82 to 885 square miles. This action, for practical purposes, wiped out some of our best study areas.

It was in this decade that a surveillance system was established to monitor: encephalitis cases, vector populations, viral infections in the vector, viral antibody development in sentinel chickens and the key environmental factors of temperature and flooding. This system provided the core of data for later evaluation of the success of control efforts and continues today.

The applications of findings from this decade of discovery can be summarized into 4 major categories:

1. A new objective and approach had been added to vector control programs -- encephalitis control.
2. A model had been developed for an integrated control program applicable to the extensive endemic area -- the Central Valleys of California.
3. New knowledge had evolved of the epidemiology of encephalitis.
4. Unresolved problems for research in the third decade were identified.

This takes us now into the third decade which I will call the "Consolidation Decade". It was important to confirm earlier findings and to extend them.

1950 – 1959 Consolidation Decade

The original questions were simple:

1. Can encephalitis be controlled?
2. Are there reliable and economical predictors for viral activity?
3. Which birds are important viral hosts?
4. What proportion of infected mosquitoes can transmit virus?
5. Are arthropods other than *C. tarsalis* important viral vectors?
6. How do WEE and SLE viruses over-winter?

It was about this time that the farmer made his classical remark, "Doc if you don't know all the answers now you never will", and while I respected, I questioned his view. However, events proved that he was right.

The principal findings are quite simple to summarize:

1. Floods in 1952 and 1958 led to epidemics.
2. Insecticide resistance, a genetic trait, made *C. tarsalis* control difficult.
3. Control of *C. tarsalis* reduced the risk of encephalitis.
4. Reliable predictors for encephalitis were *C. tarsalis* populations, water availability and temperature.
5. Common wild birds, finches and sparrows, were most important sources of infection.
6. In midsummer only, 1 in 4 infected *C. tarsalis* could transmit infection.
7. Mites, ticks and other arthropods were not important vectors.
8. Viruses might overwinger in chronically infected wild birds.

One must always realize that a summary of this type oversimplifies actuality. The occurrence of the largest epidemic in the history of the state, at a time when we thought vector control was becoming increasingly effective, was a shock. We learned that we could not cope with uncontrolled flooding of the major river systems and the miracle insecticide DDT had come to its end for *C. tarsalis* control due to genetic resistance. In years when flooding was limited or controlled the problem resolved to the control of irrigation and waste waters combined with use of selected insecticides, and *Gambusia*. This combined approach gave reasonable protection from encephalitis as a clinical disease.

We now can move into what I have called the "Decade of Encephalitis Control", 1960-1969.

1960 – 1969 Encephalitis Control Decade

The research questions regarding the vector *C. tarsalis* built upon the findings of the preceding 2 decades:

1. Can vector control be intensified to a point where human cases or viral activity cease?
2. Will resistance to organophosphorous insecticides increase sufficiently to affect vector control?
3. Is autogeny, development of eggs without a blood-meal, important in population maintenance or in dampening vector efficiency?
4. Will measurement of parity rates in the vector explain either vector or viral survival?
5. Is there a pattern to vector flight dispersal into intensively controlled areas?
6. Can an experimental model be further developed for overwinter viral survival in the vector?

A second general set of questions was of more general basic research or epidemiological interest:

1. Do tissue cultures provide a more economical and rapid technique than animals for arboviral isolations?
2. What array of arboviruses occur in the area and do they cause disease?
3. Can factors that affect epidemics be described as a critical step before epidemiologic models can be developed?
4. Will encephalitis decrease as a clinical disease as vector control increases?
5. Can we evaluate better the ability of various arthropod and vertebrate species to be vectors and hosts of arboviruses?

The findings regarding the vector have led to some new concepts and I believe the results have been gratifying to those responsible for vector control programs. First regarding findings on the diseases:

1. When New Jersey light trap indices for female *C. tarsalis* were below 10 per night, risk of WEE or SLE in man was minimal.
2. When light trap indices were below 1 per trap night, basic infection chains were interrupted.
3. In this decade WEE and SLE disappeared from most of the Central Valley, an endemic area for more than 30 years.

With reference to the basic viral cycles and vector biology:

1. *C. tarsalis* was a most efficient vector, and small birds, such as finches and doves, were the best hosts for WEE and SLE viruses.
2. *C. tarsalis* had an effective flight range of at least 5 miles.
3. Autogeny delayed blood feeding and could decrease vector efficiency.
4. Autogeny could be the basis for population maintenance without blood feeding except for the first spring generation.
5. Parity rates reflected egg laying from both blood feeding and autogeny.
6. High parity rates were associated with viral infection as this reflected blood feeding in part.
7. The most successful overwintering females were nulliparous and were not autogenous.
8. Experimentally infected *C. tarsalis* carried WEE and SLE viruses through the winter.
9. *C. tarsalis* had developed resistance to the organophosphorous insecticides over extensive areas and control with these insecticides was increasingly difficult.

The principal epidemiological findings between 1960 and 1969 were:

1. Over 50 biological and environmental factors were described that were believed to influence the probability of encephalitis epidemics.
2. WEE and SLE practically disappeared from California as public health or veterinary problems.
3. Waning of infection correlated with intensified vector control and persisted in the face of increased OP resistance and resurging vector populations.
4. Newly discovered viruses, Turlock, Main Drain and Modoc, may be associated with encephalitis in man or horse.

In 1969, there was extensive flooding in most of the Central Valley. While *C. tarsalis* populations were held down in urban areas, economical limitations and insecticide resistance precluded effective control in many rural areas. To our amazement there was no evidence that WEE or SLE viruses resurged or even occurred in the state that year. It was a relief when the summer was over and difficult to respond to the oft repeated question "What was all that nonsense about an epidemic?" I again recalled the statement from my farmer friend "If you don't know now Doc . . . ."

A few miscellaneous findings from this decade that were of interest were:

1. Tissue cultures were efficient for isolation of most arboviruses.
2. 15 arboviruses probably occurred in the region.
3. Each arbovirus had an affinity for 1 or more vertebrates or arthropods common in the region.

With the end of this decade we moved into the 1970's and I have chosen to call this "The Crisis Decade". I hope I am wrong. However, we are concerned because of the relative unavailability of new effective insecticides and the knowledge that persons living in the Central Valley must be losing the immunity that was wide spread from inapparent infections that occurred in the 1930-1959 period. Dr. Francy's presentation this morning leaves no question how serious encephalitis epidemics can be in spite of our current levels of knowledge.

1970 – 1979 The Crisis Decade

The questions which we are currently asking are:

1. Will encephalitis resurge and again be an epidemic disease in California?
2. Are there alternative approaches for control of vector populations resistant to licensed insecticides?

3. Can a knowledge of *C. tarsalis* genetics be developed sufficiently rapidly so genetic approaches can be a part of integrated control?
4. Can vector incompetence and insecticide susceptibility be incorporated into populations as genetic traits?
5. Assuming that chromosomal translocations are a feasible approach to control, can the methods be developed and isolated field areas be found for pilot studies?

I can begin to give you a progress report even though we are only half way through the "Crisis Decade". First, viral activity has stayed at a very low level and even has been absent from extensive areas of the state.

The intensive research related to the genetics of *C. tarsalis* is making excellent progress and we can say that:

1. Chromosomal translocations can be induced in *C. tarsalis* and are a promising approach to control.
2. Genetic markers have been isolated and probably represent the 3 chromosomal pairs.
3. Multiple field areas have been found that are isolated, readily monitored and suitable for pilot field releases of translocated populations.
4. Methods are rapidly developing for identification of genetic, physiological and morphological factors that control vector competence.

Later presentations by Asman, McDonald and Nelson and their associates will enlarge upon these summary statements.

Additionally, basic research and field investigations have been extended with the following accomplishments:

1. Statistical and epidemiological models have been developed for:
   A. *C. tarsalis* populations
   B. The basic infections cycle of WEE virus
   C. The patterns of WEE and SLE viral activity and *C. tarsalis* populations for a 20 year period in all of California
2. Techniques for mass rearing of *C. tarsalis* are being developed.
3. Studies in the Sacramento Valley confirmed that the findings in Kern County from 1930-1969 also apply to this area.
4. A cycle for WEE virus involving *Aedes melanimon* and jackrabbits is important in the Sacramento Valley.

As a closing note, I have looked back over a period of more than 40 years of research on encephalitis and its vectors in California particularly in Kern County. It has been my privilege to be involved in the research effort since 1940. It has been gratifying to see control agencies accept and apply the research findings. It has been encouraging to have encephalitis change from a serious and at times extensive public health and veterinary problem to one where no, or few cases can be identified in the state in recent years. Resurgence of the vector and diseases remains a possibility and you must remember the human and equine population of the state has increased several fold since our last epidemics. These host populations have lost much of their immunity. I have prepared a table that illustrates that epidemics of the intensity of that in the 1950's would result in 755 or more cases of WEE today and the rate from our highest past year for SLE would result in 166 cases. We hope to be able to prevent the suffering and headlines that would occur in such an event. My old friend the farmer is still around and still shakes his head in disbelief everytime he sees me still chasing mosquitoes and asking questions as I have proven he was right – I'll never know all the answers.

Table 1.–Attack rates of encephalitis in prior peak years in California and estimates of the number of cases those rates would produce in current populations.

Prior Epidemic Used As Base	Current Population As Base For Estimate
1952 WEE (man) 20.4/100,000 Population 375 Cases	755 Cases
1965 WEE (Horse) 80.0/100,000 Population 95 Cases	106 Cases
1954 SLE (man) 4.5/100,000 Population 99 Cases	166 Cases

PRELIMINARY OBSERVATIONS ON *CULEX TARSALIS* AND *CULEX ERYTHROTHORAX* BIONOMICS IN A FOCUS OF ARBOVIRUS TRANSMISSION[1]

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Since 1970, the Finney-Ramer Wildlife Refuge of Imperial Valley has been a focus of intensive study of many facets of mosquito vector transmission of arboviruses (Clarke et al. 1974, Work et al. 1974). Monthly vector studies to ascertain trap night yield, trap site yield, species proportions, seasonal variation and total number within the focus have shown that *Culex tarsalis* and *Culex erythrothorax* were the dominant and active species found during the summer months when virus transmission occurred. Of the approximately 96,000 mosquitoes collected during 1975, *C. tarsalis* and *C. erythrothorax* comprised about 78% and 21% respectively of the CO_2 baited CDC trap yield.

This paper reports the analysis of the consecutive monthly collection data from February, 1973, to December, 1975, with special emphasis on *C. tarsalis* and *C. erythrothorax* and their daily biting activity and attraction to CO_2 baited CDC light traps.

Using patterns established in previous years, monthly collections of mosquitoes were continued during 1975 in the Finney grid and transect (Clark et al. 1974). The data is plotted according to week of the year in which collecting was accomplished. Analysis of the average trap night yield in the grid as shown in Figure 1 reflects a marked increase in *C. tarsalis* in May, a continuation upward to a peak in July and a gradual decrease through October as the season progressed. On the other hand, *C. erythrothorax* in Figure 2, shows a sudden increase in May, peaking in June and decreasing by July to a minimal number by the middle of August. The trap night curves of both species in 1975 exhibited a close similarity to those of 1973 and 1974.

The transect pattern of Figure 3 gave an initial rise, followed by precipitous drop in *C. tarsalis* population in late July, compared to the more persistent level in the grid which peaked in July. *C. erythrothorax* presents in Figure 4, a smaller but precipitous rise and fall in the transect than in the grid, thereby indicating that the breeding sites were apparently closer to the grid than transect. The slow decline of *C. tarsalis* in the grid suggests that the grid population was maintained in substantial numbers till late October. This implies that the grid has characteristics favoring either long survival or a continuous breeding and transmission cycle.

During the months of March to July in 1975, two transects were plotted and cut through the tules and cattails of Finney Lake. As shown in Figure 5, one is parallel to the reference sites FG_3 and FG_6 in the grid and the other lies in a south-easterly direction reaching open water where the activity of various species of indigenous water birds were observed. Six CDC light traps were placed suspended over open water to sample mosquito activity. It was interesting

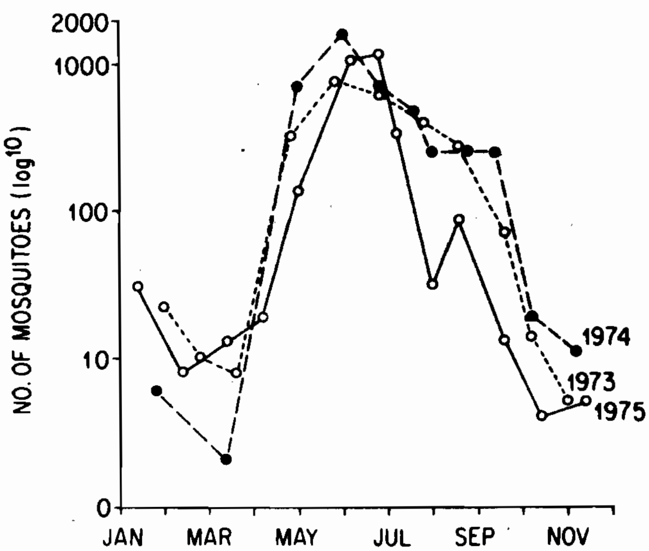

Figure 1.–Three year comparison (1973, 1974, 1975) of light trap collection of *Culex tarsalis* at Finney Lake Grid.

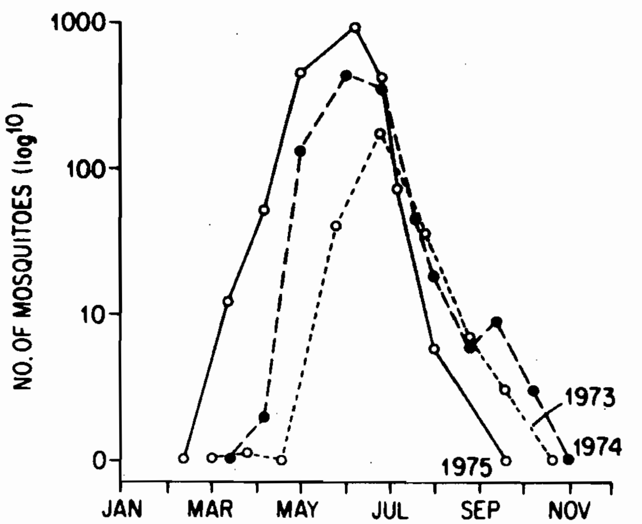

Figure 2.–Three year comparison (1973, 1974, 1975) of light trap collection of *Culex erythrothorax* at Finney Lake Grid.

[1]From the University of California Mosquito Research Program of the School of Public Health, Los Angeles, California, 90024. Partially supported by U. S. Public Health Service Training Grant AI-132-11-14.

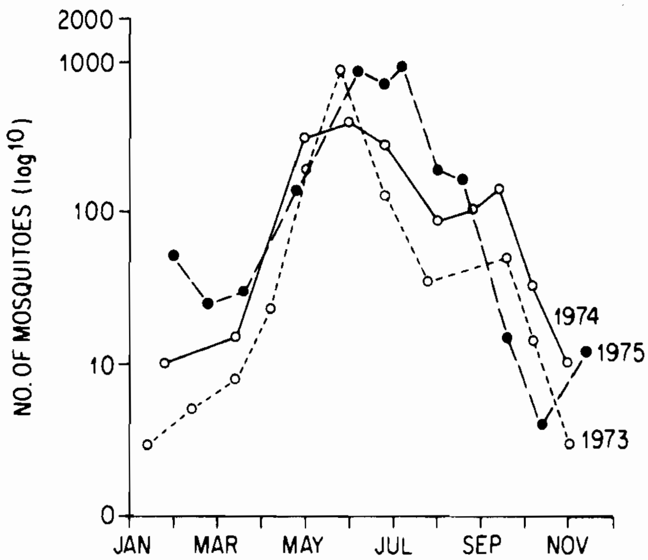

Figure 3.–Three year comparison (1973, 1974, 1975) of light trap collection of *Culex tarsalis* at Sheldon Pond Transect.

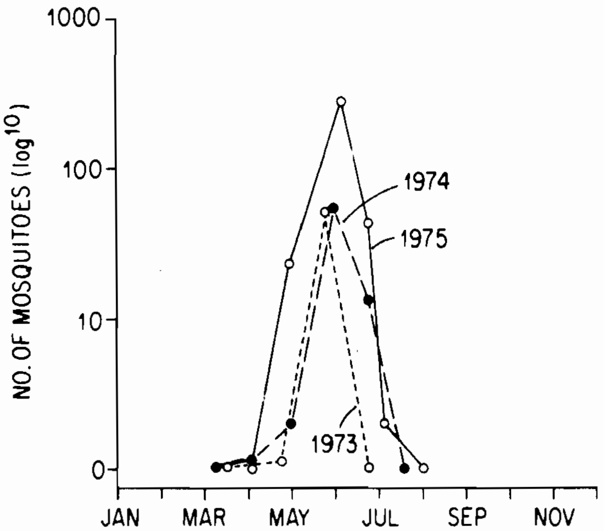

Figure 4.–Three year comparison (1973, 1974, 1975) of light trap collection of *Culex erythrothorax* at Sheldon Pond Transect.

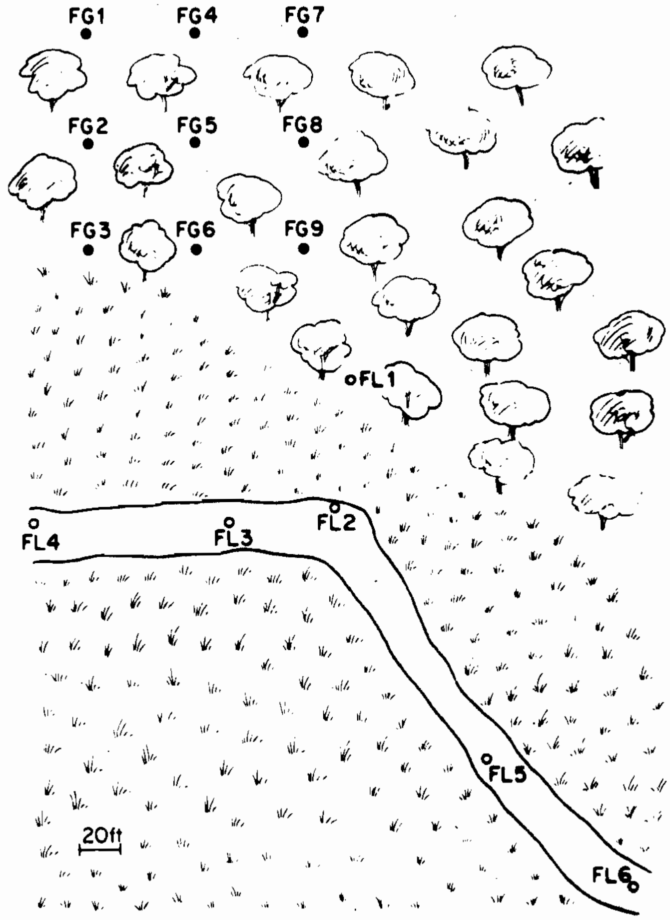

Figure 5.–Finney Lake and grid trap sites in aqueous tule and tamarisk habitat.

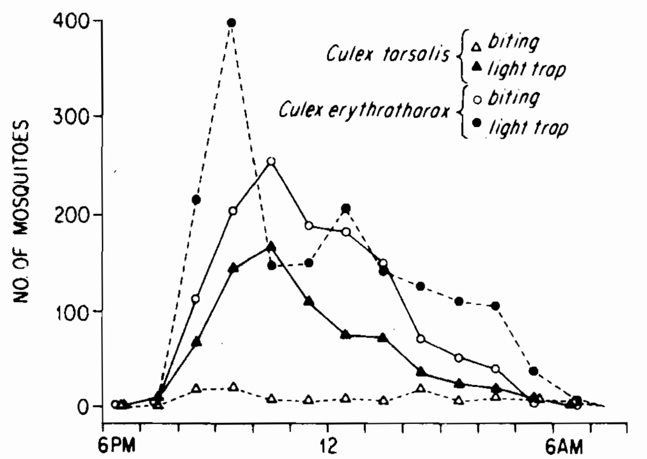

Figure 6.– Comparison of *Culex tarsalis* and *Culex erythrothorax* attraction at Finney Grid in June, 1975.

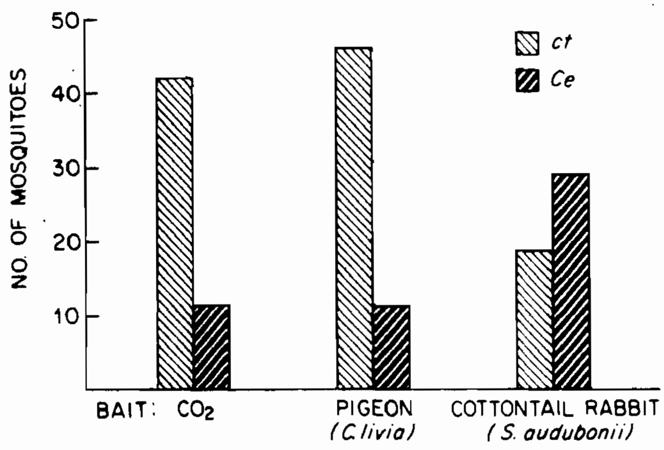

Figure 7.—Host preference comparisons of *Culex tarsalis* and *Culex erythrothorax* at Finney Lake in August, 1975.

to note that while *C. erythrothorax* decreased significantly in numbers in the grid, the population was still maintained at higher levels in the traps set over the open water channels, even exceeding the *C. tarsalis* catch in early August.

On the basis of results from studies of the seasonal activity of mosquitoes, it was decided to undertake biting and related behavioral studies of the two principal species, *C. tarsalis* and *C. erythrothorax*. Though it is known that both species are active during the early parts of the night, accurate data on their biting behavior and peaks of activity are lacking. Therefore, an attempt was made to ascertain the activity cycle of both *C. tarsalis* and *C. erythrothorax*. This information is also important because the Finney-Ramer area attracts thousands of Southern Californians for camping and fishing.

Finney grid afforded an ideal site for studying the activity of these species because they are found in large numbers from May through July. A site close to FG3 was chosen to study biting activity. Commencing at dusk the bag of FG3 light trap was replaced each hour. The mosquitoes collected were immobilized on dry ice and transferred to separate plastic containers. Simultaneously, biting collections were made adjacent to FG3 by exposure of skin of the legs below the knee. Mosquitoes alighting on the legs were collected by an aspirator throughout the night; each hour's catch expelled into separate plastic containers.

Analysis in Figure 6 of the hourly light trap collections shows that both *C. tarsalis* and *C. erythrothorax* have a distinct peak of activity in the early part of the night, gradually diminishing in numbers as the night progressed. On the other hand, while biting collections of *C. erythrothorax* showed two distinct peaks of activity (high between 9:00 and 10:00 p.m. and somewhat less between 12:00 and 1:00 a.m.) the biting behavior of *C. tarsalis* was erratic. It did not exhibit a distinct preference for human beings. Equally interesting to note were the total light trap collections; 64% were *C. erythrothorax* and 36% *C. tarsalis*. On the other hand, of all the biting mosquitoes collected, *C. tarsalis* comprised only 7%, while most were *C. erythrothorax* (93%). During the early part of the night, biting collections of *C. erythrothorax* far exceeded the light trap catch. Similar biting and light trap collections were made in the transect as well. These studies showed that *C. erythrothorax* definitely preferred humans and probably other mammals. *C. tarsalis* has repeatedly been shown to be an avian feeder as by the studies of Reeves and Hammon (1962).

In order to ascertain the biting preference, an attempt was made to study the activity of the two principal species by the lard can traps. Traps using CO_2, common pigeon (*Columba livia*) and cotton tail rabbit (*Sylvilagus auduboni*) as baits showed that while *C. tarsalis* preferred the bird, *C. erythrothorax* exhibited an attraction to the mammal. These preliminary results graphed in Figure 7 clearly showed the differential activity of these species. Further experiments in this direction are planned to extend this data.

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ENVIRONMENTAL INFLUENCES ON CDC LIGHT TRAP COLLECTIONS OF *CULEX TARSALIS* IN A FOCUS OF WEE AND SLE TRANSMISSION[1]

Gary G. Clark[2], Telford H. Work[3] and O. G. W. Berlin[3]

In 1973, monthly mosquito collections were concentrated in a focus of western equine and St. Louis encephalitis virus transmission described by Work et al. 1974.

The study area, as shown in Figure 1, is within the Finney-Ramer Wildlife area located southeast of the Salton Sea approximately thirty miles north of Mexico. It is an aqueous habitat of an arid zone receiving water from the northward flowing Alamo River and main drainage canals from the encompassing irrigated agricultural area.

Normal annual rainfall in the Valley is only 5.8 cm (2.3 in). However, water diverted from the Colorado River via the largest irrigation system in the Western Hemisphere, supplies million dollar production of vegetables, sugar beets, cotton and feed grains. Irrigation water, provides the major source of water for Finney Lake and adjacent ponds. Elevation in this locality is approximately 52 m (170 ft.) below sea level.

The permanent trap patterns designated in Figure 2 were established and operated during the 1973 phase of the study described by Clark et al. 1974. A 9-trap grid at 50-foot intervals was situated on a northern shore of upper Finney Lake. This area is characterized by a dense stand of tamarisk or salt cedar trees. In order to reach these trap sites, low trails have been carved through the dense underbrush. The 15-trap linear transect with 100-foot intervals was located directly south of the grid approximately 0.8 mi. The transect was on an east-west levee separating a drain-fed pond and the Alamo River, one of the two major water arteries to the Salton Sea. CDC miniature light traps, operated by 6-volt batteries with a carbon dioxide source (dry ice) as an attractant, were utilized throughout these studies.

Analysis of the 1973 data presented in Figure 3, revealed that *Culex tarsalis* collections rose dramatically in May and June on the transect (the broken line). However, values on the grid peaked in July. During this 3-month period, ambient temperatures (in the brackets) were also rising. These data were derived from a single 3 or 4-day field trip each month. Specific information from the 1973 phase of study was presented at the 42nd Annual Conference of the California Mosquito Control Association, Anaheim, by Clark et al. 1974.

Speculation at that time was that the more productive grid offered conditions more conducive to high mosquito numbers and survival, because of cooler temperatures and higher relative humidity. Hence, there might be fewer on the transect after mid-June because it was perceived as a hotter and drier area. Proximity to optimal breeding habitat and daytime resting sites were also factors which may have influenced mosquito collections. Furthermore, since only 3 or 4-day samples were taken, a major question arose regarding the real or artificial nature of the two curves.

Therefore, it was decided that daily collections over an extended period of time at three key sites in each pattern would be undertaken. The three most productive sites in each pattern FT1, FT5, FT6 and FG3, FG6 and FG8, were chosen for this intensive study. In order to continuously monitor the two most easily measured climatic factors, temperature and relative humidity, a Model 594 Bendix Hygrothermograph as described by Oosting (1956) was positioned adjacent to the most central of the three sites in each pattern. Hygrothermographs were housed in wooden, U. S. Weather Bureau "Cotton-region type" weather shelters as illustrated in Figure 4. This shelter provided protection from direct exposure to sun and wind. On the transect, it was stationed 100 and 400 ft from the farthest trap sites. The shelter on the grid was only 50 and 75 ft from the farthest sites. A light trap was hung at a height within 12 inches of the elevation of the hygrothermograph.

Each day, the traps were started so that all were operating at sunset. This was done to achieve maximum attraction of *Culex tarsalis*, a mosquito which is most active at dusk and at night. The carbon dioxide source was supplied in sufficient quantity so that any activity occurring around sunrise would be included. Shortly after dawn, bags were collected and placed on dry ice. Mosquito nets were emptied in the field laboratory facility, with contents transferred to plastic vials and returned to dry ice for subsequent transport to U.C.L.A. There, they were stored in a Revco freezer at -70°C until identified and pooled for inoculation for virus isolation.

It has been noted that the daily activity pattern of insects is governed by the physiological rhythm of the species. The most important influences on this rhythm are light, temperature and humidity. As quoted from Odum (1971), "Temperature and moisture are so generally important in terrestrial environments and so closely interacting that they are conceded to be the most important part of climate".

Sufficient results have accumulated to allow a preliminary analysis. The numbers of *Culex tarsalis* collected will be related to the daily maximum temperature and minimum relative humidity values in each of the two habitats. Collections are considered with temperature and relative humidity recorded continuously throughout the 80-day period from May 1 through July 20, 1974.

It is already known that the target organism in our study is most active on a circadian basis during the period around sunset. For that reason, preliminary analyses have been compared with sunset temperature and relative humidity.

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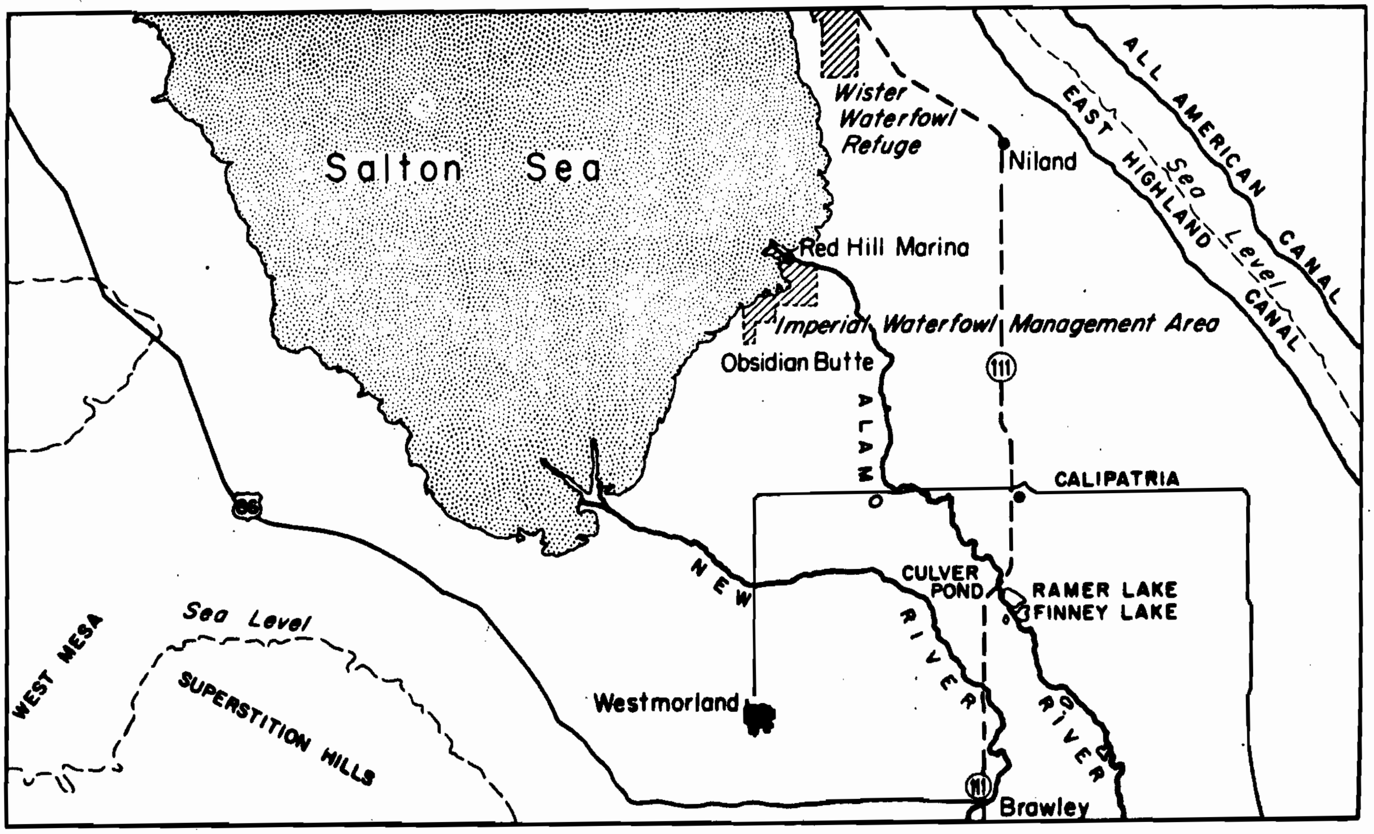

Figure 1.—Location of mosquito research areas in Imperial Valley.

By doing so, an attempt has been made to determine the effect at sunset and also to develop an index as to the influence on the remainder of the night's collection. Further, the relationship of vapor pressure deficit to collection numbers will be examined.

Figure 5 represents the average number of *Culex tarsalis* females collected in the three sites in the grid and on the transect. Each point is the value from every fifth day. A massive amount of material has been accumulated and time has not yet permitted completion of identification and processing of the samples from each of the eighty nightly collections. During certain periods, individual traps had in excess of 2,000 *Culex tarsalis*, as well as substantial numbers of *Culex erythrothorax*.

In general, the two areas had similar trends. The exceptions were the periods before and after June 20 and the interval from July 5 to July 10. The first three peaks on the grid exceeded concurrent peaks on the transect by 150, 500 and 1,000 mosquitoes, respectively. These occurred on May 11, May 31 and June 20.

In the grid, peak temperatures, above 105°F, were recorded on May 31, June 20, June 30 and July 10, as shown in Figure 6. This occurred 1 to 4 days prior to the four collections of over 1,000 mosquitoes per trap. Just prior to the last three mosquito peaks, sunset temperatures were greater than 90°F. The first "over 1,000" value was preceded by a sunset reading of 88°F. It may further be noted that with each precipitous drop in temperature there soon followed a drop in mosquito numbers. The low numbers on July 5 and July 15 follow downward temperature changes. Even though the temperatures in July ranged above those in May, the numbers are lower. Magnitude and seasonal occurrence of change may be the critical factor.

Figure 7 indicates that the transect mosquito collections consistently vacillated between 200 and 600 per trap night. The first collection above 500 was preceded by a peak of 106°F. The highest daily maximum temperature (114°F) on June 28 and 29 occurred just prior to the largest collection of *Culex tarsalis*. Once again, mosquito numbers appear to follow temperature fluctuations. However, they are not as dramatic as in the grid.

The drop in mosquito numbers in mid-July, lower than the May collections followed a sharp fall in temperature on July 14. As in the grid, the magnitude of change may have a greater influence than the actual temperature.

When considering the *Culex tarsalis* collections in relation to relative humidity on the grid as presented in Figure 8, no general relationships were observed. The minimum values (the bottom line) were fairly consistent, ranging between 10 and 36 percent, while at sunset, there was considerable fluctuation. However, on July 5 and July 15, the relative humidity at sunset was 66 and 82 percent, respectively. The values on July 15 were the upper extremes for the 80-day period.

A greater fluctuation in relative humidity at sunset on the transect, as detailed in Figure 9, may reflect its less protected location. The mosquito collections generally follow the minimum relative humidity curves through July 5. That

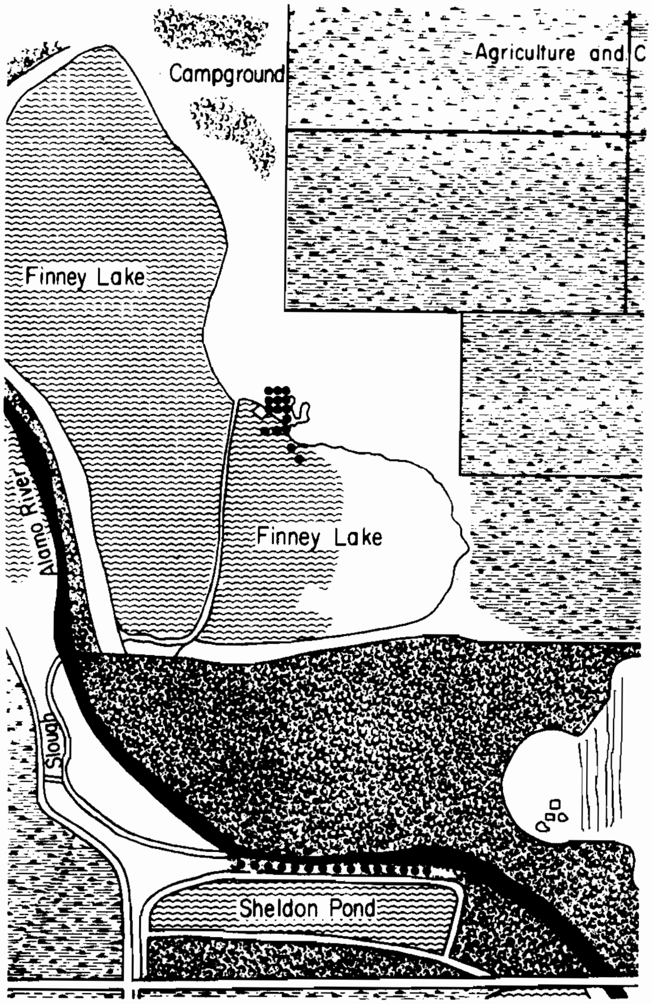

Figure 2.–CDC Light Trap locations and patterns in Finney Focus.

is to say, a precipitous decrease in number followed a similar drop in relative humidity. The collection of July 15 corresponds to the sunset relative humidity of 90 percent with the minimum for that day of 60 percent. Both readings were upper extremes for the period of study.

In an arid zone, such as the Imperial Valley of California, water, in its various forms, plays a critical role in the life of mosquitoes. This affects not only the immature forms but also the adults as they attempt to avert excessive desiccation. In general, relative humidity has been the measurement most often used in ecological work although the converse of relative humidity, or vapor pressure deficit, is often preferred (Odum 1971). This is because evaporation tends to be proportional to vapor pressure deficit rather than relative humidity.

Vapor pressure is a measure of the quantity of water vapor present usually expressed in mm Hg (Oosting 1956). Therefore, vapor pressure deficit is the difference between the amount of water present and the amount that could be held without condensation (i.e. rain) at that temperature. It is a direct indication of atmospheric moisture, independent of temperature, and thus more indicative of the potential rate of evaporation. The greater the vapor pressure deficit, the drier the air. This measurement can be calculated when both the temperature and relative humidity are known.

From preliminary analysis of the transect data in Figure 10, we can see that the lowest sunset vapor pressure deficit was in mid-May. At this time there was also a sharp decline in mosquito numbers. In Figure 11, vapor pressure deficit changes occurrred in the grid, but the drop in mosquito numbers was not as great. If it is possible, it appears that temperature from May 16 to May 21 was more influential on mosquito numbers than was humidity.

Temperature, relative humidity and vapor pressure deficit have been related to *Culex tarsalis* collections in a preliminary report. It is as yet difficult to draw definitive conclusions from the data since nightly collection values are incomplete. However, a few observations may be derived from these preliminary results. There was more variability in the nightly mosquito collections from the grid in comparison to the transect. Therefore, monthly collections of a few nights would not be truly indicative of the existing vector population.

The large numbers in the grid may be due to its proximity to the *Culex tarsalis* breeding habitat. This is further supported by the occurrence of large numbers of males in the collections (not depicted here). The mosquitoes on the transect may be wide-ranging females foraging for a vertebrate blood source. In addition, those in the grid may do as Odum (1971) stated, ". . . . . regulate their activities so as to avoid dehydration by moving to protected places. . . .". This has been observed in desert poikilotherms where behavior assisted in regulating body temperature. Furthermore, the high temperature preceding peak mosquito collections might have accelerated larval and pupal development.

In general, the grid pattern appears more susceptible to temperature changes than does the transect. However, inclusion of the remainder of the data could change the picture.

Two other factors of which we are aware but have yet to definitively resolve are wind and carbon dioxide. The effects of wind on mosquitoes directly and on the dispersion of sublimated dry ice are not known. Because of the difference in amount and directional exposure of vegetation protecting each trap site, and the fleeting gusts and eddies characteristic of a hot climate, the effect of air movement on the trap catches must necessarily be ignored. However, low catches recorded for certain dates have been associated with strong winds. Relationships of these winds to temperature humidity or vapor pressure deficit have not been examined. The relative effectiveness of carbon dioxide as an attractant at various temperatures and relative humidities has not been explored. These are some of the considerations that must be evaluated as we study the environmental influences on CDC light trap collections.

ACKNOWLEDGMENT.–The authors acknowledge Deborah Frenkel, Donald Goodwin, Clifford Howell, Marjorie Miller, Robert Nugent and William Valadez for their assistance in the field and laboratory.

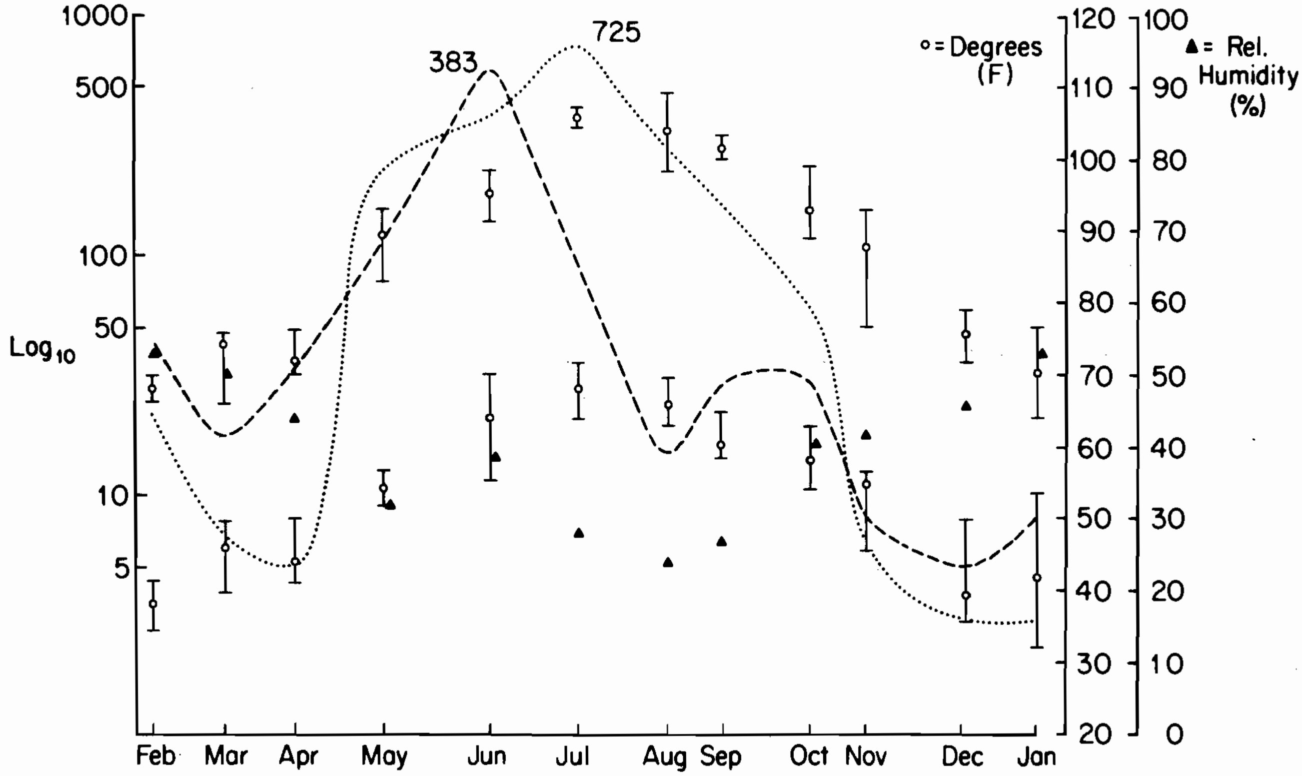

Figure 3.--Comparison of *Culex tarsalis* catch in Finney Transect and Grid, 1973.

Figure 4.--CDC light trap and hygrothermometer at FG6.

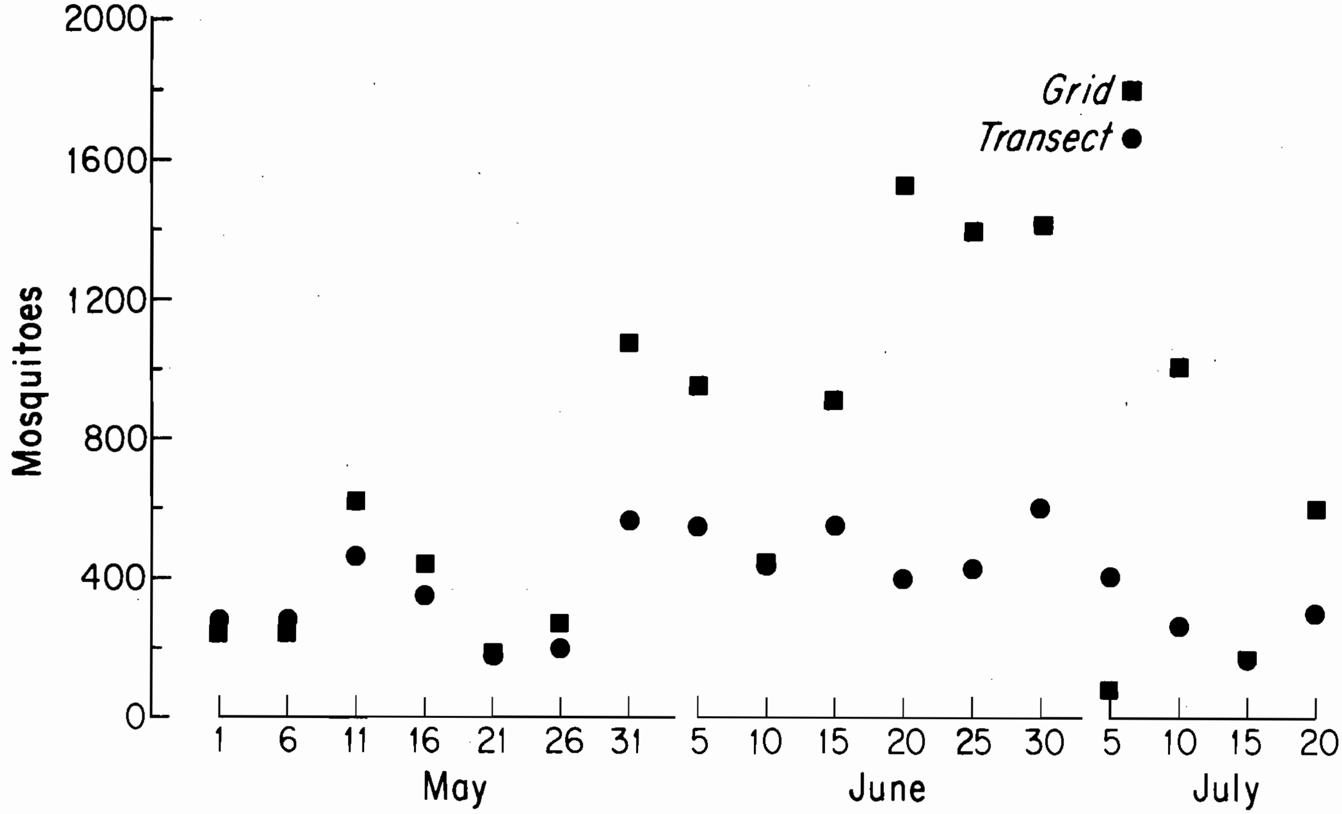

Figure 5.—Average number of *Culex tarsalis* collected per CDC Trap Night on Finney Grid and Transect.

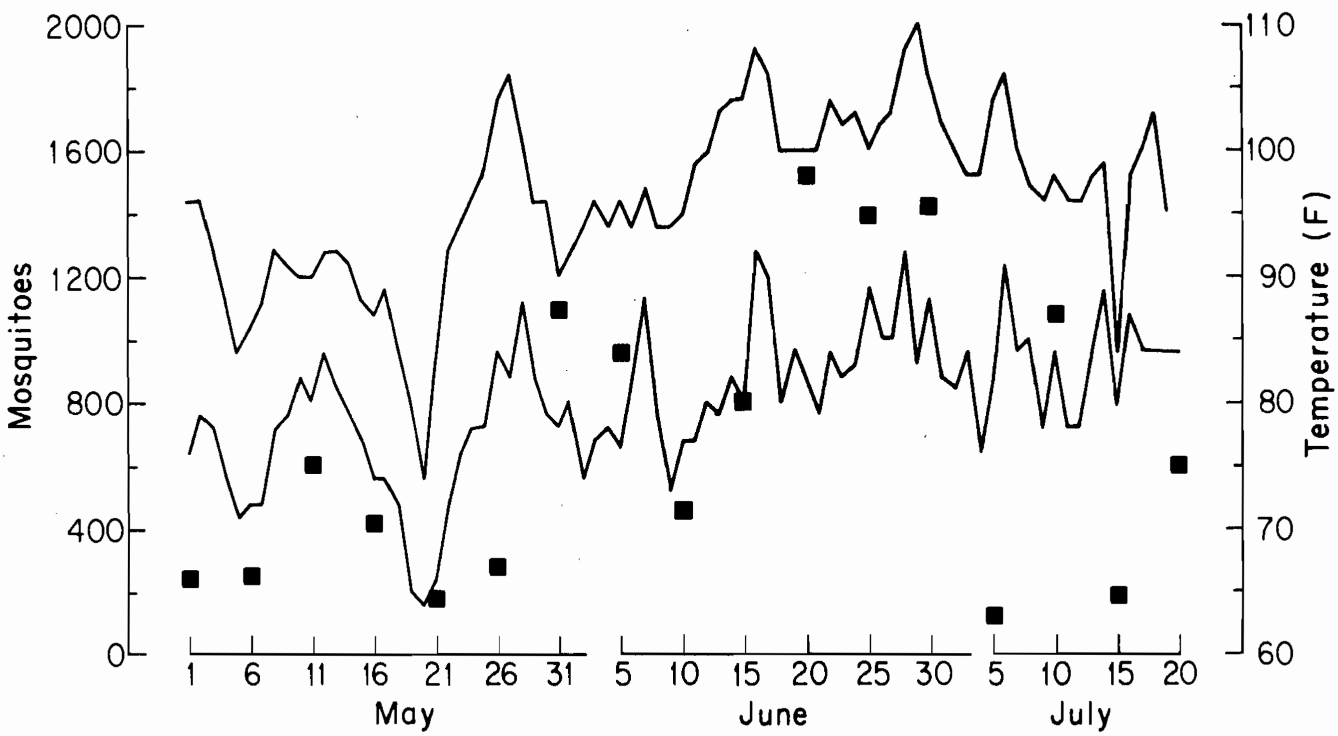

Figure 6.—Daily maximum and sunset temperatures in relation to *Culex tarsalis* collections on Finney Grid.

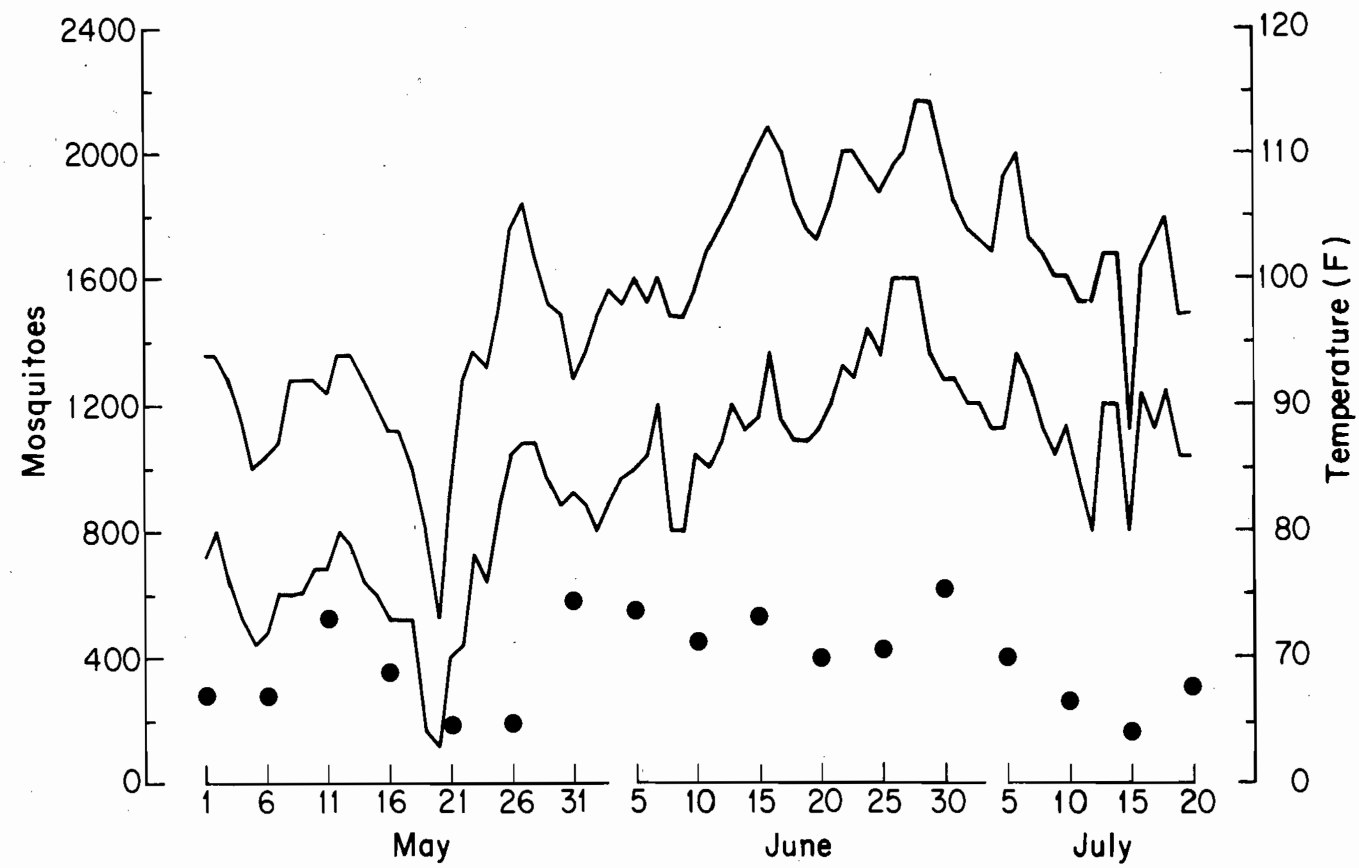

Figure 7.—Daily maximum and sunset temperatures in relation to *Culex tarsalis* collections on Finney Transect.

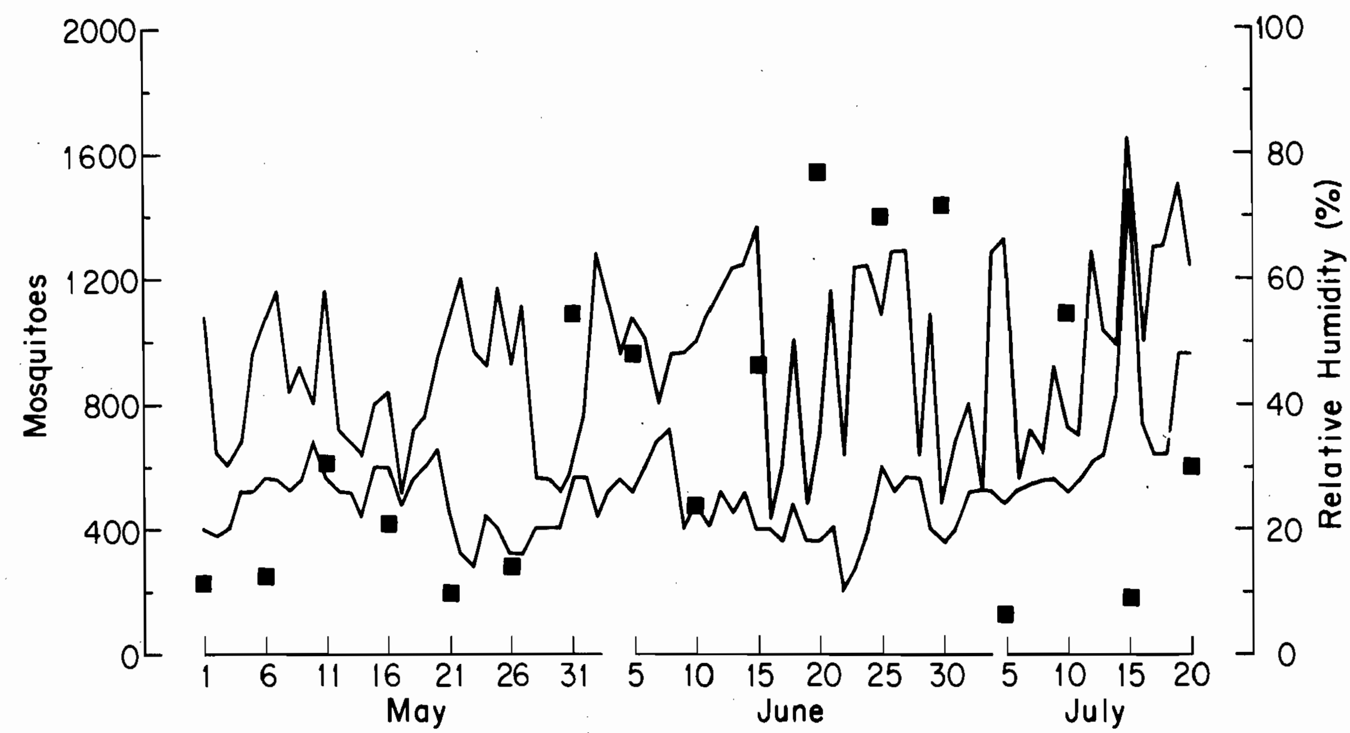

Figure 8.—Daily minimum and sunset relative humidity in relation to *Culex tarsalis* collections on Finney Grid.

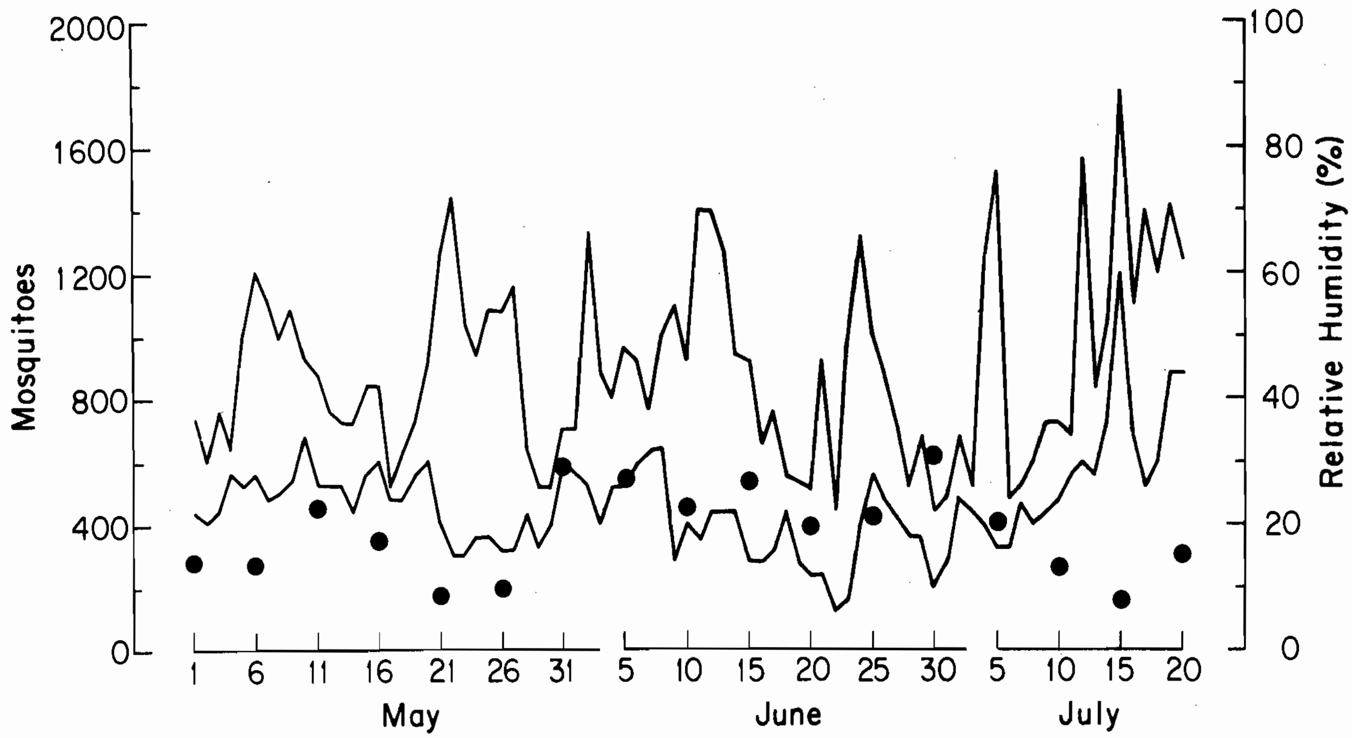

Figure 9.–Daily minimum and sunset relative humidity in relation to *Culex tarsalis* collections on Finney Transect.

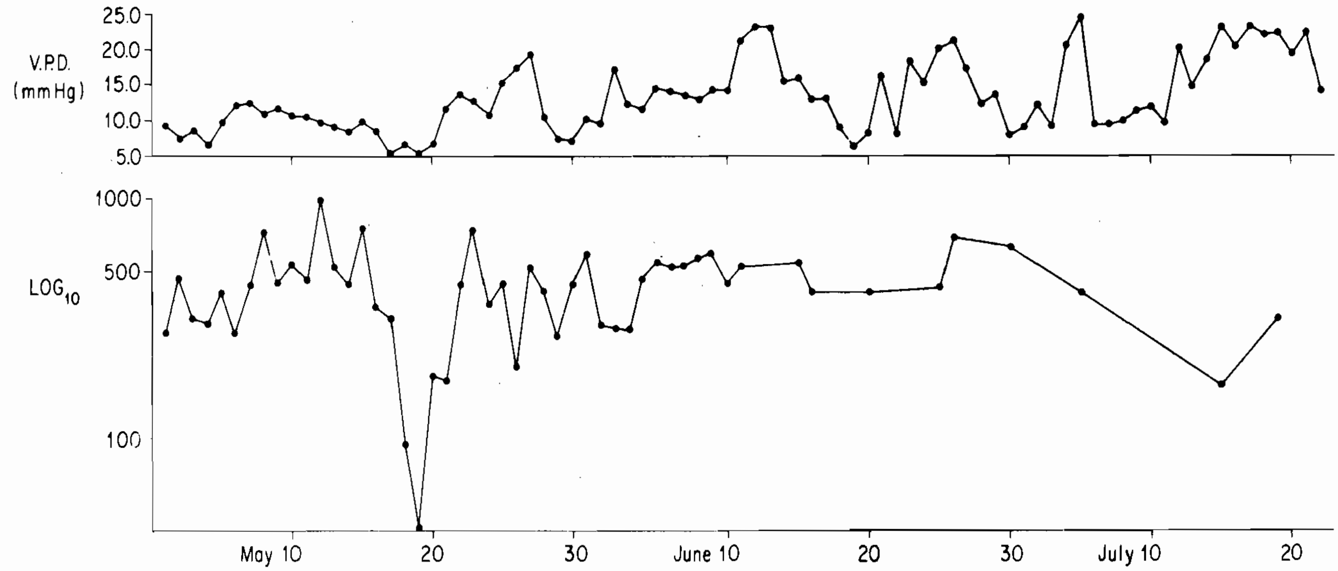

Figure 10.–Sunset vapor pressure deficit in relation to *Culex tarsalis* collections on Finney Transect.

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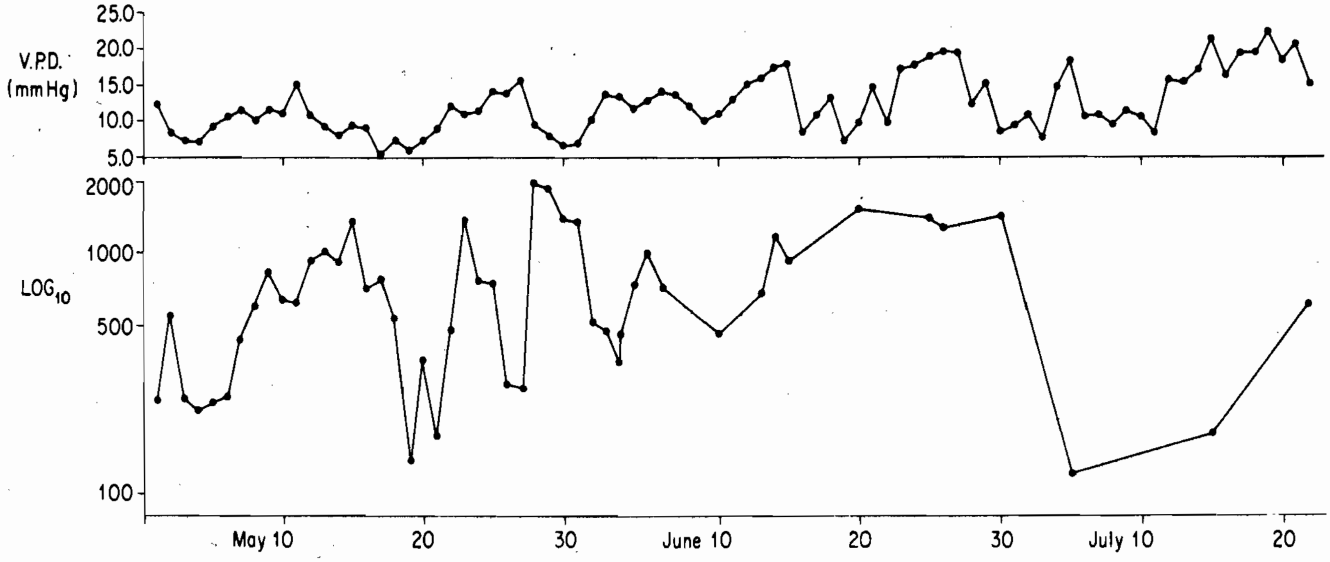

Figure 11.–Sunset vapor pressure deficit in relation to *Culex tarsalis* collections on Finney Grid.

A REASSESSMENT OF *CULEX PIPIENS* AS A POTENTIAL ST. LOUIS ENCEPHALITIS VECTOR IN IMPERIAL COUNTY

H. I. Magy[1], T. H. Work[2] and C. V. Thomas[3]

INTRODUCTION.–St. Louis encephalitis (SLE) derives its name from the devastating epidemic of 1933 where the virus was first isolated from human brain and where mosquito transmission was first considered (Leake 1935).

Rural, and geographically more extensive, epidemics in the San Joaquin Valley of California, Yakima Valley of Washington, Weld County, Colorado, and the high plains of Texas, incriminated *Culex tarsalis* as the primary mosquito vector of SLE in western United States (Hammon et al. 1941, Reeves and Hammon 1962).

Subsequent experiments by Reeves et al. (1942) and isolations from field collected mosquitoes (Reeves and Hammon 1962) established that *C. pipiens* could transmit SLE although doubt was cast upon its efficiency as a vector. Further experiments by Chamberlain et al. (1959) and series of SLE epidemics in the past twenty years involving *Culex pipiens* as the primary vector (Chamberlain et al. 1966) indicates that this species was probably responsible for transmission in the St. Louis epidemics of 1933 and 1937.

The appearance of epidemic SLE in the lower Rio Grande Valley of Texas in 1954, again incriminated *Culex pipiens quinquefasciatus* as the peridomestic vector (Beadle et al. 1957). In the past two decades *Culex tarsalis* has continued to be a vector of SLE transmission in the west, but in an increasingly sporadic distribution in time and geographical occurrence. In the same twenty year period during which *C. pipiens* complex mosquitoes have been the primary vectors, urban and suburban epidemics have increased in number, frequency and geographical extent.

The 1964 epidemic in Houston, Texas, (Luby et al. 1967) was the epicenter of an SLE virus dispersion that produced human disease in eight states, being recognized in eastern seaboard communities of New Jersey and Pennsylvania for the first time (Altman and Goldfield 1968). The 1966 Dallas epidemic demonstrated the economic consequences of an urban epidemic (Schwab 1968), and how the early detection in *C. pipiens* mosquitoes might enable intensive application of low volume malathion to abort epidemic transmission (Peavy 1967).

Evidence of annual urban transmission of SLE in Corpus Christi, Texas, (Williams et al 1975), periodic appearance in Memphis, Tennessee, the 1974 epidemic in Hermosillo, Sonora, Mexico, (Aguilera Arroyo and Miro Abella, unpublished), and most recently in 1975, the most extensive epidemic on record involving *C. pipiens* requires reassessment of *C. pipiens* activity in Southern California in 1972 (Bown and Work 1973). Here, this mosquito was found to be a significant vector of SLE along the Mexican Border of Imperial County and was associated with human disease in San Diego County in 1973 (Emmons et al. 1974).

Culex pipiens is referred to as *Culex quinquefasciatus* in an earlier work cited herein (Magy 1955). For purposes of this paper this complex is referred to as *Culex pipiens*.

DESCRIPTION OF THE AREA.–The Mexicali-Imperial Valley is an irrigated part of the Colorado desert lying south of the Salton Sea divided politically by the United States of America-Mexico border, running between Calexico on the north and Mexicali (the capital of Baja California) on the south. The Imperial Valley is about 50 miles long by 25 miles wide, and the Mexicali Valley in Mexico is about 40 miles by 50 miles. These valleys are drained by the Alamo River on the east and the New River to the west, which collect natural and agricultural drainage and community sewage waters as they flow north to the Salton Sea (Figure 1). Rainfall averages about two inches per year. The winters are mild and the summers are very hot.

Large acreages of cotton, alfalfa, vegetables and numerous cattle feedlots characterize the agriculture of the Imperial Valley which has eight urban centers with a total population of about 75,000. South of the international border, the City of Mexicali, with over 500,000 people, sprawls over an area of about 30 square miles extending into broad areas of irrigated cotton which receives water from the Alamo Canal. The Alamo Canal water derives its water from the Colorado River.

Mexicali has witnessed a phenomenal growth, tripling in population since 1960 (Table 1). This growth has outstripped the capacity of the city's sewage treatment faciltities. Partially treated sewage from about 370,000 people and untreated effluent from a population of about 130,000 is discharged into the New River, according to a report of the Regional Water Quality Control Board (1975). The New River has a flow of about 77.5 million gallons per day at the border crossing.

In contrast, the population of Imperial Valley only increased about 18% since 1952, but there are also some problems of inadequately treated sewage entering the New River from several communities.

ARBOVIRAL ACTIVITY IN BORDER AREAS.– The report of Bown and Work (1973) concerning a significant SLE infection of *Culex pipiens* collected at the Alamo River crossing at the Mexican border during the 1972 VEE surveillance suggests that SLE infection along the border is not infrequent or uncommon. The large numbers of *C. pipiens* collected may be attributed to the usual high organic content of irrigation waters accumulated in fields associated with cattle feed yards which were numerous in this area. This suggestion is further strengthened by the occurrence of SLE in Hermosillo, Sonora, Mexico in 1974.

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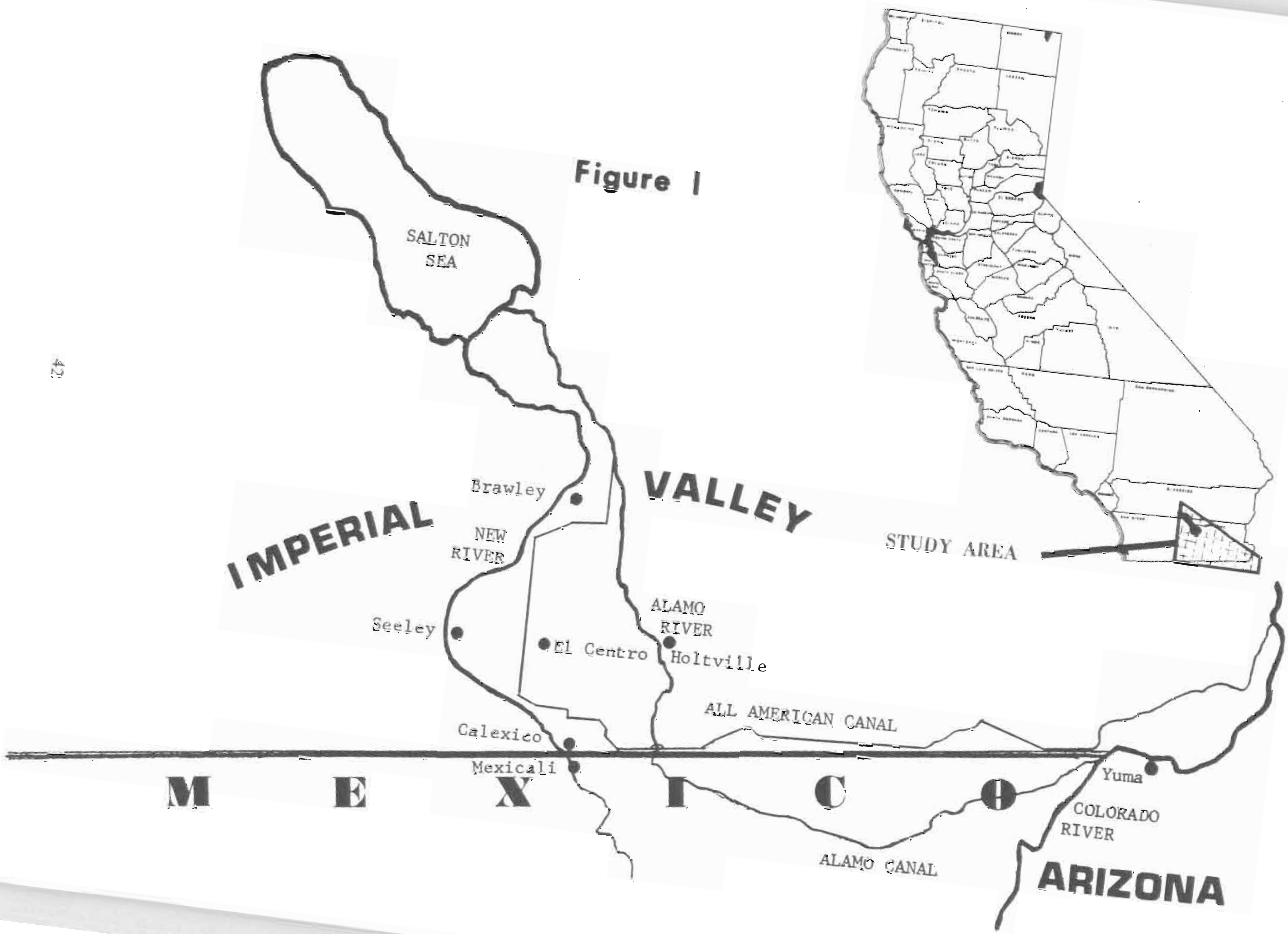
SALTON
SEA
IMPERIAL
VALLEY
Brawley
NEW
RIVER
Seeley
El Centro
ALAMO
RIVER
Holtville
ALL AMERICAN CANAL
Calexico
Mexicali
M E X I C O
ALAMO CANAL
Yuma
COLORADO
RIVER
ARIZONA
STUDY AREA

Figure 1

Table 1.–Mexicali-Imperial Valley population data.

Year	Mexicali	Calexico	Imperial County
1920	6,200		
1930	14,842		
1940	18,775	5,415	59,740
1950	64,701	6,433	62,975
1960	171,535	7,992	72,105
1967	225,000		
1972	350,000+ 1970	10,625	74,492
1974	500,000		
1980	750,000		
1990	1,650,000		
	Sewer Connected Population (estimated)		
1946	12,000		
1951	20,000		
1962	30,000		
1974	375,000		

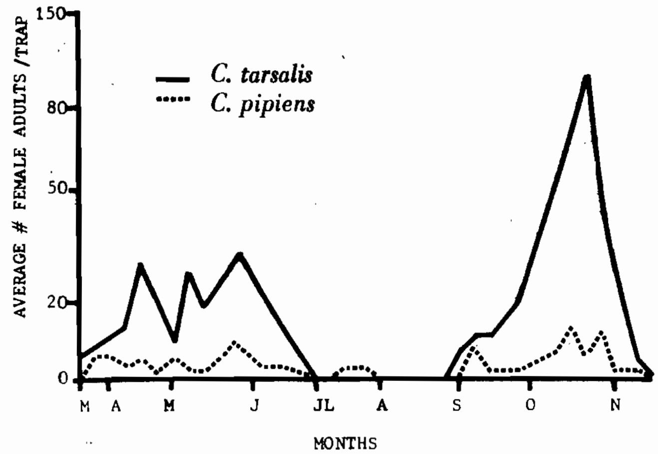

Figure 2.–New Jersey light trap collections, 1954 Imperial County (Brawley, El Centro, Holtville).

Reports of virus recovery from a generalized survey of Imperial Valley reported by Madon et al. (1974) revealed that 1.1% of *C. pipiens* pools and 4.7% of *C. tarsalis* pools were positive for SLE in 1972, providing further support for the existence of a natural mechanism for virus-crossover between these species.

To add credibility to the role of *C. pipiens* as a potentially significant source of SLE infection in Southern California, in 1973 (Emmons et al. 1974) the California State Department of Public Health in San Diego County isolated SLE from a single pool of 10 *C. pipiens* two weeks prior to onset of SLE in a 10-year old boy who had been fishing at the same site on the San Diego River Channel. This channel had been heavily polluted with inadequately treated sewage effluent from the suburban community of Santee. The prevalence of SLE virus in the Santee area was further demonstrated by the results of HAI antibody tests on domestic fowl within a mile of the positive mosquito pool.

ENTOMOLOGICAL CONSIDERATIONS.–In reevaluating records of *Culex pipiens* mosquitoes collected in Imperial County, the data of a California State Health Department report of an extensive mosquito survey of Imperial Valley prepared 20 years ago (Magy 1955) was compared with published and unpublished data collected from 1972 through 1974. This encompassed data collected by members of this department (Madon et al. 1974) (Emmons et al. 1974). It also included records of the Imperial County Health Department which began routine control operations in 1975. For the purpose of this paper only collections of *Culex pipiens* and *Culex tarsalis* are discussed.

In the 1955 study adult mosquitoes were collected weekly with New Jersey light traps which placed a trap at each of the peripheries of Brawley, El Centro and Holtville (Figure 2). For determining the magnitude of mosquito larval breeding as it might influence the adult counts in the light traps, a breeding index was used (Figure 3). This breeding index (Surface area x average/dip x positive dips + total dips x 100 = breeding index) was a modification of that developed by Belkin (1954). Larvae were collected within a one mile semicircular radius around each light trap during every fifth week for 13 months. Larval source information from these and other collections outside these areas were also made (Table 2). Data from CEC CO_2 baited light traps for 1972, 1973, 1974 is presented in Figure 4. The average number of female *C. tarsalis* per trap night for May through November is given, representing 6 through 17 trap settings each month.

Table 2 indicated that *Culex pipiens* and *C. tarsalis* were distributed throughout Imperial Valley, occurring together and separately in agricultural and urban type sources. It also suggests that *Culex pipiens* is found more frequently in domestic sources, which is characteristic for this species in most areas of the U.S.A. (Carpenter and LaCasse 1955). The more recent larval collection data, Table 3, submitted by the new mosquito program of the Imperial County Health Department, although limited to November and December, 1975, suggests that the types of sources of *C. pipiens* have not significantly changed in 20 years.

A comparison of the 1954 New Jersey light trap collection results, Figure 2, with the larval breeding index, Figure 3, indicates that the lower numbers of *C. pipiens* in light traps do not reflect *C. pipiens* larval breeding within the flight range of the light traps. On the other hand, the *C. tarsalis* larval index corresponds closely with the trap collections. This data also demonstrates a bimodal occurrence in the spring and fall of these species. The bimodal occurrence is less pronounced for *C. pipiens*, which had a higher breeding index throughout the year than *C. tarsalis*.

The 1972-74 CDC light trap data demonstrated the same difference in attraction and bimodalty, Figure 4. *C. pipiens* were trapped significantly less frequently than *C. tarsalis* although more specimens of both species were collected than with the New Jersey light traps. Although these traps were usually located in irrigated areas compared to the suburban locations of the 1954 New Jersey traps, the general reluctance of *C. pipiens* to enter either trap is clearly indicated in comparison with *C. tarsalis*.

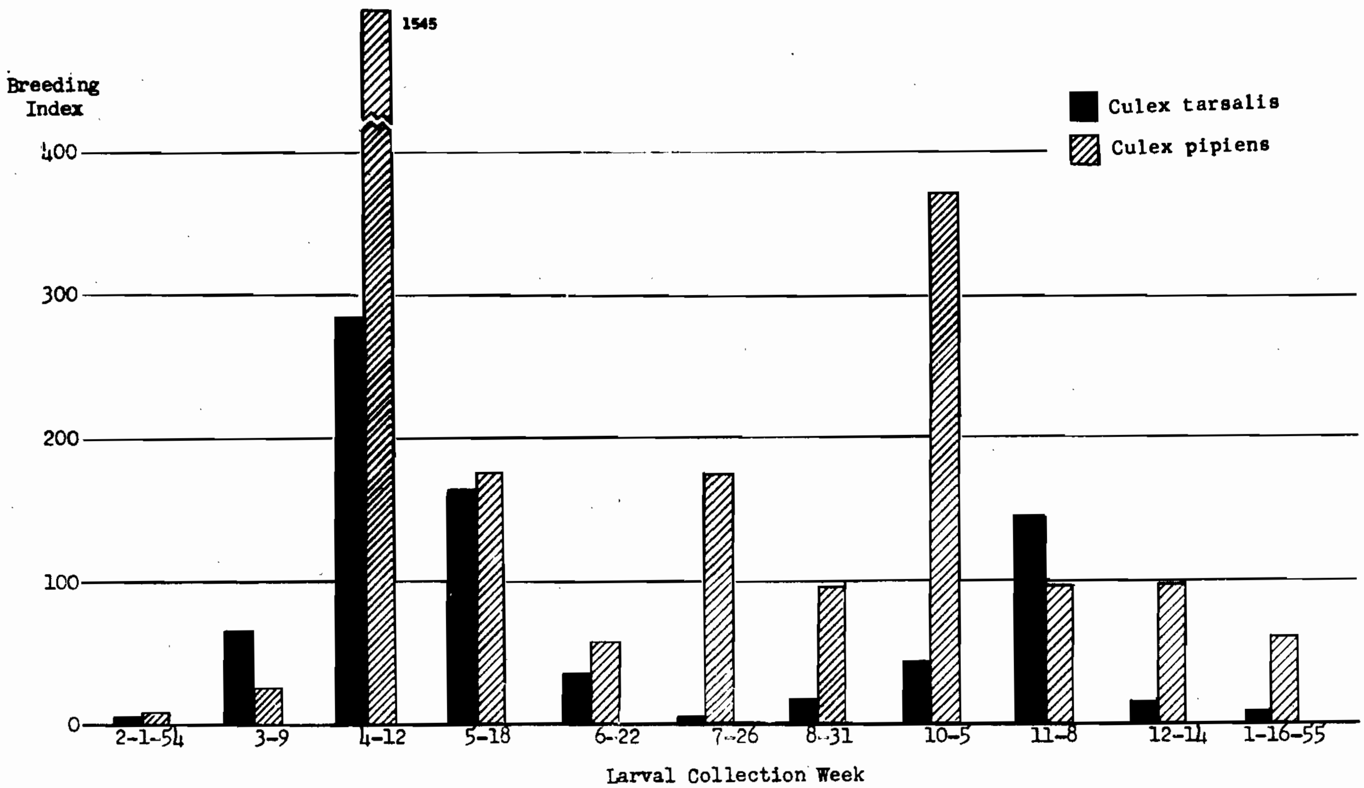

Figure 3.--Breeding index of two common species of mosquitoes in Imperial County, 1954-1955.*

*Adapted from: Harvey I. Magy, 1955. A mosquito survey of Imperial Valley, Imperial County, December, 1953 – January, 1955 (Mimeo), State of California, Department of Public Health.

Table 2.–*Culex pipiens* and *Culex tarsalis* larvae by sources, Imperial County, December 1953 – January 1955.

	Numbers of Sources			Percentage of Sources		
Sources	*Culex tarsalis*	*Culex pipiens*	*Culex tarsalis & Culex pipiens*	*Culex tarsalis*	*Culex pipiens*	*Culex tarsalis & Culex pipiens*
Irrigation Practices	96	60	45	52	35	38
Animal Practices	33	59	39	18	34	29
Industrial	19	20	12	10	11	10
Domestic	36	35	27	20	20	23
TOTAL	184	174	118	100	100	100

The preceding data suggest that neither light trapping method accurately measures *C. pipiens* produced in the areas sampled. Additional adult sampling measures should be used, such as biting, animal bait traps, or resting collections in order to sample accurately female *C. pipiens* for determining the rate of arbovirus infection in this species.

CONCLUSIONS.–

1. In the light of last years epidemic activity of SLE in 26 states and Canada, extensively involving the *Culex pipiens* complex mosquito as a vector, and recognition of persistent *C. tarsalis* and *C. pipiens* transmission of SLE in Imperial County, these events stimulated a reexamination of accumulated data on *C. pipiens* mosquitoes.
2. This reexamination has resulted in recognition that *Culex pipiens* is a common and extensively distributed mosquito in the Imperial Valley. The data also reflects limitations in assessing the true picture because of the reluctance of *Culex pipiens* to enter light traps relative to the ease of capturing *C. tarsalis* by this method.
3. When larval data is examined it is evident that the population is much larger than indicated by light trapping of adults and indicates that other adult collecting techniques should be developed.
4. A significant infection of *Culex pipiens* collected in an area with numerous feedlots at the Alamo River Mexican Border site during the 1972 VEE surveillance suggests that

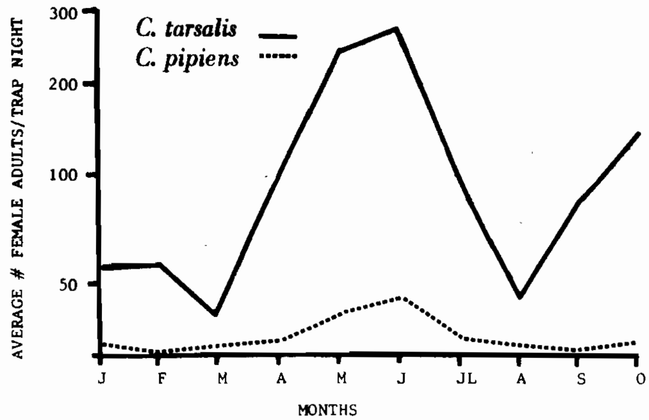

Figure 4.–CDC-CO_2 light trap collections Imperial County, 1972-73-74.

Table 3.–*Culex pipiens* and *Culex tarsalis* larvae, Imperial Valley, Nov.-Dec., 1975.

Source	Tl. No.	*C.p./C.t.*	*C.p.*	*C.t.*
Natural	5	0	0	5
Domestic	18	3	7	8
Irrg.-Drng.	29	1	12	16
Ind.-Mun.	3	0	2	1
TOTALS	55	4	21	30

SLE infection of *Culex pipiens* along the border is not infrequent or uncommon.

5. The explosive growth of Mexicali's population, which now exceeds a half million people and the recent report of the California Regional Water Quality Control Board of the exceptionally high organic content of the New River, which is conducive to *Culex pipiens* breeding, suggests increasing concern regarding SLE virus transmission potential.

6. There emerges from this combination of observations and information a potential situation for rapid and explosive peridomestic transmission of SLE arbovirus in Mexicali and Imperial Valleys.

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NATURAL ATTRITION IN LARVAL POPULATIONS OF THE TREEHOLE MOSQUITO *AEDES SIERRENSIS* (LUDLOW)

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ABSTRACT

Methods were described for determing population density and natural attrition in immature populations of *Aedes sierrensis* in an oak-bay woodland in Marin County, California. A 97.5% mortaltiy was recorded between the first instar larval stage and the emerging adult population for the 1974-75 season. This was much higher than the 1973-74 season which was established at about 85%. The vast majority of treeholes followed produced few adult mosquitoes (less than 200 each) while a relatively few holes produced the majority of emerging adults.

THE EVOLUTION OF BIOLOGICAL CONTROL FOR MOSQUITOES

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Biological control of mosquitoes as used by different investigators is a broad and complex area dealing with a large accumulation of published information. In the space permitted I intend to redefine and describe the scope and concept of biological control; to review briefly some of the organisms that have been used successfully in control programs and to discuss the major technical obstacles that have hampered the development and use of a wider variety of biological control organisms.

The origin of the field as it relates to mosquitoes can be traced to the beginnings of organized mosquito control, although the term per se was not applied to this area until much later. The term biological control to some workers includes practically every control technique used against mosquitoes. For instance, as described by the World Health Organization, biological control is a specific sub-component of naturalistic control, and is defined as the "direct or indirect manipulation of the natural enemies of pest species in such a way as to increase mortality among the pest populations" (Anon. 1973). WHO also includes autocidal control, which is the manipulation of individuals of a pest species through sterilization, genetical procedures or other means to harm their own kind. Others have included almost any manipulation, component or product of a biological system as biological control, such as juvenile hormones, growth inhibitors, seeds, extracts of plants, water level manipulation, etc. (Chapman 1974).

The inclusion of all these techniques and methods under the heading of biological control is cumbersome and confusing and is not consistent with terminology applied by agricultural workers. To avoid these difficulties the definition as used by Stern et al. (1959) and DeBach (1964) will be proposed with minor modification. It is defined simply as the action of predators and pathogens in maintaining another organism population density at a lower level than would occur in their absence. The term parasite was omitted from the Stern et al. and DeBach definition to avoid confusion between true parasites and entomophagous parasitoids commonly referred to as "parasites" by agricultural workers. The term pathogen is used in its broadest sense and thus includes the true parasites as a component of the total complex of disease causing organisms. An important point in the above definition is that it includes that aspect of natural control which occurs through the action of indigenous natural enemies. It therefore does not imply that man must be a manipulator in the process, but certainly leaves the opportunity open.

In reviewing literature published before the turn of the last century it was apparent that an appreciation for the possible role of natural enemies for the control of mosquitoes existed even at that time. Lamborn (1890) had been impressed by various reports on the elimination of adult mosquitoes by dragonflies. Much credit had been given to the large colorful dragonflies for the demise of hordes of adult mosquitoes particularly on the western plains of the United States. This led Lamborn in 1890 to offer a series of prizes for essays dealing with methods for destroying mosquitoes and houseflies, especially pointing out the dragonfly for careful consideration. It is of interest that the investigative procedures suggested for a chance to win one of the prizes was very logical and would still be appropriate today. The suggested research approach was as follows:

1. Observations and experiments upon various insects that destroy mosquitoes and houseflies, stating the methods and capacity of destruction.
2. Observations and experiments to determine the best dragonflies to be artificially multiplied for the two above named objectives (method and capacity of destruction).
3. Give detailed statements of the habits and life history of the species.

The prizes were awarded; however, none of the winners was able to solve the problem of mass breeding on a large scale. Like then, this obstacle of mass rearing is a major problem confronting the use of insect predators as an applied technique. Of the over 200 insect predators that have been recorded attacking mosquito larvae, only a few show any promise at all for mass production and inundative release against mosquitoes (Jenkins 1964).

The predaceous mosquito *Toxorynchites* was one predator that seemed to possess promise along the lines of classical biological control against certain container breeding *Aedes*, particularly on Pacific Islands. Several releases of this mosquito were made and it did establish in several areas. However, the impact on the *Aedes* populations was not considered significant (Steffan 1968). Recently, further attention has been given to this predator for mosquito control through the mechanism of inundative releases timed with the seasonal cycle of *Aedes aegypti* in selected urban areas of Africa. Although insect predators are not readily amenable to manipulation, their natural occurrence in certain aquatic systems probably plays an important role in regulating mosquito populations. For this reason, mosquito breeding habitats should be evaluated carefully before applying a control procedure which might disturb this component of natural control.

In L. O. Howard's classic pamphlet of 1910 entitled "Preventive and Remedial Work against Mosquitoes", a section was devoted to the "Practical Use of Natural Enemies of Mosquitoes". The opening sentence reads . . . . "Almost no practical use has been made artificially of the natural enemies of mosquitoes, except with fish." This is unfortunately about the current state of conditions for the biological control organisms for mosquito control. However, it should be pointed out that more interest has been shown and research progress has been made over the last several years with several pathogens and other predators. Nonetheless, fish represent the only major manipulative organism for use in control programs.

Their use dates back to the beginnings of organized mosquito control. One of the first published accounts was one described by Howard (1910) in which small fish were used for control of mosquitoes in a livery stable cistern in Georgia in 1854. The name of the fish was not given, however, it could well have been *Gambusia affinis*.

Of the various fish used for mosquito control, *Gambusia affinis* is by far the best known. Over the past 70 or more years it has been transported throughout most of the warmer geographical areas of the globe, including California. This wide distribution has led one fish ecologist to state that *G. affinis* is the most widely disseminated natural enemy in the history of biological control (Wilson 1965). According to Gerberich and Laird (1969) there are some 265 species of fish that have been employed in one capacity or another against 35 species of mosquitoes in over 40 countries. From the turn of the century to the present, the use of fish for control of mosquitoes has had varying degrees of success. Fish are certainly not a panacea for mosquito control, but, on the other hand, are a useful control agent for certain aquatic habitats.

One of the first and most successful uses of fish for mosquito control was through a recommendation by Smith (1904). A system was described for salt marshes of the eastern seaboard for the naturally occurring salt marsh killifish to intrude into the major mosquito breeding areas. The program was successfully carried out and after some 60 years it is still functioning (Anon. 1973).

Fish have been used for malaria control in special situations for many years before World War II (Hackett 1937; Herms and Gray 1940; Kliger 1930; Covell 1941). With the introduction of DDT after this period their use was de-emphasized until failures with the insecticide promulgated their recent reinstatement into control programs. WHO now reports some 30 countries are using fish for the control of *Anopheles* (Anon. 1973). A recent report from the Peoples Republic of China indicates that fish are an important component of their malaria control programs as well (Kung and Huang 1975).

I would now like to conclude this brief review with a reference to the use of other invertebrate predators and pathogens. Although no widespread use of these biological control organisms has yet been practical, work in the laboratory, and field trials with several of these agents have been very encouraging. Again I am not talking about a future panacea for mosquito control but only as useful components under the preamble of integrated control. Two invertebrate predators that show promise both from a manipulative view as well as natural control are *Hydra* and the planarians. Both groups appear to be amenable to mass production and storage techniques. (See Bay 1974 for review.)

In the area of pathogenic organisms in which I include viruses, bacteria, fungi, protozoa and nematodes there has been substantial progress with several of these agents over the last several years. Some unresolved problems in the biology, mass production, impact on non-target organisms and efficacy remain unresolved. However, practical applications with some of these agents appear relatively close at hand. (See Chapman 1974 for review.)

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OBSERVATIONS ON GROWTH, BREEDING, AND FRY SURVIVAL OF *GAMBUSIA AFFINIS AFFINIS* (PISCES:POECILIIDAE) UNDER ARTIFICIAL REARING CONDITIONS

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ABSTRACT

Growth of *Gambusia affinis* was rapid; some of the fish born in May had reached sexual maturity by mid-June. Most of the females over 22 to 23 mm standard length had eggs or embryos. Most of the females born in May were producing young by fall. Growth rate slowed significantly in male *G. affinis* upon reaching sexual maturity. Mean female post reproductive growth ranged from 10.0 to 11.4 mm by October. Slightly greater growth was found to occur with wider fluctuations in temperature and *G. affinis* apparently shows adaptation toward fluctuating temperature environments.

The numbers of fry per brood ranged from 13 to 64 (x = 39.1, N = 57). Cannibalism was significantly less in water high in algal content as compared with clear water conditions. Survival of fry to 14 to 16 mm (SL) averaged 5.4 fry per brood, and although low, survival rates were high once fry surpassed 16 mm.

INTRODUCTION.–Many aspects of the ecology, biology, and behavior of *Gambusia affinis* populations have been studied. The studies of Hildebrand (1921), Samokhvalova (1941), Krumholz (1948, 1963), Maglio and Rosen (1969), Hoy and Reed (1970), Hubbs (1971), Challet et al. (1974), Wu et al. (1974), and Martin (1975) relate to growth, numbers of fry per brood, and cannibalism in gambusian fishes.

In this investigation preliminary experiments were conducted to provide information on growth, numbers of fry per brood, and survival of *Gambusia affinis affinis* under artificial rearing conditions.

METHODS AND MATERIALS.–Growth--In May samples (1005 and 1451) of fry, 4 to 11 mm in standard length (SL) were placed in 7.3 and 4.8 m diameter portable pools, respectively. The pools were filled with 24.5 m^3 and 16.3 m^3 of water, respectively. The depths were kept constant during the study, as a means of regulating temperature ranges. Both pools were similar as to algal content and feeding rate. The feeding rate (using Purina trout chow, size no. 1) was kept at 3% of body weight of fish per day, as with all experimentation in this study, exclusive of cannibalism investigations. On the 14th or 15th of each month, fish were netted at random, measured to the nearest mm, and then returned to the pools. Temperature (2 cm below the surface) was monitored with a Comark electronic thermometer at 0900, 1200, 1500, and 1800 hrs each day for at least 10 days each month in each pool. Growth calculations were determined from the above information.

Numbers of fry per brood.--Wooden breeding trays with screen partitions were constructed (Figure 1). The trays were constructed .6 m square and .3 deep with fine mesh screen attached to the bottom and 3 mm mesh screen dividing each tray, the top half for adult fish and the bottom half to collect fry when dropped. These trays were floated on the surface in another 4.8 m diameter pool and weighted so that only the top several cm remained above the surface (Figure 1). Gravid females (30-50 mm in SL) were placed in the trays immediately prior to their giving birth to young. Daily inspections were made of the trays, and after the young had been dropped, they were counted and removed to other tanks for survival studies. Additional gravid females were then placed in the trays, after the spent females had been removed, and the experimentation repeated.

Survival of fry.--Polyethylene trash containers were filled with 35 l of water, clear in some (24 experiments) and high in algal content (22 experiments) in others. The relative opacity of algae was less than 2 to 3 cm. Fifty fry were placed in each container. Ten adult fish (35-50 mm in SL) were placed in the containers with clear and algal water. The fish were not fed during experimentation. Controls without adult fish were also established in two containers in which 105 fry were kept in algal water and 114 fry were kept in two containers of clear water. Both control experiments were maintained for 11 days. The number of fry alive in each container were counted at 24 hr intervals. Cannibalism experiments were maintained for only two days. From these data the number of fry eaten per adult female per day could be calculated.

Individual broods (6) of fry of 4 to 10 mm were removed from the breeding trays and placed in a 1.8 m^3 tank containing algal water (each brood was treated individually). The fry were fed at the usual feeding rate. When the fry in the brood reached standard lengths of 14 to 16 mm they were removed and counted. These 14 to 16 mm fry were then transferred to another 1.8 m^3 tank and held and fed to determine survival rates for larger fish.

All experimentation was conducted at a University of California research facility established at the Bollman Water Treatment Plant near Concord, California from May through October 1975.

RESULTS AND DISCUSSION.–Growth.--Figure 2 compares growth data from the two pools which were maintained at different temperature regimens. Although growth appeared slightly greater in the pool maintained at the daytime ranges of 18.1°C to 29.1°C than the one maintained at 18.1°C to 23.9°C, the differences were not significant (5%, overlap of 2 S_x). Growth appeared to be rapid at both temperatures. Some of the fish had reached sexual maturity as

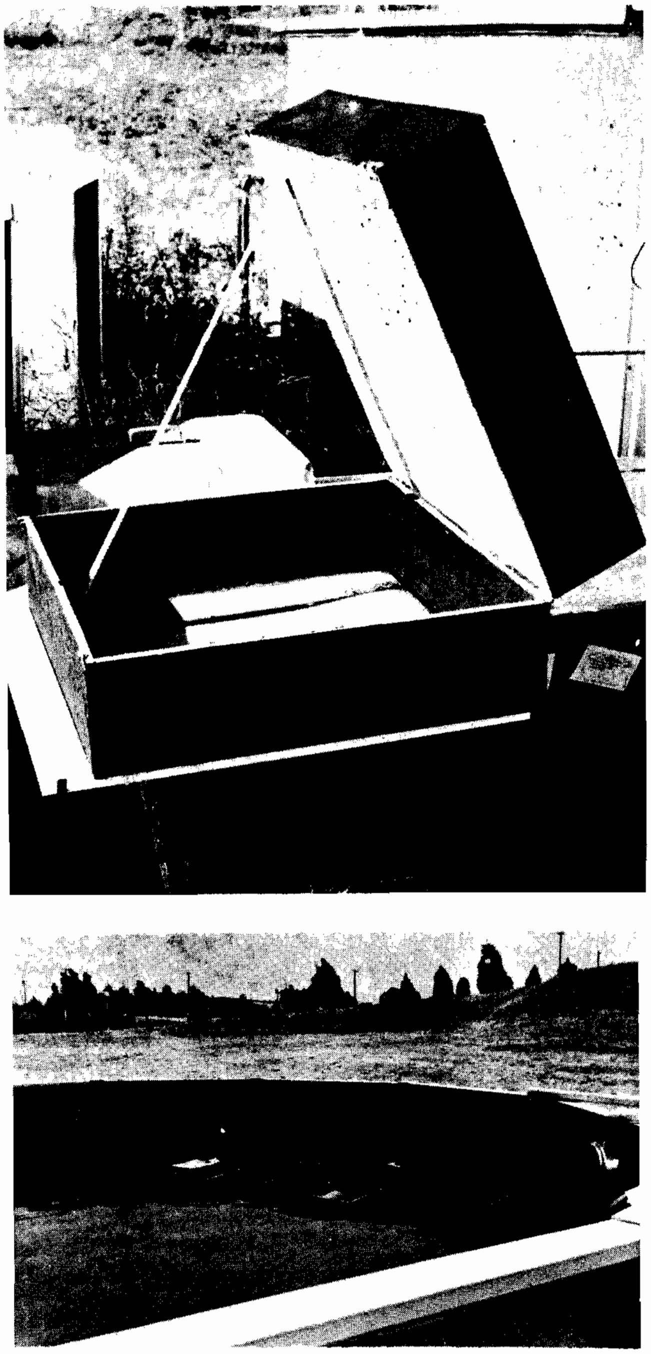

Figure 1.–Top photograph shows the construction design of the wooden breeding tray and the bottom photograph shows a series of these trays floating in one of the portable pools.

early as the month of June. Examination of 22 to 23 mm (SL) females revealed that most had eggs or embryos. Upon external examination, females at 26 to 27 mm (SL) displayed the characteristic gravid condition. Most of the females born in May were producing young by fall and in September and October fry were abundant in both pools. Similar results were found by Hubbs (1971) for wild populations of *G. affinis*. Growth was minimal in male *Gambusia affinis* upon reaching sexual maturity and few males exceeding 25 mm (SL) in length could be found. Both Krumholz (1948) and Hubbs (1971) reported cessation of growth in sexually mature males. Mean female post reproductive growth ranged from 10.0 to 11.4 mm by October. Hubbs (1971) found that one year old females ranged from 35 to 36 mm (SL) which indicated that 14 mm of post reproductive growth had occurred in the field. Hubbs (1971) found that the growth rate and fecundity were reduced in thermally constant environments and that populations were most abundant in fluctuating temperature environments. In this study a slightly greater growth rate was found under the more widely fluctuating temperature regimens. *G. affinis* apparently shows adaptation toward fluctuating temperature environments. Further support has been found in a study showing that increased heat resistance occurred during the midday hours in *G. affinis* (Johnson 1977, in press) which coincides with the activity peak of 1100 hrs as found by Hubbs (1971).

Numbers of fry per brood ranged from 13 to 64 ($\bar{x}$ = 39.1, N = 57). These data are in close agreement with some aquarium studies where the numbers of young ranged from 16 to 73 ($\bar{x}$ = 41.9, N = 33) (Johnson 1975). Other workers have reported average numbers ranging from 40 (Hildebrand 1921) to 104 (Hoy and Reed 1970). Challet et al. (1974) found that fry per brood ranged from 25 to 60 based upon a small sample. Brood numbers as high as 240 have been predicted for a 5 gm female *G. affinis* (Wu et al. 1974).

Survival of fry.--Figure 3 depicts cannibalism on fry under the two conditions of clear and algal water. Significant differences were found between the two water conditions in both 24 and 48 hr intervals (5%, non-overlap 2 $s_{\bar{x}}$). Cannibalism was significantly less in the algal water condition (Table 1). The reduced water clarity due to the high algal content appears to aid the fry in avoiding adult cannibalism since *G. affinis* is probably predominately a sight feeder. Figure 4 describes percent mortality due to cannibalism for the first and second day. Although the number of fry consumed per adult per day decreased significantly during the second day, no significant differences were found in percent mortality due to cannibalism between the two days.

Table 1.–Number of fry consumed per adult per day for 24 and 48 hr periods under clear and algal water conditions. N = number of tests.

	24 hr				48 hr			
	$\bar{X}$	Range	N	2 $S_{\bar{X}}$	$\bar{X}$	Range	N	2 $S_{\bar{X}}$
Clear water	2.66	1.22-4.53	24	0.54	1.15	0.10-1.65	24	0.36
Algal water	1.59	0.00-3.98	22	0.42	0.16	0.00-0.34	22	0.06

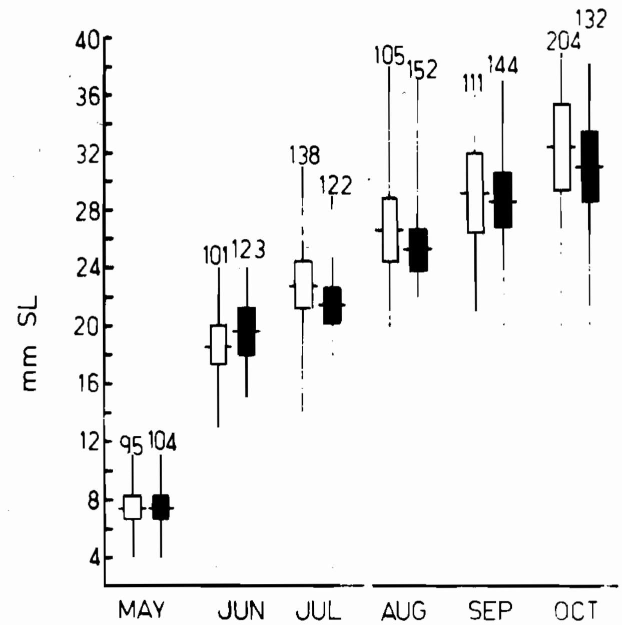

Figure 2.–Growth of *Gambusia affinis affinis* under artificial rearing conditions. Open rectangles, data taken within the temperature ranges of 18.1°C to 29.1°C; dark rectangles, data taken within the temperature ranges of 18.1°C to 23.9°C; vertical lines, ranges; horizontal lines, means; rectangles, twice the standard error on each side of the mean; numbers above refer to sample sizes.

Natural mortality of fry was less in algal water than in clear water for the first 3 days (algal water = 0.9% mortality; N = 105; clear water = 2.6% mortality, N = 114), but after 11 days mortality was approximately the same (algal water = 7.6% mortality; clear water = 6.1% mortality). Survival of fry to 14 to 16 mm (SL) averaged 5.4 fry per brood as determined from breeding tray data, a mean survival rate of 13.8% (range 2.5%-33.2%) was calculated. Survival of fry from 16 mm to sexual maturity was excellent; a mean survival rate of 96.2% (range 92.4%-100%) was determined based upon 6 broods.

ACKNOWLEDGMENTS.–This research was supported by California State Legislature Special Allocation for Mosquito Research funds. Robert van den Bosch, Richard Garcia, and William G. Voigt, University of California, Berkeley, offerred suggestions concerning the manuscript.

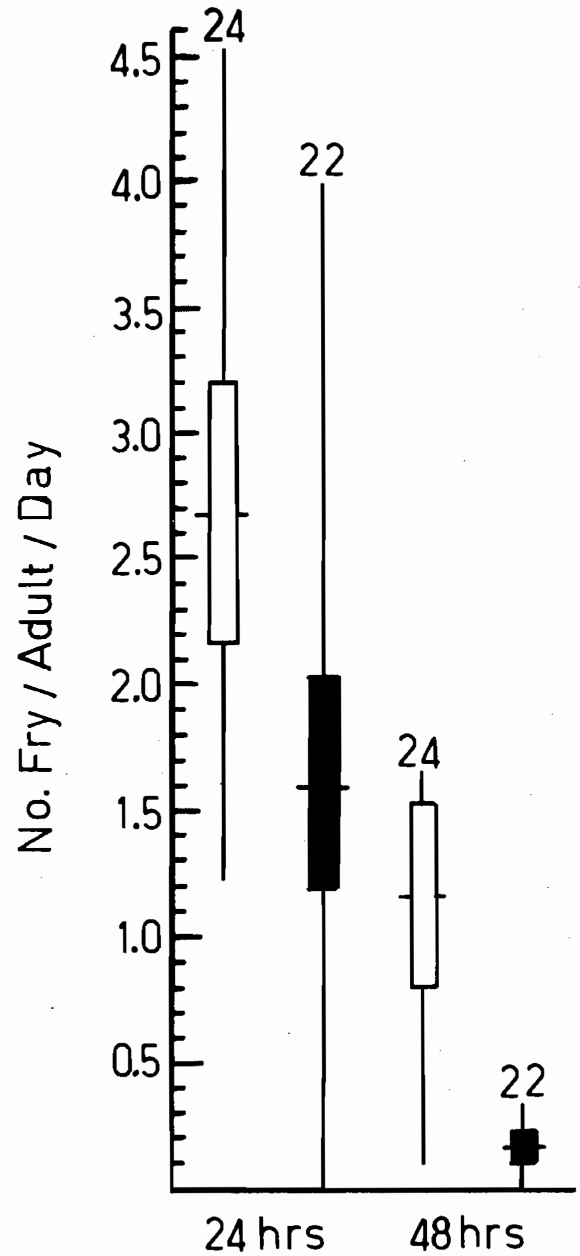

Figure 3.–Number of fry consumed per adult per day for the first and second days. Open rectangles, clear water condition; dark rectangles, algal water condition; vertical and horizontal lines, rectangles and numbers the same as in Figure 2.

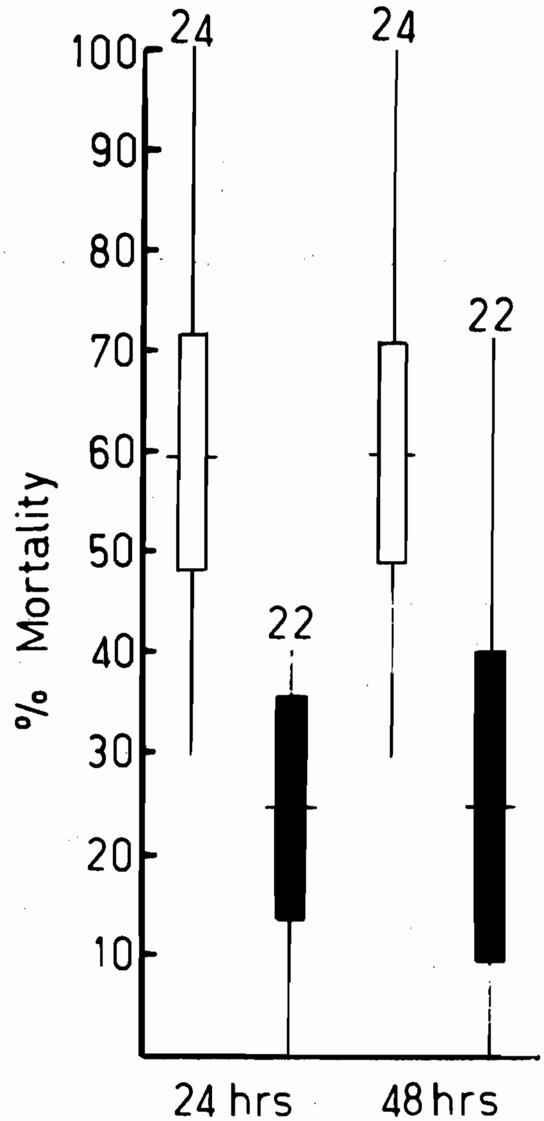

Figure 4.—Percent mortality of cannibalism during first and second days. Open and dark rectangles same as in Figure 3; vertical and horizontal lines, rectangles and numbers same as in Figure 2.

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THE ESTABLISHMENT OF *LAGENIDIUM GIGANTEUM*, AN AQUATIC FUNGAL PARASITE OF MOSQUITOES, THREE YEARS AFTER FIELD INTRODUCTION

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ABSTRACT

Lagenidium giganteum was recovered in 1975 from test sites in Colusa County, California, where it had been introduced on an experimental basis 3 years earlier (1972). In contrast to 1974, the fungus in 1975 exhibited: (1) high levels of infection in the site which previously exhibited the highest infection rate in 1972 and was the only site which yielded fungal isolates in 1974; (2) normal infectivity pattern (in contrast to the erratic and low order nature of 1974 isolates); (3) its initial recovery in two sites 3 years after introduction and where only low levels of infection was noted during the 1972 study.

We have tentatively concluded that (1) survival through seasonal droughts can be accomplished over a 2 and possibly 3 year period; (2) dispersal from inoculation sites are probably minimal; (3) high temperatures and possible excessive salt concentrations can be a limiting factor under field conditions.

FURTHER INVESTIGATIONS INTO THE CULTURE AND WINTER MAINTENANCE OF *GAMBUSIA AFFINIS AFFINIS* (PISCES: POECILIIDAE)

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ABSTRACT

Pond culture studies were carried out at Modesto and Concord, California. At Modesto 9 ponds were established under different rearing conditions of fertilization (chicken manure), vegetation density and feeding. The ponds were seined in March to determine the quantity of fish present in each individual pond and again in November to determine the effects of different treatments. In the ponds with monthly applications of chicken manure, the yield was twice that of the unfertilized ponds. These ponds are being fertilized throughout the winter months to determine if mortality can be lessened by providing higher productivity during the colder months. A sampling program will commence this spring to determine the effect of chicken manure fertilization on the biota of the ponds. At the Concord facility between May and October, 445 lbs of fish were reared in four large concrete basins at a cost of $2.62/lb.

Several small warm-water production rearing systems have been set up at various sites. Systems using commercial pool heaters supplied by both propane and natural gas are being tested. Some of these systems are designed to heat the water directly and other systems heat the water indirectly through heat exchange in circulation through a submerged network of pipes. The costs and efficiencies of these systems are being compared. Modified heat pump systems also have been designed and tested on a small scale. Energy expenditure was minimal and the operational costs were less than $3.00 per month. The effectiveness of solar panels is being tested both on a small scale with portable pools and on a large scale at Coachella, California for a 30,000 gallon pond. This larger system is composed of 17 FAFCO solar panels. A heating system has been designed to utilize the heat available in large industrial cooling ponds based upon a modified heat pump-heat exchanger system. Installational costs are reasonable and operational costs are minimal. This system has great potential in that extremely large ponds can be heated cheaply. The use of heat from geothermal sources is being investigated and tested to rear fish near Calistoga, California.

The biological phase of investigation consists of studies on the numbers of fry per brood, fry survival rates and cannibalism, and growth under artificial rearing conditions. Studies on the thermal physiology of *Gambusia affinis* indicated that a diel fluctation in heat resistance occurred which coincided with an activity peak during midday hours. Studies have been made on the effects of subacute concentrations of organophosphorus insecticides on physiological functions. Tests were conducted with Abate®, methyl-parathion, chlorpyrifos-ethyl, fenthion, and malathion at concentrations of 1, 5, and 10 ppb for 24 hours exposure and at 5 ppb for 48 hours exposure. Reflexes, orientation, and thermal tolerance were significantly effected.

POPULATION STUDIES OF *GAMBUSIA AFFINIS* IN RICE FIELDS: SAMPLING DESIGN, FISH MOVEMENT AND DISTRIBUTION

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ABSTRACT

The minnow trap was found to be an efficient sampling device for *Gambusia affinis affinis* (Baird and Girard). The catch response was positive and linear with no evidence of trap-induced attractancy or repellency at all densities of fish tested.

At field stocking rates of 0.4, 1.0, 2.0 and 3.0 lbs fish per acre the minnow traps showed good correlation between number of individual fish caught and stocking rate. Sampling over a 2-month period indicated that stocking rate is not the determinant of ultimate population density in all cases. Food supply is likely the limiting factor in the control of population size. Distribution and movement within the field are influenced by physical characteristics of the field itself, particularly the density of the rice. Trap layout is critical for monitoring population trends. Traps should be placed in the 3 distinct areas of the paddy, i.e., the middle, along the border and at the inlet box.

Rice production has increased significantly in Kern County from 1500 acres in 1973 to 10,000 acres in 1975. This increase with its concomitant mosquito problem has necessitated a closer examination of the population dynamics and behavior of the mosquito fish *Gambusia affinis affinis* (Baird and Girard), our major rice field mosquito control agent. Most studies of mosquito fish in rice fields have focused on feeding patterns (Washino and Hokama 1967) and on determining the optimum stocking rate for maximum larval suppression (Hoy and Reed 1970, Hoy et al. 1971, Hoy et al. 1972). Only a few studies have dealt with the population dynamics of the fish itself in rice fields. Reed and Bryant (1974) stocked paddies with mature females and followed population trends by means of minnow traps. This work was followed by studies to develop a population increase curve (Reed and Bryant 1975).

The purpose of the following study was to design an accurate and efficient mosquito fish sampling technique and to then elucidate fish movement, distribution and growth patterns in the rice field.

MATERIALS AND METHODS.– Trap efficiency – Trap responses to varying densities of fish was studied at district headquarters in concrete holding tanks (9 x 9 x 2½ ft). Tanks were filled to a depth of 1 ft and stocked with known amounts of a mixed population of field-caught *Gambusia affinis* (approximately 70% female, 30% male and immature females). Eleven densities ranging from 0.01 to 0.55 lb/sq ft water surface were employed. For convenience fish were measured volumetrically and these figures converted to weight. Eight-mesh/in. wire minnow traps were suspended on wires from poles placed diagonally across the top of the tanks.

The opening of each trap was located 2 inches below the surface. Five traps were used per tank, arranged as 1 trap in each corner on the diagnal pole 2 ft from the corner and 1 trap in the center. Several hours after stocking the tanks the traps were lowered simultaneously into the tanks. Twenty-four hours later the traps were lifted from the water for catch evaluation.

The effect of trap contents (fish) on trapping efficiency was studied by placing pre-filled traps into tanks stocked with known amounts of fish. The same diagonal pole design was employed as in the first study. Four replicates each of densities of 0, 0.3, 0.9 and 1.9 lb fish/trap were placed in each of 3 tanks. Prior to introduction of the traps the tanks were stocked at rates of 3, 15, and 30 lb fish respectively. The total amount of fish per tank after introduction of the pre-filled traps was 15.5, 17.5 and 42.5 lbs respectively. Traps were arranged in a manner that would equalize the positional influence on all traps. Twenty-four hours after introduction traps were retrieved and evaluated.

Field Studies – Five rice fields, ranging in size from 15 to 80 acres, were selected for study. All met the following requirements: 1. small size 2. irrigated only with well water 3. no drainage outlet. Mature female mosquito fish were separated from collections obtained from natural breeding sites, weighed, and transported in an oxygenated tank truck to the field release point. One field each was stocked at the rate of 0.4, 2, and 3 lb fish/acre respectively and 2 were stocked at 1 lb/acre. At the time of release during the first week of July the rice ranged in height from 1 to 1½ ft throughout the 5 fields. The fish were introduced into every paddy along one entire edge of each field. Approximately 2 weeks after the releases were made fish populations were sampled by means of the 8-mesh minnow traps. The uppermost and lowermost paddies in each field were sampled by placing replicates of 3 traps each in the center of the paddy, near the water inlet box and along the border. Twenty-four hours after traps were set they were retrieved and evaluated. Each field was trapped for 3 consecutive days and the average catch per trap for 3 days taken as the final figure. Four trappings were carried out over a 2¾ month period.

RESULTS.– Trap efficiency – The catch response of the minnow traps at varying fish densities is illustrated in Figure 1. Over a 55-fold range of 0.01 to 0.55 lb of fish per sq ft of water surface the response remained linear. The regression line shows a correlation coefficient of 0.98 and the slope is significant at the 95% confidence level.

In a linear relation of the kind shown by the line in Figure 1 the traps would normally be expected to catch a fairly constant proportion of the population. However, as expressed in Figure 1 the percent of the population trapped rises dramatically at the lower densities. That the points of interception of X on Y could remain within the limits of the regression line is due to small increments of change in catch having greater influence on the proportion of catch than on scale distance at lower densities. The interpretation and significance of this response will be considered in the following discussion of the second test.

The study utilizing pre-filled traps was designed to elicit information about the effect of various densities of fish inside the trap on its subsequent catch. This design was also intended to reveal the degree of movement of fish into and out of traps. Unfortunately, data from the high density tank (41.5 lb total) was unusable because of high fish mortality both inside and outside the traps. Mortality was caused by failure to properly recondition the fish after stress induced when transported from the field. At the 2 lower densities results were informative. Twenty-four hours after introduction of the pre-filled traps each had readjused so that all held nearly equal volumes of fish. The appearance of the data indicated that the traps would have been even more equalized within several hours. Moreover, when plotted against their respective tank densities the mean catch of the 16 traps in each tank agreed with results of the previous test. This is shown in Figure 1 as the square points plotted along the solid regression line.

The results of the pre-filled trap test show no evidence that the contents of the trap influences subsequent catch through factors of attractancy, repellency or exclusion. Fish move in and out of the traps constantly but do so in a manner that maintains within the traps a constant proportion of the population being sampled. It appears that at least 24 hours are required for the traps to come to equilibrium with the outside population. Variation in the percentage of the population trapped (Figure 1) is likely due to changing behavior patterns at various population densities. From approximately 0.19 lb of fish/sq ft water surface the percent catch becomes constant to the highest densities studied which suggests that individuals move about at approximately the same velocity within this density range. The percent of fish trapped probably levels off again at some point below 0.02 lb/sq ft water surface. Densities encountered in the field would ordinarily be far below the lowest tested in the tanks and it is doubtful that variations in velocity of movement would influence catch size.

Field Studies – Figure 2 illustrates the catch of mature females 2 weeks after stocking. The mean catch from the two fields stocked at 1 lb/acre was 4.9 and 3 fish respectively and these were combined and plotted as 4 fish/trap. The correlation between catch and stocking rate is good at the densities sampled. Trap placement is critical to catch results and this aspect will be considered in detail in later discussion.

The longer-term population growth trends of *Gambusia* in the 5 rice fields are illustrated in Figure 3. Fields C, D and E were owned by the same grower and appeared quite similar in most physical respects. Fish populations in these fields displayed comparable growth patterns. Due to a schedule problem field C was sampled 1 week later than the other two in July. The July C sample implies that the first generation offspring that were too small to be captured 1 week earlier were now of catchable size. If fields D and E were also in this condition, the population in all 3 fields were supporting the maximum number of fish with the first generation offspring by the first week in July. The second generation offspring, had there been any, should have shown up in the traps by at least the last trapping in September. That they did not, coupled with the evidence that all three fields peaked and declined at similar population levels suggests that some limiting factor or set of factors is at work to maintain a carrying capacity for each field.

Fields A and B shared 2 characteristics that were absent in the other three. They both had wide (6 ft), deep (2½ ft) borders and both were nutritionally poor in terms of types

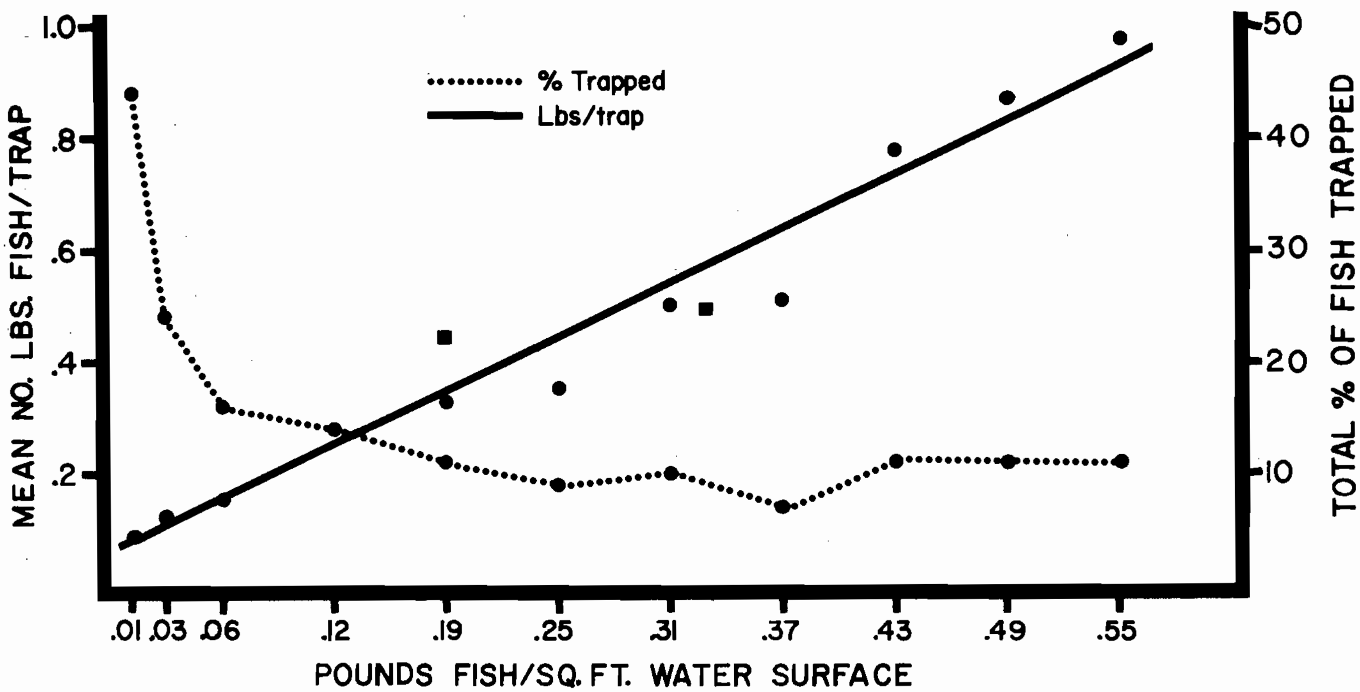

Figure 1.–Minnow trap catch of *Gambusia affinis* in concrete holding tanks.

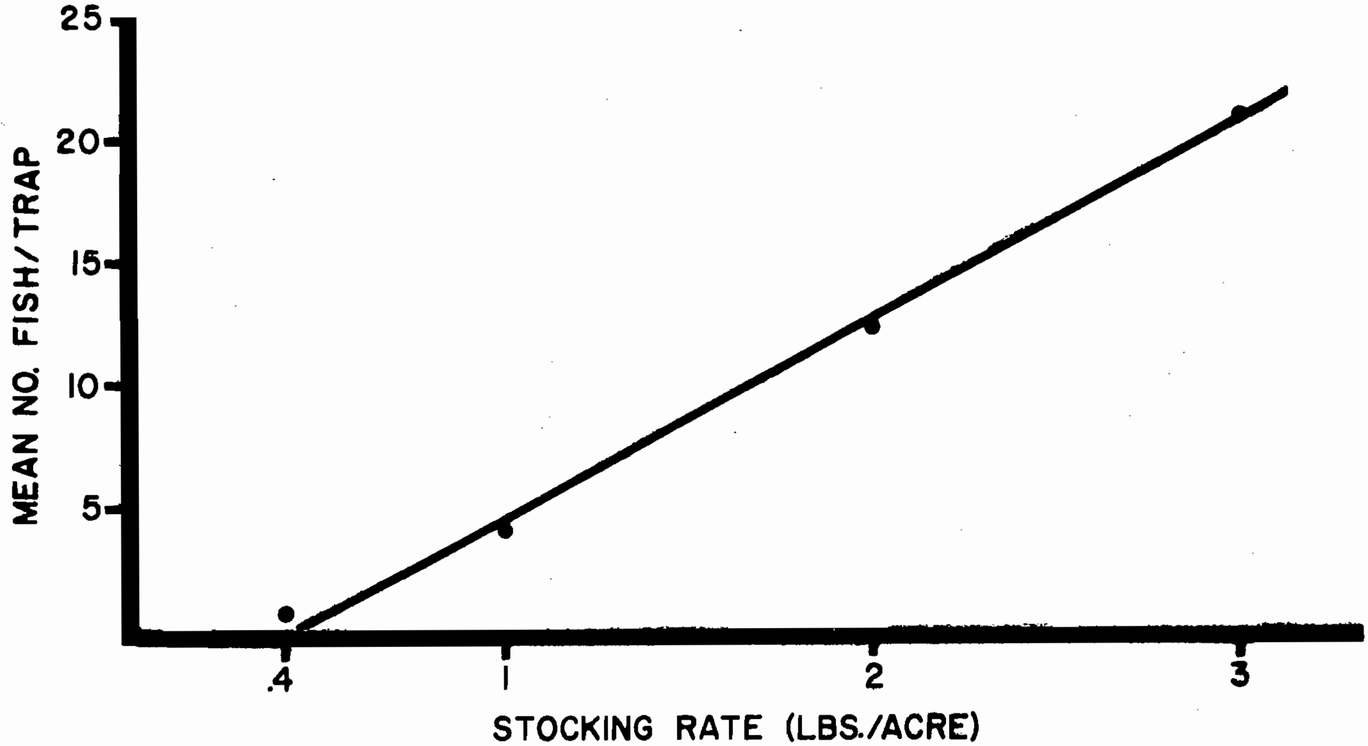

Figure 2.--Minnow trap catch of mature female *Gambusia affinis* in rice fields stocked at varying densities.

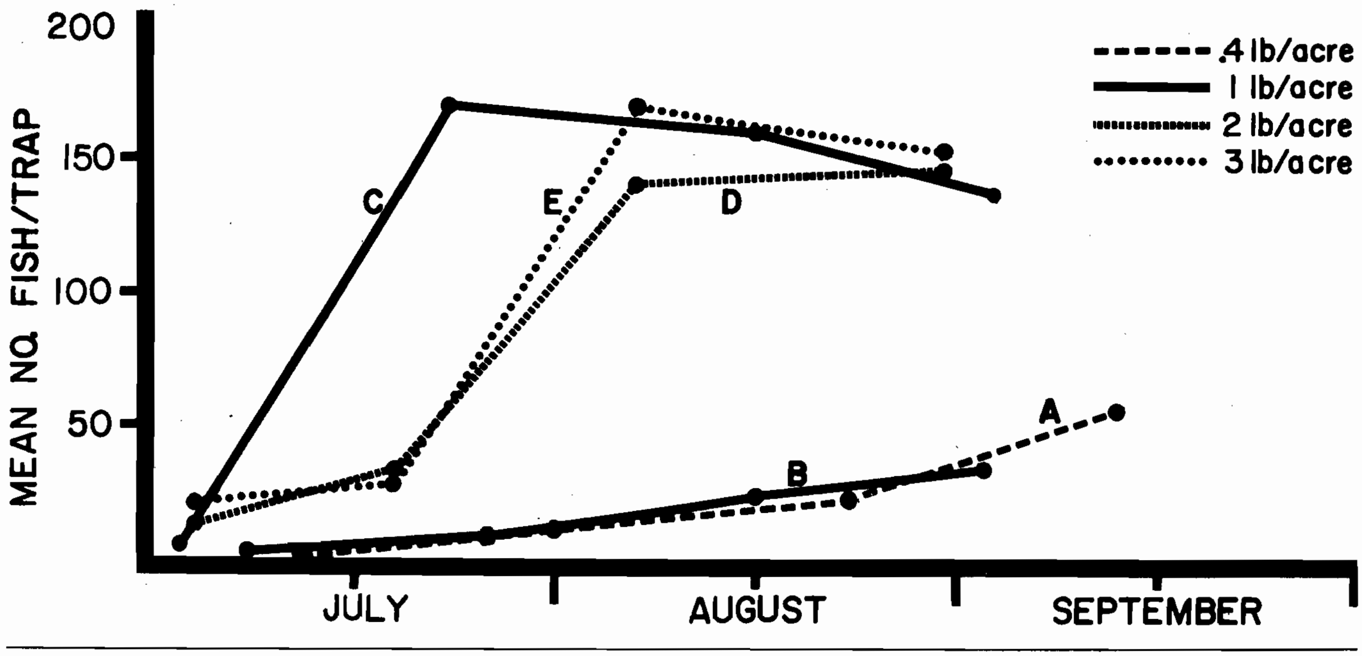

Figure 3. Population growth of *Gambusia affinis* in 5 rice fields stocked at varying densities.

and amount of arthropod food. Nutritional factors are perhaps responsible for densities in B and C, both stocked at 1 lb fish/acre. Careful studies will be conducted next season to determine the relationship between arthropod and fish density at carrying capacity. The possible influence of border width on fish abundance will be considered in the discussion of movement and distribution.

The distribution pattern of *Gambusia* in a rice field may also be significant in the level of larval control obtained. Table 1 shows the results from first and last trappings in the 5 fields. Traps from along the borders and at the inlet boxes were combined (perimeter) in the table for good reasons. When traps from the inlet boxes and along the borders were examined separately there was no consistent pattern

Table 1.–Comparison of % catch of *Gambusia affinis* in the center and along the perimeter of paddies within rice fields over a 2-month period.

Field	Lbs/acre	Perimeter First Trap	Perimeter Last Trap	Center First Trap	Center Last Trap
A	0.4	50	75	50	25
B	1.0	23	45	77	55
C	1.0	84	74	16	26
D	2.0	78	74	22	26
E	3.0	89	71	11	29

of distribution when compared to the center traps. However, the center traps demonstrated low variability between catches and showed strong correlation with stocking rates. Observations in the field showed that *Gambusia* moved along the borders and sometimes congregated at the inlet boxes but neither pattern was predictable or consistent. The data indicate that the center and the perimeter represent distinct zones of fish activity within the individual paddy, and the inlet box and border areas comprise sub-zones of the perimeter. Each sub-zone should be represented in the trap layout but catch should be analyzed by zones.

The data in Table 1 indicate a slight shift in distribution in fields C, D and E toward the end of the season as the proportion of fish increases in the centers. Although the distribution of *Gambusia* in field A at last trapping was similar to C, D and E, both A and B showed dramatically different patterns in the beginning. At first trapping A and B had a very sparse rice stand which might have enabled greater fish penetration into the center of the paddies. At last trapping the vegetation pattern of A was similar to C, D and E. However, the rice stand in field B was different from the other four at last trapping due to a difference in cultivation practice. The harrow used to prepare field B for planting had the teeth set widely apart. The rice usually grows in rows created by the harrow teeth which resulted in the rows of rice being spaced 3-5 in. wider in B than A, C, D and E. It is possible that this permitted a greater proportion of fish to occupy the center of the paddies in field B. There is no evidence that border width influences fish distribution.

The role of food availability in fish distribution is not totally clear. Preliminary studies indicate fish abundance is controlled by food supply, as would be expected, and also that food animals, mainly arthropods, are clustered throughout the fields (unpublished). Recent work by Case and Washino (1975) indicates that mosquito density and survivorship are positively correlated with levels of Ca^{++} ion in the rice field water. The relationship of water and soil chemical parameters to the entire arthropod community and the distribution and predation effectiveness of the fish population needs further clarification. Since fields C, D and E supported 3 times as many fish as field A at last trapping (Figure 3) yet had identical fish distribution patterns at that point (Table 1), the inference is that food supply governs fish abundance and physical field characteristics determine distribution. The data for field B also supports this conclusion. The level of fish predation on mosquito larvae may well not be dependent upon fish abundance alone.

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LABORATORY RATE OF PREDATION OF SEPARATE AND MIXED SEXES OF ADULT *NOTONECTA UNIFASCIATA* (GUERIN) ON FOURTH-INSTAR LARVAE OF *CULEX PEUS* (SPEISER)

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INTRODUCTION.–Recent investigations have demonstrated the capability of various species of backswimmers as effective predators of immature mosquitoes (Lee 1967; Ellis and Borden 1970; Sjogren and Legner 1974). Field experiments on naturally recruited and introduced populations of backswimmers suggests they might be used in special or limited mosquito breeding habitats (Garcia, Voigt, and Des Rochers 1974; Hazelrigg 1974). Hazelrigg (1974) has shown that mated pairs of *Notonecta unifasciata* Guerin adults are capable of making a numerical response[1] to sufficient numbers of immature mosquitoes as prey. The following laboratory study was performed to determine at what rate, if any, *N. unifasciata* would make a functional response[2] to various densities of immature mosquitoes for separate and mixed sexes of adult backswimmers. DeBach (1971) has stated that combined functional and numerical response are required of potential biological control agents, particularly predators, if they are to be implemented successfully in control programs.

PROCEDURES.– The predation rates of adults of *N. unifasciata* were determined for predator groups of: 1) single males, 2) single females, 3) single pairs of males, 4) single pairs of females, and 5) single mated pairs all exposed to prey densities of 10, 25, 50, and 100 fourth-instar mosquito larvae (*Culex peus* Speiser) weighing an average of 2.6 ± 3 mg/larva. All backswimmer adults recruited for this study were laboratory reared (Hazelrigg 1975). The males were virgins and seven to nine days old; females were virgins and nine to twelve days old. Each predator-prey density combination was replicated six times for only five days to minimize the possible effects of predator aging on the predation rate. Backswimmers were not starved prior to beginning each rate determination.

The predation rate determinations were conducted in quart, polystyrene containers as shown and described in Figure 1. Each predation unit was connected to a circulating, closed water system with a central reservoir (Bay, unpubl. data) and held approximately one-half quart of water, equalizing the conditions and prey-searching area in all units.

RESULTS AND DISCUSSION.–The effects of several combined *C. peus* larval prey and adult *N. unifasciata* predator densities on the rate of predation of this backswimmer are summarized in Table 1. All backswimmer adults, singly or in combination as identical or mated pairs, daily consumed a correspondingly greater number of prey with an increase in prey density (Figure 2), demonstrating what is characterized as the predator functional response for prey density (Solomon 1949; Holling 1961. and DeBach 1971). Of the predator arrangements listed in Table 1, only single paired males failed to show a daily significant functional response between adjacent prey densities of 25 and 50 and 50 and 100 fourth-instar mosquito larvae per unit.

No significant differences occurred among the daily predation rates of the backswimmers at prey densities of 10 and 25 mosquito larvae per unit. This was expected, as most larvae at these densities were consumed within the 24-hour predation rate interval. However, at higher prey densities (50 unit 100 larvae per unit) total larval consumption rarely occurred within the 24-hour predation rate interval, except in single and paired females. At a density of 50 fourth-instar mosquito larvae per unit, paired females consumed significantly more than any of the other predator densities, whereas single and paired males consumed significantly fewer larvae than any of the other predator densities. At 100 fourth-instar mosquito larvae per unit, both paired females and single-mated pairs showed no significant difference in their predation rates, killing more larvae than the remaining predator densities tested. At this density, paired males continued to consume the fewest larvae.

There was no overall significant difference between the predation rates of single male and single female backswimmers (Table 1), yet the latter consistently consumed more larvae at the four levels of prey density tested. This greater predation rate of females presumably was linked to their need for more food to sustain a higher rate of metabolism associated with egg production.

From studies conducted on other insects, data have been obtained evidencing that as predator density increases at any given prey density, corresponding declines usually occur in the number of prey consumed per predator (Holling 1961). Such declines typically associated with increased predator densities are considered depressions in the predator functional response and are believed to result from the coupled actions of at least two components: 1) prey exploitation[3] and 2) predator interference[4] (Holling 1961).

In *N. unifasciata* a definite depression in the functional response occurred when the adult backswimmer densities were increased from one to two individuals per predation unit. When compared to the mean larval consumption of

[1]Predator-parasite capacity to increase in number with a corresponding increase in prey density.

[2]Predator-parasite capacity to increase prey consumption with a corresponding increase in prey density.

[3]The chance that any individual predator will discover a prey that has not already been discovered as the density of predators increases.

[4]Increasing frequency of interaction and contacts of predators as the density of predators increases.

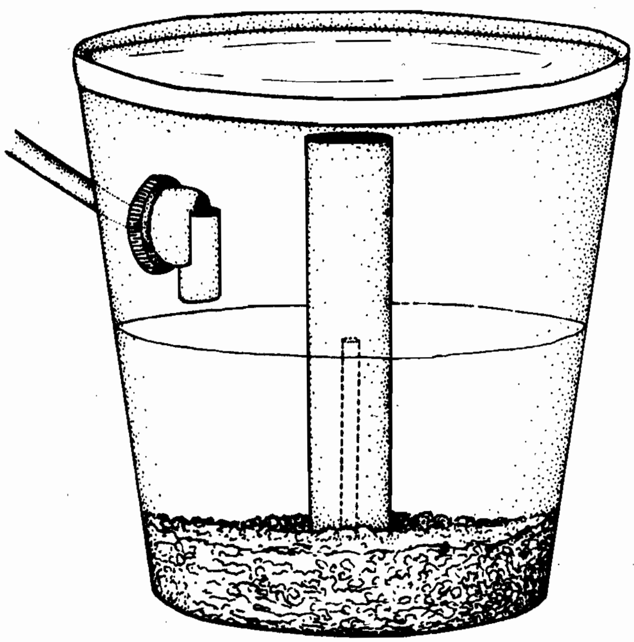

Figure 1.–Illustration of one of the quart polystyrene containers used in the predation studies of adult *N. unifasciata* on fourth-instar larvae of *C. peus*. The bottom of the container was covered with coarse aquarium gravel as filter material. The drain was constructed of a plastic drinking straw (dotted-line cylinder) and protected by a larger cylinder of polyethylene plastic tubing notched at the bottom to permit water flow through the gravel (not seen).

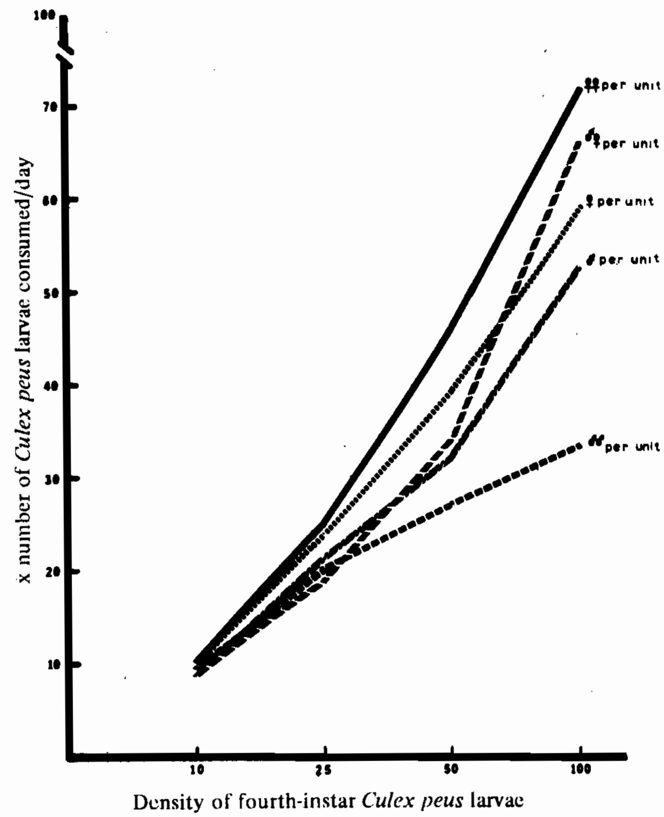

Figure 2.–The functional response of various adult combinations and densities of *N. unifasciata* fed 10, 25, 50, and 100 fourth-instar larvae of *C. peus*. Adult backswimmers maintained in quart polystyrene containers containing equal volumes of water (Figure 1). Individual mosquito larva average weight was 2.6 ± .3 mg. Water temperature was maintained at 21°C.

Table 1.–A nested analysis indicating the effect of predator types and density and prey density on the number of fourth-instar larvae of *C. peus* consumed by *N. unifasciata* at 21±1°C.

Predator Types and Density	Mean No. of Prey (Fourth-Instar Larvae) Consumed per Sample Density: 10	25	50	100	Predator Mean
Single ♂	9.3	22.0	31.6	52.1	28.8
Single ♀	10.0	24.2	39.3	57.9	32.9
Two ♂♂	9.8	20.5	26.3	33.4	22.5
Two ♀♀	10.0	24.7	45.8	72.3	38.2
♂♀ Pair	9.7	19.4	34.5	65.6	32.3
Prey Mean	9.8	22.2	35.5	56.3	

Least Significant Differences	$P \leq 0.05$	$P \leq 0.01$
1. Between predator means	5.46	7.43
2. Among prey means	3.76	5.03
3. Among predator types and density x prey means:		
a. Among prey means for same predator type and density	8.41	11.25
b. Between predator type means for same prey density or among prey means for different predator types	9.09	12.25

the single male and female predators at the four prey densities, respectively doubling the densities of backswimmers resulted in a decline of the number of prey consumed per predator in all cases.

Among all the backswimmer densities tested, paired males significantly and consistently consumed the fewest mosquito larvae every 24 hours (and failed to exhibit a functional response between successive increments in prey density) due to presumably three interacting factors: 1) a generally low rate of predation in individual males; 2) mutual interference and prey exploitation, and 3) behavioral interaction – specifically, frequent attempts by both males to copulate. Mated pairs did not differ significantly in their rate of predation from single males or females. Presumably, this result was associated with prey exploitation, mutual interference, and time spent in copula rather than prey finding and feeding interacting in the mated pairs, factors not operating to lower the rate of predation at the single predator densities. Among all doubled predator densities, paired females showed the highest daily rate of predation. This was expected, as single females, compared to single males, tended to show a higher intrinsic rate of predation, and no factors other than mutual interference and prey exploitation were operating to lower their rate of predation.

That pairs of *N. unifasciata* did not act synergistically in reducing mosquito larvae probably has little effect on the biological efficacy of this predator, except during predator overcrowding. Holling (1961) has commented similarly on the reduced predator efficiency associated with increased predator densities for other insect predators.

Encouraging in terms of using *N. unifasciata* in the control of actual mosquito populations, was the finding that at increasing prey densities mated pairs of backswimmers consumed nearly as many mosquito larvae as the highly predaceous paired females. Field-released mated pairs of *N. unifasciata* should as rapidly and effectively reduce mosquito populations as non-mated females. This is fortuitous, as there are several benefits associated with introducing mated pairs. The sexes need not be separated prior to introduction, and the progeny provide additional advantages such as habitat colonization, and accelerated and sustained mosquito reduction.

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MULTIPLE-MARKER LINES FOR GENETIC STUDIES IN *CULEX TARSALIS*[1]

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Projections of using genetic autocidal systems and/or the substitution in native populations of selected genotypes related to the control of *Culex tarsalis* depend on the development of the basic formal genetics for this species. Such development in turn is contingent on an accumulation of viable phenotypic mutations with which to ascertain such genetic information.

Mutations can be spontaneous or are induced. The former are sought by screening isolated populations which possibly have genotypes specific to themselves, and by inbreeding these to bring to light the hidden and recessive variables. Mutations are also induced by exposure to some mutagenic agent such as radiation or a specific chemical. The morphological mutations described to date for *C. tarsalis* are: yellow larvae (*v*) and white eye (*w*) (Barr and Myers 1966); black eye (*ble*) and carmine eye (*car*) (Asman 1975).

This paper lists additional monofactorial mutations found to date, and stocks that have combinations of these as multiple-marker lines to be used for the identification of chromosomal aberrations known as translocations or chromosomal interchanges.

Fifteen different geographic strains of *C. tarsalis* from various California counties and outside the state were screened for several generations for spontaneously occurring inherited morphological variations. In addition a Co-60 source and the chemical, ethyl methane sulfonate (EMS), were used as mutagenic agents. In the former case young adults were exposed to approximately

Table 1.–Monofactorial mutations that have been established as laboratory colonies.

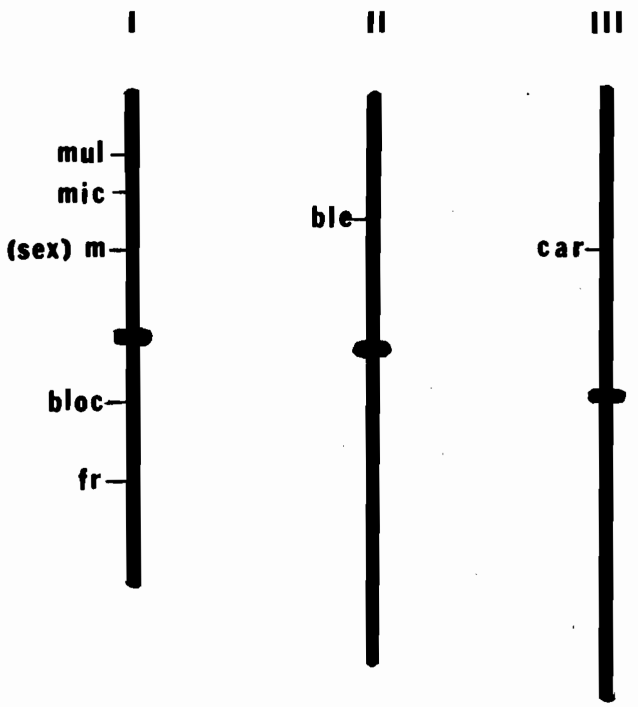
Mutant (symbol)	Mutagenic agent and colony source	Description	Linkage*
Black eye *(ble)*	Spontaneous Hart Park Strain	Black pigment - actually dark green under high magnification but black to naked eye - good penetrance and in both sexes	II or III recessive +
Mulberry *(mul)*	Ethyl methane sulfonate (EMS) Berkeley Strain	Facets of compd. eye irregular in shape - giving convexity	sex-linked (I) recessive +
Microcephalic *(mic)*	EMS Berkeley Strain	Many individual facets of compd. eye completely absent -	sex-linked (I) recessive +
Carmine *(car)*	Spontaneous Yuma Strain	Dark red pigmented eye, seen in larvae, pupae and adults	II or III recessive +
Setaceous palps *(sp)*	Spontaneous Dewarts Strain	♀♀ have 1 or 2 setae on each apical sement of palps, parallel to prob.	linkage (?) recessive +
Bleached ocelli *(bloc)*	Spontaneous Presidio Strain	Ocelli of larvae and pupae light pink	sex-linked (I) recessive +
Fringe wing *(fr)*	Co-60 irradiation Berkeley Strain	Wing scales heavy and ruffled giving fringe appearance	sex-linked (I) +
Charcoal *(char)*	Co-60 irradiation Berkeley Strain	White scales on proboscis legs and antennal pedicel missing - also reduced white on abdomen	II or III recessive +
White tarsomere *(wt)*	Spontaneous West Poso Creek	Distal segment of hind tarsi with white scales only	II or III recessive +

* + or - value for linkage studies.

[1]This research was supported by U. S. Army Grant/Contract No. DAMD-17-74-C4128 U. S. Army Medical Research and Development Command, Wash., D. C.

1200 r, and in the latter instances young adults are fed on a .5% EMS solution using a 10% sugar solution as the solvent. Over 20 mutations were isolated, and of these 9 have been recovered in sufficient numbers after outcrosses to successfully establish laboratory colonies (Table 2). By making the appropriate crosses of the mutants with "wild-type" and gathering the segregation data over several generations, the correlation of the mutation to one of the 3 linkage groups was established in 6 instances (Figure 1a).

a) Linkage groups



b) Multiple - marker stocks

1. sex — ble — car
2. mul — ble — car
3. mic — ble — car
4. bloc — ble — car
5. fr — ble — car

Figure 1. -Relationships of mutants.

C. tarsalis like other *Culex* species described cytologically has 3 pairs of chromosomes with no distinguishable differences in the karyotypes of the 2 sexes. Therefore, there are 3 linkage groups, and until linkage group-chromosome correlation studies are established, the chromosome holding the sex-determining locus is here tentatively designated as linkage group I, while the 2 autosomes are designated II and III. Since sex is determined by a single gene or small segment of a chromosome (Barr and Myers 1966) this locus can also be used as a genetic marker. Females are homozygous (m/m) for the recessive gene, and males are heterozygous or M/m.

Four of the new mutants here listed are sex-linked; that is, they are on the same linkage group as M or m, and barring exceptions due to crossovers between their position and the sex locus, they do not segregate independently of the sex-determining factor. The two eye-pigment mutants are not sex-linked and also give no evidence of being linked to each other. The remote possibility that they are on the same linkage group but are widely separated cannot be genetically ascertained without more autosomal markers. Thus *ble* and *car* are considered to be on linkage groups II and III; black eye *(ble)* has been arbitrarily assigned to linkage group II and carmine eye *(car)* to linkage group III.

Fortunately the 2 eye-color mutants have the unique relationship that both can express themselves in the same individual. Neither is fully epistatic to the other. Females and males homozgous for both *car* and *ble* have carmine eyes as larvae and pupae; however, when adults emerge the anterior portion of the eye shows the red pigment while in the posterior part black pigment is expressed (Asman 1975). As the adults age the carmine almost completely disappears and the compound eye is difficult to distinguish from those in adults homozygous for black eye only.

The combination of these 2 eye-color mutations in the same organisms already gives us one multiple-marker line if we use the sex locus as the marker for linkage group I. Having successfully interbred each of the sex-linked mutants with the line homozygous for *ble* and *car* gives us 5 multiple-marker stocks to date (Figure 1b). While in preliminary studies with this species it was only possible to identify translocations by cytological means (Asman 1976) genetic identification is now possible using these multiple-marker stocks.

The weakness of these stocks lies in the fact that all have the same autosomal markers while the sex-determining linkage group holds the only variables. Having only one marker on each of the 2 autosomes limits the 'capture' by genetic means of translocations involving either or both of the autosomes to those that by chance include the ble or car loci in the interchange. The following paper (McDonald, Terwedow, and Asman 1976) helps clarify this point.

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TRANSLOCATIONS CAPTURED BY GENETIC MARKER STRAINS FOR GENETIC CONTROL OF *CULEX TARSALIS*[1]

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ABSTRACT

The isolation and maintenance of radiation-induced chromosomal translocations has been achieved through the use of a genetic marker strain. Confirmation has been made on the basis of inherited partial sterility and cytology. The capture of translocations is the first step in the development of genetic control strains of *Culex tarsalis*.

INTRODUCTION.–Genetic control is the employment of a species for its own control (Whitten and Pal 1974). There are two approaches for genetic control, autocidal control through sterility, and replacement of one genetic characteristic in a population with another. Chromosomal translocations have potential for both approaches to the genetic control of *Culex tarsalis*.

Reciprocal translocations occur when two non-homologous chromosomes of the complement are broken (as by irradiation) and the broken ends reattach to nonhomologous chromosomes. When the gamete containing such an interchange fertilizes a normal gamete, a translocation heterozygote results (Figure 1), The heterozygote has two important features in terms of genetic control. First, it is partially sterile because unbalanced gametes result from meiotic segregation of the interchange complex in the heterozygote. Second, the translocation is transmitted to one-half of the viable progeny resulting from the balanced gametes. When individuals with more than a single translocation are produced, the sterility is greater (Serebrovskii 1940). Thus, translocation heterozygotes have potential for genetic control by sterility.

It is possible to isolate translocation homozygotes (Figure 1) by selecting this type from the progeny of two heterozygotes. When the fitness of homozygotes (theoretically 1.0) is actually greater than heterozygotes (theoretically 0.5) then these homozygotes can be used as a genetic wedge, driving into a population the genes with which the homozygote is genetically linked (Curtis 1968). If a virus refractory character could be linked with a homozygote, then the homozygote could drive that character into the population and replace the ability to transmit with refractoriness to transmission.

Translocations involving the sex chromosome have a special characteristic in that they are transmitted from the male to: (1) predominantly or exclusively males (*M*-linked), or (2) predominantly or exclusively females (*m*-linked). The translocations not involving the sex chromosome are autosomal and transmission is similar in both sexes.

Our goal has been to induce, isolate and characterize translocations, ultimately for use in either sterility or replacement approaches to genetic control of *C. tarsalis*. Until now it has been necessary to proceed without the genetic markers in the search for useful chromosome rearrangements. With the development of mutant multiple marker stocks, it is now feasible to isolate and maintain large numbers of translocation stocks (Asman 1976). This is necessary for preliminary screening of useful translocations.

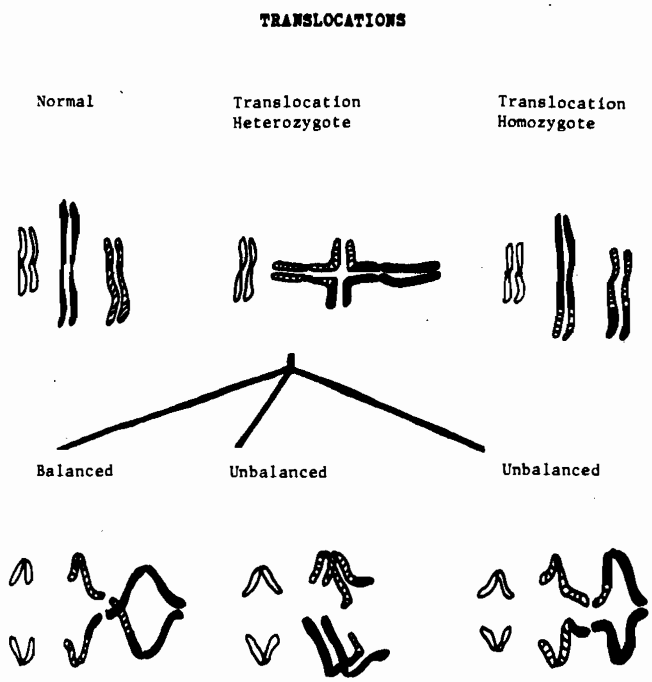

Figure 1.–Chromosme pairing in mosquitoes. Meiosis in translocation heterozygote leads to partial sterility.

MATERIALS AND METHODS.– One-day-old adult males of the BERKELEY strain were irradiated in various experiments at 1000 rads, 2000 rads and 3000 rads from a Co-60 source at the Lawrence Berkeley Laboratory. These males were mass mated with mutant marker females (homozygous for the recessives carmine (*car*) eye on linkage group 3 and black eye (*ble*) on linkage group 2 and *m* at the sex locus on linkage group 1). The resultant F_1 males were backcrossed in single-pairs to the marker stock as in Figure 2. The genetic scoring of the phenotypes in the progeny of a partially fertile F_1 reveal a new genetic linkage (pseudolinkage) if markers known to be on different linkage groups are associated by a translocation. Since the three pairs of

[1]This research was supported by U.S. Army Contract/Grant No. DAMD-17-74-C4128, U.S. Army Medical Research and Development Command, Wash., D. C.

Table 1.–Translocation in *Culex tarsalis.*

Translocation	Pseudolinkage	Number Examined	Egg Rafts from Males Semisterile/Normal	Females Tested
T(1:2)a	1.1	1112	124/0	+
T(1:2)b	22.5	520	32/1	+
T(1:2)c	1.3	76	19/0	
T(1:2)d	4.7	43	9/0	
T(1:2)e	21.7	23	2/3	
T(1:2)f	33.3	33	4/8	
T(1:2)g	30.7	163	15/0	+
T(1:2)h	29.9	117	16/13	
T(1:2)i	8.6	93	6/0	
T(1:3)a	18.2	77	15/4	
T(2:3)a	13.2	38	14/8	+

chromosomes are the physical entities of the three linkage groups, new relations between chromosomes are detected with new relations between the markers on linkage groups. Those lines showing the genetic evidence of a translocation were maintained by further backcrossing to the marker stock and culling each generation. Cytological study of the translocations was done by making aceto-lactic orcein squashes of the testes of young pupae.

RESULTS AND DISCUSSION.–Radiation dosages of 2000 rads and 3000 rads yielded translocations that could be detected with the mutant marker stock. A listing of the translocations obtained to date and their characteristics is given in Table 1. Ten are sex-linked and one is an autosomal translocation. Variable pseudolinkage between the markers for the different linkage groups indicated unique breakpoints for each of the translocations. All sex-linked translocations were isolated as *M*-linked translocations because of the nature of the scheme. Genetic cross-over females were tested in 4 lines to get the *m*-linked translocation necessary for female heterozygotes as the first step in producing homozygotes.

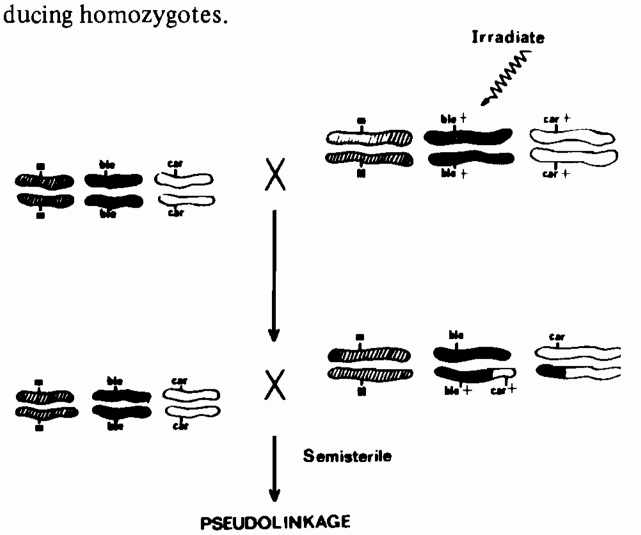

Figure 2.–Scheme for capturing a radiation-induced translocation in F_1 backcross by use of a multiple marker stock.

Male translocation progeny were used to maintain the translocation and the egg rafts fathered by the males were scored for partial sterility vs. fertility (>80 percent hatch). The data indicated a particularly great potential of the T(1:2)a translocation, because by checking markers this translocation can easily be maintained. We are now in the process of determining if this line would perpetuate the translocation from father to son without culling each generation.

Cytology has been done of the T(1:2)a translocation. The evidence for a translocation came from the presence of a chain of four chromosomes at metaphase I of meiosis, which resulted from the interchange complex of a translocation heterozygote (Figure 3).

In the near future our research will move to the area of multiple translocation heterozygotes and translocation homozygotes. When promising candidates for genetic control are produced, these will be tested in laboratory cages and ultimately at our field site at West Poso Creek near Bakersfield.

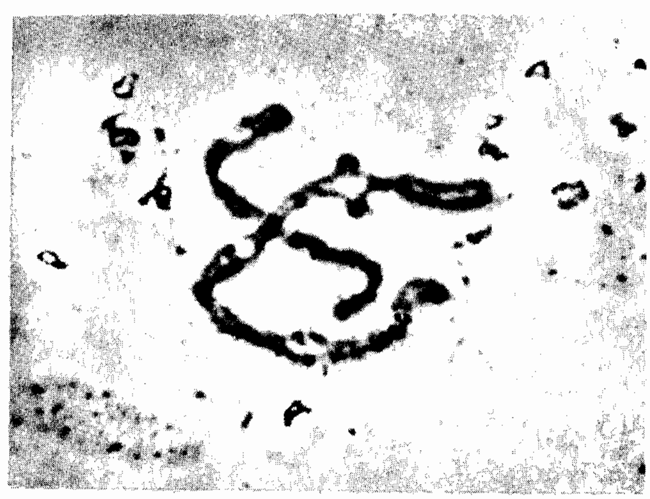

Figure 3.–Meiotic metaphase in T(1:2)a, showing a chain of four chromosomes plus a bivalent pair.

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AGRICULTURAL WATER USE AND MANAGEMENT[1]

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Twenty-four years ago, more or less, some influential mosquito control experts apparently believed that there was no significant relationship between mosquito control operations and agricultural drainage. My first appearance before the members of the California Mosquito Control Association, in 1952, was an attempt to outline the mosquito control benefits of agricultural drainage and to emphasize the need for becoming thoroughly familiar with agricultural water management practices and problems.

During those early days, we made good and rapid progress in developing information useful to mosquito abatement districts. It was relatively easy, for example, to point out the fact that most irrigated crops are damaged by water that stands long enough to allow mosquito production. We got more irrigation engineers and the American Society of Agricultural Engineers into the act. More mosquito abatement districts began emphasizing source reduction involving drainage construction and the improvement of irrigation systems. Source reduction specialists were hired who were, or became, highly knowledgeable about agricultural water management systems and practices. These things are history, and some of you know a lot more about it than I do. I am sure you are more interested in the present and in the future.

One of the most serious problems facing irrigation farmers is the high cost of skilled labor required to efficiently operate farm irrigation systems. Such skilled labor is often not available at any price. Accordingly, research engineers have been working for about the past 15 years to develop reliable automatic irrigation systems. Automatic systems have been available for several years to operate valves in sprinkler pipelines. Developing automation for gates and valves in surface irrigation systems was more difficult, but Agricultural Research Service engineers have finally achieved a system that has, for over 2 years, reliably operated large gates in open ditches and valves in large diameter concrete pipe. This system should begin to be adopted by farmers on a non-experimental basis within the next year or two. These systems will increase irrigation efficiency and reduce water waste.

Drip irrigation systems apply water to plants at a slow rate from applicators or small holes in plastic tubing laid along the crop rows. Properly designed and operated, these systems can apply irrigation water with high efficiency. Drip systems compete cost-wise with sprinkler systems in the irrigation of row crops. You should know, however, that there are problems that should be recognized before drip systems are adopted by any grower. Dr. Falih Aljibury has summarized these rather well. Because of the low application rates, daily water application must be monitored carefully, and quick adjustments in scheduling must be made to avoid water stress damage to the crop. Among the problems remaining to be solved are the day-to-day management of drip systems, the need for more reliable drip system components, reduction of clogging problems, and better procedures for applying chemicals such as herbicides and fertilizers through drip systems.

Perhaps the most important current development is public concern for environmental protection. A good example is the fate of the master drainage channel, extending from Buena Vista Lake in Kern County to Suisun Bay, that was included in the original California Water Plan. The present status of that drainage channel is briefly outlined in the 1975 report of the Westwide Study entitled "Critical Water Problems Facing the Eleven Western States," conducted jointly by a number of state and federal agencies:

> "Waste water and irrigation return flows with high concentration of nitrates and salts contribute to the salinity levels of the San Joaquin River and the Delta. The river systems and delta are presently suffering from high levels of nutrient pollution, low dissolved oxygen levels, and high levels of total dissolved solids. Original state and federal plans included the collection of returned drainage and disposition in the delta, but this could result in adverse environmental effects in the delta without adequate management practices. To date, no widely accepted plan for collection and disposition of drain water has been developed. Ponding of saline return flow is being practiced as a temporary measure and has resulted in the establishment of a substantial wildlife habitat."

At the present time, it is obvious that wildlife and preservation of the delta environment have higher priority than the San Joaquin master drainage channel. In a similar vein, we have been recently told that funds for designing the Delta Bypass Canal have now been diverted to studying alternatives to that Canal. Concern for environmental protec-

[1]Contribution from the Agricultural Research Service, U. S. Department of Agriculture.

tion includes protection from unnecessary pollutants such as smoke, odors, and noise. This developing concern can be beneficial to our programs aimed at reducing environmental mosquito pollution caused by mismanagement of agricultural water supplies.

You are undoubtedly interested in the current legal battle between the State of California and the Federal Government over legal ownership of water rights within the State. This has been a bone of contention for as long as I can remember, with acrimony and dire predictions coming from both sides. Until now, both sides have avoided a showdown, but it now appears that a final court battle may be in progress. I cannot predict the decision or the year in which that decision will be made. Knowing the endless delays our legal system permits, the odds are that the final decision is still many years away. I would also suspect that, whatever the decision may be, the results will be nowhere near as dire as the losers will have predicted.

Predicting the future is an uncertain process. Nevertheless, there are some future developments that seem reasonably certain. One of these is the increased use of vegetable protein to extend or replace meat products. Imitation sausage and bacon are now being marketed. Powdered and granulated vegetable protein extenders for ground meat have become widely available within the past year. Increased use is certain as people become familiar with them, and the price of meat continues to increase. The use of animal protein will decline. This, plus increased demand for grain and legume products in the international market, will result in conversion to other crops of many irrigated pastures now used for beef production. The attendant increase in irrigation water management efficiency will materially benefit the operations of many mosquito abatement districts.

I have commented on delay in constructing the San Joaquin Valley master drainage channel because it would cause undesirable pollution of downstream water resources. The Research Committee of the Irrigation and Drainage Division, American Society of Civil Engineers, has written a relatively comprehensive report on agricultural water management. One of the statements in that report follows:

> "The day is rapidly approaching when some irrigated regions will operate as an essentially closed system. Thus, all (or nearly all) return flows would be collected and recycled or treated. The management in such regions will be highly complex. Additional technology regarding treatment processes and the deleterious effects of poor quality water upon plant growth will be extremely important."

Most of the irrigated areas in California will ultimately operate as closed systems. Some are closed systems now, even though system design and management practices do not yet recognize that fact. Salinity and drainage problems are increasing. Long-term survival of these areas depends on recognizing the fact that these are and will be closed systems. System designs and management practices, including precise water handling control, must be developed to cope with the complex problems that will be encountered. Adoption of these systems and practices will be highly beneficial to mosquito control.

One final, safe prediction for the future is the very real effect that public concern for environmental protection will have on all our activities. This concern is being increasingly reflected in laws and regulations that not only affect future planning but are causing us to re-think plans we have already made. Recognition of that fact by state and federal agencies concerned with water management is indicated in this statement from the Westside Study:

> "While irrigation projects are and will remain important to water resources conservation and development, current trends and planning are placing greater emphasis on much broader objectives attuned to meeting a wide variety of human needs. Under the new principles and standards, environmental considerations as well as those of economic efficiency are valid objectives of water resources planning, and alternative plans to stress these differing values are required. Many existing authorized but unconstructed projects are being, or will have to be, reevaluated with these new objectives in mind."

Management of our vast public rangelands is undergoing a rapid change as the result of recent laws and court decisions. Management for cattle and sheep production is no longer the only concern. Consideration of recreation, wildlife, water quality, and even scenic values, is now mandatory in the development of public range management plans. We can expect that one of these days the spotlight of environmental concern will focus on damage created by faulty agricultural water management. Sloppy water management practices, resulting in mosquito pollution, will no longer be tolerated. I may not live to see that day, but it will come.

I have tried to touch briefly on some past history, a few things that are happening today, and some reasonably safe predictions of the future. The predictions related to reduction in irrigated pastures, development of closed irrigation systems, and the increasing effect of environmental concern. The latter is obviously the most important because it relates directly to your own objectives. You are charged with improving the environment as it relates to public comfort and health. While you do this, you can have a beneficial effect on the management of our water resources, improved agricultural production, and the economy of the area in which you work. You are potentially important allies of the agencies responsible for managing our water resources. Development of an effective alliance will take considerably more time and effort on your part, but that time and effort can pay great dividends. During this current era of increasing regulation by agencies and agents having no real experience with the things they are required to regulate, you know better than I that you need all the help you can get.

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THE LAW AS A TOOL IN PHYSICAL CONTROL

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The legal aspects of source reduction, mosquito control and related subjects, have been discussed several times. I'm sure this entire assembly has read and studied the law, and, in some cases, have used it. So, rather than burden your tired ears with worn phrases, I would like to illustrate how the law has been used to obtain the desired results in physical control.

I think perhaps the best way to do this is to acquaint you with two cases that occurred in our district over the past two seasons. However, I would first like to stress that before any legal action is initiated in our district, every effort to solve the problem by other means is expended. This includes meeting with the landowner at his convenience, even outside working hours or on weekends. The results are often worth the extra effort, as the majority will be convinced of your sincerity in helping him and the district. He realizes that you are not out to crucify him on what he may feel is the cross of legal maneuvers and, sometimes impossible, expense. However, this is not always the case!

The first case in point involved a vineyard located on the eastern border of our district. It had been a problem for sometime. The owners, a local winery, had been contacted several times in an attempt to solve the problem. The vineyard was in non-cultivation and there was no provisions for drainage in three of four eighty-acre blocks.

On August 8, 1974, a warning was issued to the owners as the first step in possible legal action. A letter accompanied the warning outlining our policy and making it clear that we wished to avoid legal action, but would take the proper steps if necessary. The warning was acknowledged, but no changes were made.

On August 21, 1974 the citation was issued requiring the owners to appear at the District office on September 3, to show cause why a service charge of twenty dollars should not be assessed against them. This is a minimum charge to cover costs of inspection, transportation, personnel, administration and conducting the hearing.

Both pieces of correspondence were sent by certified mail. Receipts were received for both, though no one appeared at the designated place and time.

A result of this was a letter to the owners from the Hearing Officer, in this case the District Manager. They were informed that the service charge was due and payable and that all future costs would be refunded.

Several days later a check in the amount of twenty dollars was received, also a call to the effect that they wished to cooperate. However, the vineyard had received it's last treatment for the season, as the water was shut off and the vineyard prepared for harvest, but the stage had been set for the following year.

Before the past season began, some ditching was done, but was totally inadequate. The owners were informed of this but they disagreed. In order to show that we still wished to cooperate, we agreed to give it a try, with the provision that if it failed to work, other methods would be employed. As was expected, there was no improvement, so *Culex tarsalis* and *Aedes nigromaculis* began to breed.

The first treatment this past season was on July 9, 1975 by aircraft, as was the following treatments. On the tenth a statement in the amount of $176.44 was sent to the owners. On July 16 a check in that amount was received. The next treatment was on July 30. A second flight was necessary on July 31. After a statement, $279.36 was received. Treatments on August 13th and 19th cost them $350.68. This made a total of $806.48. Obviously, they felt it was less expensive to defray the costs of service than to hire personnel to do what was necessary. About this time water was greatly reduced, due in part to lessened irrigation requirements. Naturally, this was the time they chose to call, complaining that while they wished to do their part they felt that they were paying for other people's problems. They were informed that this was not the case as their neighbors were also paying taxes but were not allowing mosquitoes to breed. Also they were informed that the costs were for the vineyard alone and not the surrounding areas, whose problems were caused by them.

They now have requested that they be allowed to meet with our board to "work things" out. They have been told the Board's meeting schedule and that they were welcome to appear at any meeting. To date they have not done so.

Chapter 5, Section 2270, paragraph K of the Health and Safety Code states that a District Board may assess civil penalties as determined by the discretion of the Board, but not to exceed $500. per day for each day that a notice or hearing order to abate a nuisance has not been complied with. This has been useful to us.We have used it several times, always with success. We have not assessed any daily penalties, but passing on the knowledge that we are able to do so has served our purpose.

The law was used again, this past season as a quick solution, to a real problem that existed outside, but adjacent to, the district.

Immediately across the road from a permanent pasture, residents inside the district were being severely bitten by *Aedes nigromaculis*. The pasture was checked and samples taken. The owner was contacted and a meeting was arranged at the site. I had hoped to discuss the problem with him and to come to some understanding. He arrived at the meeting with reinforcements in the form of his family and his foreman.

Just prior to the meeting he had looked at several areas within the district and immediately became quite vocal to the effect that the district was not caring for it's own problems. He thought we were harassing him because he was instrumental in an annexation being denied several years before. Tempers became short at this point, so I informed him that I was not there to defend the district, but to solve this particular problem and that I would do so by whatever means necessary. The problem source was low areas and a mile of ditchline that was so overgrown with weeds and algae that *Gambusia* could not function.

Immediately after returning to the office, County Counsel was contacted and a restraining order was obtained. It specified that the areas would be corrected in a manner satisfactory to the District and in a time set by the District.

I delivered it to him for his signature and to establish a time limit. He requested 30 days to complete the project. I allowed 45, to show that we would cooperate but would also get the job accomplished.

I feel sure that he had contacted legal counsel, as approximately half the ditch was completed and work was in progress at that time.

While I definitely do not believe that legal procedures are the best, or only way, they certainly are an excellent tool when used properly and wisely. After all other measures are exhausted, if the steps are taken in proper sequence, we have found them to be effective. I'm sure that while there are districts that do not agree with this policy, it is ours and we will continue to use it when necessary.

A REFORMATION OF THE CALIFORNIA MOSQUITO ABATEMENT PROGRAM

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A review of the past 27 years produces the conclusion that very little change has actually occurred in our overall California mosquito abatement program during this period. It appears that our emphasis has been misdirected through employing too many scientists, who have tried too hard to devise more complicated ways to continue killing mosquitoes with repetitive sprays, rather than to seek a logical alternative.

While it is true that we are engaged in an entomological endeavor, it nonetheless appears that we have failed to recognize that it is NOT the mosquito that should be our prime target, NOR the water that causes the mosquito, but rather the PEOPLE who cause the water that causes the mosquitoes.

During these 27 years we have worn out numerous pesticides as we sprayed the same mosquito breeding sources until we encountered resistance time and again; yet we have not realistically analyzed our plight and changed our course. In alarm we concluded that public education was the only long-range solution, meanwhile disregarding the fact that our records of mosquito breeding contained the names of the people and locations of the areas responsible for all our woes.

Regrettably, we overlooked (or ignored) the only key to a successful alternative program based on direct education of the very people who are, and who have been, producing the mosquitoes. This direct education program includes the necessary clarification and allocation of responsibility for causing a public nuisance, consistent with the State Health and Safety Code.

With this alternative approach, a foundation is prepared for a reformation of the California mosquito abatement program, and the creation of a statewide concept of mosquito prevention. A new era of communications and cooperative assistance can and will replace the time-worn procedure of our past, which made varied claims of accomplishment, and included such grandiose terms as "balanced", "integrated", and "comprehensive", but which actually have been the cloak for what has been essentially control and more control.

With a California mosquito abatement program dedicated to mosquito prevention, we can collectively fill a more meaningful role in this environmental age. Instead of our prime effort being directed at pesticide pollution of waste water, we can accomplish our purpose and make a major contribution toward the conservation of water previously of concern solely because it produced mosquitoes. Through the elimination or reduction of mosquito breeding sources, we will be effectively aiding in future economics, since the result will provide more available land for the land-owner and reduced pesticide costs for our agencies.

In summary, it is suggested that we broaden our scope and align our public service effort in a direction which provides constructive assistance and worthwhile environmental change for our constituents, rather than continuing a desperation program of mosquito killing, which involves greater costs, at increasing odds against continued success.

For the skeptical, it should be pointed out that a legal abatement program is not being suggested. Rather, it should be recognized that we will be involved in a direct education function which is supported by principles consistent with good agriculture, proper waste disposal, and the desired environment. Our law should be used as an educational assist: meanwhile, it should be apparent that there is a major sales job to perform as we apply common sense with a common tongue in meeting with and assisting people to resolve their problems and assume their (not our) responsibility for proper water usage.

As previously stated, our procedure for tomorrow is based on effective communications (both written and verbal) and the object is to reach agreement with each and every mosquito producer relative to water management and/or accomplishing such corrective work as may be necessary to prevent future mosquitoes.

Try it . . . . . . you'll like it . . . . and it works!

AB 2862, WILL IT DO THE JOB?

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ABSTRACT

Mosquito control has changed remarkably in California's Central Valley in the last few years. Most of the changes are directly attributable to the gradual failure of pesticides to control mosquitoes. Source reduction programs tied to a strong legal abatement procedure have been developed by many mosquito abatement districts to replace the chemical control programs that are proving less effective each year.

Pest abatement districts engaged in mosquito control have been unable to develop a strong legal abatement procedure because of a lack of statutory authority.

The Eastside Pest Abatement District developed a progressive source reduction program in 1973. It was apparent, however, that mosquito resistance to pesticides was progressing with greater speed than was the source reduction program in eliminating sources. It was felt that a legal abatement procedure was necessary to facilitate the source reduction program.

Examination of the Health and Safety Code revealed that the legal abatement powers of a pest abatement district were implied, but not specified. (State of California Health and Safety Code, 1969). Due to the fact that pest abatement districts did not have strongly stated legal powers, it was necessary to seek a change in specific sections of the Health and Safety Code pertaining to a pest abatement district.

The desired changes to the Health and Safety Code were determined. Assemblyman John E. Thurman (27th District) was contacted; the changes were drafted into AB 2862. Resistance to passage of the bill was minimal. It was signed into law on July 11, 1974.

The passage of the new law has enabled Eastside to strengthen and facilitate its source reduction program. However, it is felt that the law lacks "absolute" powers to prevent mosquito production, and that it may be necessary in the future to ask the State for stronger legislative power.

RESISTANCE IS A STIMULANT TO REDUCING SOURCES

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Mosquito control over the last 30 years has been heavily committed to chemical control and rightly so. How else could the public have been provided with the relief that they voted for when they accepted and agreed to pay for mosquito control.

For this reason any district that attempts to reverse directions and de-emphasize the use of chemicals in their program needs a strong justification to make this change.

What better reason could any district have than resistance. Its the best thing that has happened to mosquito control since DDT, if you still consider DDT very great in 1976. In 1943 it was fantastic and even now in some areas in the world it is the only way to control the malaria vector, but here again resistance is wiping out the tremendous progress made in the control of malaria all over the world. If major changes aren't made soon, the clock will be turned back to 30 years ago when misery and suffering from mosquito-borne disease was common in many countries.

With a cheap, effective insecticide available, the only incentive to reduce mosquito sources was to save water and increase crop production. This was the approach I used over the last 20 years and it had been very successful with the more progressive farmers but had little appeal to the large mosquito producer who usually was not very progressive. He could have been farming alkali ground, usually had poorly levelled pasture, didn't believe in drainage and had little financing with which to make improvements.

Resistance has been the key to a complete changeover in policy and program in the Delta VCD. Resistance we have now, nobody can argue with that fact; it is real and it won't go away. We can't go back to our old program of spray, spray, spray. New materials like Altosid or certain larvicidal oils are too expensive for this and the timing involved in their use is too precise to be permissive in their application.

We still work cooperatively with the more progressive grower but we take a much stronger approach with the large mosquito producer. We expect him to reduce the problem to a size that we can control, by relevelling the land, by drainage and/or a return system or by better water management. Most of these large mosquito problems can be vastly improved by one or all of these suggestions.

If the grower agrees to follow one or more of the preceding suggestions, and then doesn't live up to the committment he has made within a reasonable time limit, we make him aware of the fact that our Board of Trustees expects results from our contacts or the grower will be invited, and I do mean invited – not forced, to a board meeting to discuss the problem and potential solutions. If this doesn't stimulate action and the invitation is not accepted, a legal citation is issued and the grower is charged for spray work on his property. Since bringing in a group of 32 growers to discuss their problems with the board, under legal citations, our contacts have been much more productive: the grapevine among growers is fast and effective. Once it is accepted that certain cultural practices which were common in the past are not acceptable in this day and age, we have a good strong base to operate from. The holdouts and the hardheads get little sympathy from the growers who have already moved on to a more progressive type of farming.

Of course this program does not develop quickly. We have been actively pursuing this new policy and program for 5 years and I feel we have been very successful.

The success that we have achieved is due to the complete commitment of all concerned; starting with the Board of Trustees. They must have complete confidence in the integrity and competence of the staff and be willing to commit themselves to the new policy and program, for if they don't stand 100% behind the staff there is no program.

The responsibility for the production of mosquitoes is directly on the shoulders of the growers and it is not the responsibility of the district to take up this burden by spraying, but rather to stimulate and help the grower to find ways not to produce mosquitoes.

The operators also have a critical role to play. They must keep the grower constantly aware of the problem without causing any more friction than is necessary. This is not an easy job and there are bound to be personality clashes. Some operators will do a fine job and others will be very poor. This is to be expected and planned for.

In 1969 we sprayed 144,000 acres. This includes air and ground spray for larvae and adults. This last year, 1975, we sprayed 4,772 acres. If anything, we had better control in 1975 than we did in 1969.

I won't insult your intelligence by claiming we accomplished all of this with our new approach but we certainly want some of the credit for this 90% or better reduction in acreage sprayed while achieving what we consider to be excellent control.

EVOLUTION OF CHEMICAL CONTROL STRATEGIES FOR MOSQUITOES CONVENTIONAL, NOVEL AND NATURAL CHEMICALS

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It is indeed an honor and pleasure to be asked to discuss with you today the "Evolution of Chemical Control Strategies for Mosquitoes." Scientists and researchers within and outside the University of California have made significant contributions to this area of research. I will, therefore, be drawing upon the efforts and accomplishments of many colleagues and workers who have pioneered in the field of mosquito control programs. Since this field is so vast, it is inevitable that some studies and programs will be overlooked by necessity and not intentionally. Because I have been with the University of California for 20 years and have served mosquito abatement districts for this period, our moderator thought that I should render this presentation, even though there are others amply qualified to do so.

INTRODUCTION.–In a treatment of this subject, "chemical control agents" are taken in the broadest context including natural products, autoregulating and transspecific chemicals and finally processed or synthetic inorganic and organic chemicals. To advance a meaningful discussion of this topic, it should be understood that chemical control agents play an important role in an integrated management of mosquito populations, their effectiveness governed by proper and judicious use when combined with source reduction, biotic control agents and legal and preventive measures. Used in this manner, chemical control agents have and will offer unique and promising opportunities for the suppression of many species of mosquitoes in diverse habitats. The list of successes of chemical control programs is so vast that neither time nor space permits full enumeration and discussion here. In the past decade, the negative aspects of chemical control programs have been focused upon more extensively and a great deal of erroneous interpretations and misguided notions have been incorporated into recent literature. In the last address entitled "Looking Back Half a Century for Guidance and Conducting Mosquito Control Operations," Professor William B. Herms in 1949 (Herms 1949) advised members of CMCA to look for specific Culicides that can be employed in mosquito control, along with other techniques. A year later, Arthur F. Geib (Geib 1950) stated that chemicals with their inherent problems are indispensable for mosquito control. These assertions hold true even today.

In discussing the evolution of chemical control technology for mosquitoes, I would like to briefly cover the period of recorded human history and then focus on the development and implementation of our modern day chemical control strategies. As we go from ancient times to the modern era, we will see a tremendous diversification in the development of chemical control techniques, accompanied with outstanding increase in their effectiveness, and last but not least, decrease in cost of mosquito control technology when adjusted for the rising cost of other goods and services.

The evolution of research on chemical control strategies is governed by the availability and use pattern of chemical control agents and the general trend in other areas of scientific research. Some of the major factors which stimulate continued research on the development and application of mosquito control chemicals are:

1. Biological Activity. The effectiveness of chemical control agents against mosquitoes is an important consideration in the evaluation, selection and development of mosquito larvicides and adulticides.
2. Specificity. Researchers and users have always sought after chemicals which possess narrow spectrum activity, inducing mortality or response in target species only. In this context, the economic benefits have to be carefully weighed against specificity, as highly specific chemicals by necessity are more costly.
3. Acquired Resistance. This feature of the biological species is probably the most important consideration in the development of substitute materials and alternate vector control technologies.
4. Safety. The extent of toxicity and hazards of chemicals to man and his domestic animals determines the use pattern of chemical control agents under various conditions. Chemicals having high toxicity with LD_{50} of 5-20 mg/kg, although developed and used during the past 30 years, are no longer considered desirable substitutes for current mosquito control agents.
5. Hazards to Nontargets. Harmful effects of chemical control agents on nontarget organisms and wildlife have been the subject of much research and discussion within the past 2 decades. Researchers and personnel dedicated to effective mosquito control have given and always will give due consideration to this aspect in the development of chemical control agents.
6. Environmental Impact. Recently, this has become a magic expression, leading to confusion in the area of research as well as operational vector control programs. The objectives of EI studies are not well defined and the ecological implications are not recognized by many of those who play a role in decision-making process.

   Researchers and mosquito control personnel have been aware of the side effects of chemical control agents for decades and have given due consideration to environmental quality in the development and selection of Culicides.
7. Economics. Cost of chemicals plays an important role in the selection of pesticides in general and mosquitocides in particular. Very costly materials are not generally developed for pest control measures. In recent years, the cost of developmental research to facilitate registration of a chemical has skyrocketted, which renders the cost of substitute materials much higher than the one they replace. Notwithstanding this, the cost of chemical control

agents has surprisingly remained low, rising no more than the inflationary rise of materials and services in most situations.

HISTORICAL.—From the dawn of civilization, a variety of pests and noxious arthropods has been associated with man, taking a toll of his crops, stored foods, household goods and many even feeding on man himself and transmitting pathogenic organisms, spreading pestilential diseases in the process. To protect health and crops, the ancient societies resorted to a variety of religious practices, folk magic and some crude cultural, biological and chemical control strategies (Smith and Secoy 1975). The earliest written records dealing with pest and vector control are those of the Greek writers such as Democritus, Xenophon and Theophratus (4-5th century B.C.), and the Roman writers such as Cato, Varo, Virgil (these 3 writers lived between 17-234 B.C.), Pliny, Columella and Palladius (these 3 writers lived in 1-4 century A.C.). Unfortunately, no adequate records of pest control methodologies are avilable for the more ancient civilizations in China, Egypt and Mesopotamia.

The Geoponika (XIII, 10, 14) or agricultural pursuits mentions the remedy for bugs and ants to consist of a mixture of sulfur with oil or origanum. The same volume provides information on poison baits used for killing flies. The most effective bait used by the early Romans for flies consisted of an infusion of bay and black hellebore (powder from roots and rhizomes of *Helleborus* herb containing alkaloids) in milk or sweet wine. When hellebore and arsenic were macerated in milk, the mixture proved fatal to flies (Smith and Secoy 1975). Some 2000 years later, we are still using protein hydrolysates (of plant and animal origin) and stale beer as attractive baits for housefly control.

The art of fumigation was also employed by the early societies. For examples, gnats (probably Mycetophilidae) infesting gardens could be repelled by the fumes of burning galbanum resin (Pliny, XIX, 58) obtained from several species of Asiatic plants. Locusts and ants were killed or kept away by the odors from the burning of their own kind. Smoke and odors from burning of animal dung, bones, horns, ivory, garlic, cedar gum and other plants and roots (Geoponika XIII) were useful techniques against a variety of insect pests (Smith and Secoy 1975). These crude but time-tested pest control measures were practiced by man until the mid 19th century when the new era of scientific pest control was initiated.

In mid 19th century, some of the techniques recommended for housefly control indoors was the habit of settlers to hang hornet nests inside their homes to get rid of flies. This indeed is not a hearsay or wild imagination, because B. D. Walsh, an American Entomologist in 1869, wrote that the pioneers capitalized on the work of wasps in driving away noxious flies (Teale 1962).

MODERN TECHNOLOGY.—Systematic experimental research on vector control, especially that of mosquitoes, did not take hold until the turn of the century when the pioneer entomologist L. O. Howard observed larvicidal activity of kerosene spread over water harboring larvae (Howard 1892). From that earlier discovery to the present time, petroleum hydrocarbons have been researched extensively for use against preimaginal mosquitoes, and these natural products have played an important role in mosquito control programs.

PETROLEUM OILS.—Research on petroleum hydrocarbon larvicides was intensified after World War I (Freeborn and Atsatt 1918, Gray and Bent 1938, Herms and Gray 1944, Powers and Headlee 1939, Stage 1952), and again during the 1960's when widespread resistance in 2 species of mosquitoes against organophosphate insecticides became prevalent. The major thrust of the investigations dealt with the mode of action of larvicidal oils, increasing their biological activity through additives such as surfactants, spreading agents and toxic substances or finding biologically active fractions (Darwazeh and Ramke 1972, Hagstrum and Mulla 1968, Micks et al. 1967, 1968, Mulla and Chaudhury 1968, Mulla and Darwazeh 1971, Murray 1936, Stage 1952). The outcome of these investigations has been the development of more potent formulations, thus decreasing the volume of oils used per acre from 8-20 gal/acre (Herms and Gray 1944), or as much as 50 gal/acre (Mulhern 1968) to about 1-4 gal/acre (Darwazeh and Ramke 1972, Mulla and Darwazeh 1971). The cost of material and labor for larvicidal oils in 1930's ranged from $2/acre for special oils (using 6 gal/acre), $2.80/acre for diesel oil (using 9 gal/acre) to $6.85/acre for lubricating crankcase oils (using 21 gal/acre as reported by Herms and Gray (1944). The cost of mosquito control with larvicidal oils is no more per acre now than it was some 40 years ago. Although the cost of oils, per unit-volume, has increased 5 to 10 fold in this period, the decrease in cost of application and reduction in dosage has compensated for cost of materials. Were it not for continuous research to find more effective and economical substitute oil formulations, the cost of petroleum oil larvicides would be beyond the reach of many districts.

Accurate records for the use of larvicidal oils in California prior to 1950 are not available. With the compilation of information on the use of mosquito control chemicals in California, reasonably good, although not complete data, have been reported in the Year Books published by the California Mosquito Control Association. A steady increase in the use of petroleum hydrocarbon larvicides has been recorded during the 2-decade period of 1954-74, where 100% increase in larvicidal oils (from 300 to 600 thousand gallons annually) has occurred. The larvicidal oil formulations currently employed in mosquito control are more active than earlier formulations, therefore the acreage now treated with oils is much more than in the 50's. It should be noted that part of the increase is due to the formulation of additional mosquito abatement agencies in California, providing mosquito control service to additional areas of the state. A decrease in the use of petroleum oils after 1965 (despite increased coverage of additional areas for mosquito control) is mostly due to the development of more effective formulations of larvicidal oils (Hagstrom and Mulla 1968, Micks et al. 1967, 1969, Mulla and Darwazeh 1971, Mulla et al. 1971) and the resultant decrease in rate of oils used per unit area (Table 1).

Future research programs on the development of larvicidal oils will primarily deal with increasing the biological activity of cheaper fractions of oils. As the chemical composition of oils available for larviciding varies from area to area and time of extraction, continuous research on oil formulations has to be expedited.

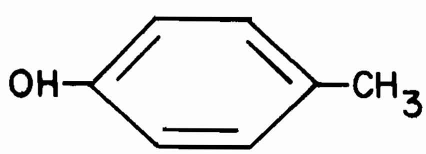
Looking to the future, it is very likely that the total volume of oils used in California for mosquito control will

Table 1.—Approximate relative cost and rates of application of mosquito control agents during the past 5 decades.

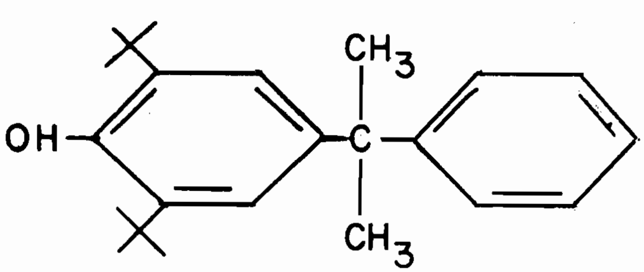
		Dosage/Acre		Cost $	
Material	Period	Gals	lbs	Material labor	Material
Special oils	1920-40	6	-	2.00	-
Diesel oils	1920-40	9	-	2.80	-
Lube oils	1920-40	21	-	6.85	-
FLIT MLO	1968-76	2 - 4	-	-	1 - 3
Cresylic acid	1925-40	4	-	-	2.25
Paris green	1920-40	-	.5 - 1.0	-	2 - 4

remain below 1 million gallons per year. This prediction is prompted by the fact that the efficacy of larvicidal oils can be further increased and that highly specific and effective compounds are yet to be developed for mosquito control.

OTHER LARVICIDES. —Soon after World War I, 2 additional mosquito larvicides were developed and employed by control agencies. High boiling fractions of crude cresylic acid or carbolic acid (a mixture of cresols from wood tar, also containing phenol) when mixed with soap or other surfactants and caustic soda, could be diluted with water and sprayed onto mosquito breeding sources. This was known as Panama larvicide (Herms and Gray 1944). In 1939, this material cost about $.45/gal, the rate of application being 5 gal/acre, thus costing $2.25/acre for material. This material was reported to be highly effective against larvae of tree-hole mosquitoes (Herms and Gray 1944). Recently, a relative of these cresols was tested as an IGR and it was coded as MON 585.



Cresol (Cresylic acid)



MON 585
IGR

Paris green (copper acetoarsenite) also joined the ranks of the other larvicides in 1920. Research showed that Paris green, diluted with moist sand and other diluents, proved effective against *Anopheles*, *Culex* and *Aedes* species (Cook 1931, Cushing 1957, Griffith 1927). However, this larvicide did not find widespread use in California as it did elsewhere in the country and overseas. The dosage of this compound necessary for effective mosquito control, ranged from 0.5-1 lb/acre, costing more than competitive available larvicides.

Pyrethrum extracts and phenothiazine were also used as larvicides in eastern United States, but very little, if any, in California. Phenothiazine was reported to be especially effective against container breeding mosquitoes such as *Aedes aegypti*. Borax (sodium borate) and nicotine sulfate (Black Leaf 40) were used as mosquito larvicides but with little success. These 4 larvicides (pyrethrins, phenothiazine, borax and nicotine) were either unstable or were needed in high dosages.

ORGANOCHLORINES (OC). —During World War II, the advent of DDT enhanced the effectiveness of mosquito control programs on a global basis. Its high larvicidal and adulticidal activity was reported by several workers (Bohart and Murray 1950, Hoskins 1948, Lindquist and Yates 1950, Metcalf et al. 1945, Yates 1948 and others). The biological activity of DDT was very high, its LC_{90} ranging between 4-6 ppb against larvae of various species of mosquitoes. DDT was instituted almost as the sole mosquito control agent in California in 1945. Its appearance sparked the institution of many mosquito control programs in California and elsewhere, and it was one of the main reasons for the organization of additional mosquito abatement districts in California.

Concern over the residue, magnification and ecological implications of DDT were voiced after a 3-year use of this material, before the annual CMCA meetings in 1948 and 1949. Both Hoskins (1948) and Herms (1949) warned of the consequences of relying on one material and limiting research to only one compound or its relatives.

The new era of chemical control of mosquitoes was thus initiated. Spectacular results were obtained with DDT. Although resistance to DDT in some mosquitoes appeared fast, this material nevertheless was employed for mosquito control in California for almost 2 decades, its peak usage occurring during the early and mid 50's.

With the appearance of acquired resistance, research on substitute materials was expedited by the U.S. Dept. of Agriculture, State Dept. of Public Health and University of California and others. The first group of effective substitutes were those related to DDT, such as methoxychlor (LC_{50} of 2-4 ppb) against various species of mosquitoes), TDE or DDD (activity similar to DDT) and others (Deonier et al. 1946, Prill et al. 1946, Yates 1948).

The high usage of OC insecticides continued into the early 60's, their use becoming greatly reduced by 1965 and coming to almost nil by 1970 (Table 2). Perthane, a relative of DDT, although used in small amounts, outlasted DDT, but it also disappeared after 1970.

Two factors accounted for continued research to find specific and desirable OC substitutes. Development of resistance in mosquitoes and biological magnification for the most part accounted for OC replacement with other materials. Occurrence of residues in forage crops also contributed to this. Surprisingly, methoxychlor, which does not have these undesirable properties, did not find a place in mosquito control programs. It is possible that its relatively high cost played a part in this.

Table 2.–Use of organochlorine insecticides by California mosquito abatement agencies for larvicidal and adulticidal operations during 1954-74 (10 years selected)[1].

Year	No. Reporting/Total Districts	Amount (pounds X1000) active ingredient DDT	DDD	Perth-Tox[2]	Total
1954	33/49	176	54[3]	37	267
1955	43/50	210	19	16	245
1958	45/53	186[4]	5	130	321
1960	50/55	72	26	14	112
1961	46/54	51[4]	0	35	86
1962	50/54	44	0	31	75
1965	52/60	7	0	20	27
1968	54/60	<1	0	10	<11
1970	53/60	0	0	2	2
1972	60/60	0	0	0	0

[1]California Mosquito Control Association Year Books. Rounded to the nearest 1000.

[2]Over 80% constituted perthane, 15% toxaphene and the remaining were: lindane, BHC, aldrin, dieldrin, chlordane and heptachlor.

[3]Last time treatment made with this material against *Chaoborus* in Clear Lake.

[4]Additionally, about 200 and 300 thousand pounds, respectively, were used for eye gnat control in the Coachella Valley.

ORGANOPHOSPHATES (OP).– Research on the development of substitute materials was enhanced during the early 50's. Several researchers and workers, well-known to members of this Association, played a role in the progress of chemical mosquito control technology in this state. Although time and space do not permit giving credit to all the workers, a few of those involved in active research and institutions of programs were:

C. M. Gjullin, U. S. Department of Agriculture
Art W. Linquist, U. S. Department of Agriculture
Richard F. Peters, Calif. Dept. of Public Health
Lawrence L. Lewallen, Calif. Dept. of Public Health
W. Donald Murray, California Mosquito Control Assoc.
Arthur F. Geib, California Mosquito Control Assoc.
Gordon F. Smith, California Mosquito Control Assoc.
Lewis W. Isaak, California Mosquito Control Assoc.
William M. Hoskins, University of California, Berkeley
Richard M. Bohart, University of California, Davis
Robert L. Metcalf, University of California, Riverside

In the late 50's, the bulk of the chemical control research was conducted by the U. S. Dept. of Agriculture, California Dept. of Public Health and University of California, Riverside (Robert L. Metcalf and Mir S. Mulla). By this time, OP insecticides were discovered and developed at a rapid rate for the control of all kinds of pest and vector insects. A very large number of promising OPs were screened and evaluated during the next decade against mosquitoes (Isaak 1953, Lindquist 1953, Mulla et al. 1960, 1961, 1962, 1964, 1966, Mulla 1967). Most of the effective OPs showed activity in the range of 2-50 ppb, between the ranges of activity of parathion and malathion. Today, if necessary, a substantial number of OPs from this list of already tested compounds can be developed.

The use of malathion and parathion, starting in the early 50's, continued into the 70's, but their use started to dwindle when the effectiveness of fenthion was established (Mulla et al. 1960) and this material became operational in 1962 (Table 3). The use of malathion and parathion reached a peak in 1960-62 and from then on it started to decline, with only small quantities of these 2 materials being employed after 1972. The most effective substitutes for malathion and parathion in the late 60's were fenthion and highly effective formulations of petroleum hydrocarbons. Additionally, the OP chlorpyrifos, highly active against mosquitoes, also joined the ranks of effective chemical mosquito control agents (Mulla 1967). However, this material has not found significant use in California mosquito control programs. A maximum of 5-8 thousand pounds has been used to date annually.

ORGANOCARBAMATES.–Organocarbamates have not displayed a prominent role in California Mosquito Control programs. Although some effective carbamates have been studied (Georghiou and Metcalf 1961), very few are used in mosquito control programs. In the late 60's, it appeared that the carbamate RE-11775 with high larvicidal activity (Schaefer and Wilder 1970) might control OP resistant mosquitoes. However, this material, for various reasons, was dropped from further development. One carbamate insecticide, propoxur (Baygon), having high adulticidal activity (Georghiou and Metcalf 1961, Mulla et al. 1973a, Sjogren et al. 1973), has been used since 1968-69, but its usage has been less than 11,000 pounds in any given year.

ALIPHATIC AMINES.– During the late 60's, in conjunction with oils, the biological activity of aliphatic amines was established against preimaginal mosquitoes (Mulla 1967, Mulla and Chaudhury 1968, Mulla et al. 1970). They were found to kill the larvae as well as pupae, the younger larvae being more susceptible. Since then, these materials have been used as additives to petroleum oil larvicides (0.5-3%) for the past decade or so. Recently, concentrate formulations of these amines have been developed, which could be

Table 3.–Use of organophosphorus insecticides by California mosquito abatement agencies for larvicidal and adulticidal operations during 1954-74 (10 years selected)[1].

Year	Districts reporting	Amount (pounds X1000) active ingredient[2] Mala	Para (Eth)	Para (Meth)	Fenth	Other[3]	Total
1954	33/49	23	16	0	0	3	42
1955	43/50	51	34	0	0	<1	86
1958	45/53	107	74	0	0	3	184
1960	50/55	200	120	<1	1	7	329
1961	46/54	150	116	0	2	<1	269
1962	50/54	134	142	0	4	1	281
1965	52/60	106	107	0	40	1	254
1968	54/60	16	38	47	96	11	208
1970	53/60	10	72	28	110	10	230
1972	60/60	11	19	12	51	7	100
1974	50/60	10	15	9	51	10	95

[1]Source: California Mosquito Control Association Year Books.

[2]Rounded to the nearest 1000.

[3]Includes Metacide, naled, dichlorvos, Abate®, EPN and chlorpyrifos.

Table 4.–Relative effectiveness of various synthetic insecticides against mosquito larvae in California.

Material	Lab activity ppb	Effective rate[1] lbs/A
DDT	3 - 6	0.5 - 1.0
Malathion	20 - 50	0.5 - 1.0
Parathion	2 - 4	0.05 -0.1
Fenthion	3 - 5	0.05 -0.1
Chlorpyrifos	1 - 3	0.02 - 0.05
Abate	2 - 4	0.05 -0.1
Methoprene	1 - 4 Ae.	0.05 - 0.25
Dimilin	1 - 4	0.001 - 0.025
Petroleum oils	20 - 50 ppm	1 - 5 gals
Aliphatic Amines	1 - 3 ppm	0.5 - 1
Overcrowding Factors	0.5-1ppm	-

[1]These rates are not necessarily registered rates.

diluted with water and applied to non-vegetated breeding sources of mosquitoes (Mulla and Darwazeh 1975, 1976). The aliphatic amines provide relatively cheap and available preimagocides which can be employed in nonagricultural breeding sources. Further research on their formulations is needed.

INSECT GROWTH REGULATORS. Research on chemical control agents against mosquitoes emphasized the development of specific, less hazardous and novel types of compounds. In the late 60's, as the spectrum of mosquito resistance to OPs broadened, compounds with novel modes of action were identified, evaluated and put into operational programs. Evaluation of compounds possessing growth modifying characteristics offered promising potential for mosquito control in California. Research on these new chemical control agents was rapidly advanced at U.C. Fresno Lab and at U.C. Riverside (Mulla and Darwazeh 1975, Mulla et al. 1974, 1975b, Schaefer and Wilder 1972, 1973) and elsewhere. As a result, the first IGR was developed and put into operational programs with great success in 1974 when 1000 pounds of the IGR methoprene were used in California (Table 4).

OVERCROWDING FACTORS OF MOSQUITOES.–The use of natural products for pest control has captivated the imagination of man for many centuries. However, it has been only during the past decade that sophisticated technology for the isolation and identification of auto- and transspecific regulating chemicals has become available. This technology made it possible for us to isolate and identify chemicals produced by mosquito larvae under overcrowded conditions, and these chemicals play an important role in the self-regulation of larval populations of mosquitoes (Ikeshoji and Mulla 1970 a, b, 1974 a,b). These compounds and their analogues have availed candidates which possess biological activity much greater than that of petroleum oils or aliphatic amines (Hwang et al. 1974, Hwang and Mulla 1976). The ecological and practical significance of overcrowding factors of mosquitoes (OCF) have been fully reviewed elsewhere (Hwang and Mulla 1976).

SYNTHETIC PYRETHROIDS.–Recently, some highly active synthetic pyrethroids have been evaluated as mosquito larvicides and adulticides. It has been only in the last 2-3 years that pyrethroids were studied extensively (Mulla et al. 1973b) and the synthetic pyrethroid FMC-33297 was found to control mosquito larvae at rates as low as 0.025 lb /acre under field conditoins (Mulla et al. 1975a). Other synthetic pyrethroids are now being studied for efficacy as mosquito control agents. These novel compounds will very likely include condidates with high efficacy against larvae and adults.

ADULTICIDES .–As resistance to conventional synthetic larvicides increased, adulticidal programs became more popular in California. Here again, research came up with the development of some effective adulticidal compounds such as synthetic pyrethroids and propoxur (Mulla et al. 1972, 1973a, Sjogren et al. 1973). Among the newer adulticidal compounds, propoxur was added in 1970 to the list of already available compounds such as dichlorvos, naled, malathion and chlorpyrifos. Propoxur has been used in the amount of 6-11 thousand pounds per year (1970-74) and chlorpyrifos in the amount of 5-8 thousand pounds during the same period. Although chlorpyrifos has been on the scene for some 8 years, it has not found a significant market as yet in California mosquito control programs.

SUMMARY. The evolution of chemical control strategies in California over the past half a century has been in the right direction. Cost of materials and application for mosquito control has been lowered, held low or risen no more than the inflationary trends of the economy. This has been made possible through continuous research on the development of integrated management of mosquito populations by any and all practical and available means.

Looking over the past 5-6 decades, we have seen a tremendous reduction in the volume of petroleum oil larvicides applied per unit area. Where 20-50 gallons per acre were employed a few decades ago, the rate of application is now reduced to 1-5 gal/acre. The diversity of mosquito control agents has been greatly enhanced. A few decades ago, only 2 or 3 chemical control agents were at hand, but today the choice is much better. A selection can be made from some 3 dozen compounds or formulations (Table 4).

Most of the mosquito control agents in operational use today possess greater margins of safety to man and beneficial life than those materials which were in use 2 or 3 decades ago. This has been achieved through the development of some effective and specific mosquito control agents and the availability of compounds having novel modes of action.

Through the effort of academic and industrial research, highly specific and effective mosquito control agents have been developed and put at the disposal of mosquito control personnel (Table 4).

Through sustained and systematic research, we can very likely in the future produce a safe, economically feasible and environmentally acceptable mosquito control technology. The research has to be balanced, encompassing all aspects of mosquito biology, ecology and management. It is only through such a comprehensive research program that the use of pesticides for mosquito control has been greatly reduced over the past few years in California. As alternate control methods are developed, and as more effective and safe mosquito control agents are discovered, the decline of less desirable pesticides in mosquito control programs will undoubtedly result.

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EFFECTS OF NONTHERMAL AEROSOL APPLICATIONS OF CHLORPYRIFOS ON NON-TARGET ARTHROPODS, AND PEOPLE IN AN URBAN AREA

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INTRODUCTION.–During the past four years, experimental nonthermal applications of various insecticides have been made in several of the mosquito abatement districts of California. Some were in urban situations and others in rural areas. Success as measured by mortality in caged mosquitoes exposed during the tests and by public reaction has generally been excellent provided certain conditions of weather, swath width, discharge rate, and dosage rate were met. Equally spectacular failures have occurred when some or all of the conditions were quite not right. These experiments have been previously reported by Whitesell (1973), Womeldorf et al. (1973), and Townzen & Whitesell (1975).

During the summer of 1975, the California Department of Health, the University of California at Davis, Departments of Entomology and Agricultural Engineering, the California Department of Food and Agriculture and the Colusa Mosquito Abatement District cooperated in an additional series of three nonthermal chlorpyrifos applications in the village of Colusa. The experiments were planned to provide information on three specific questions. These were:

1. What level of control could be achieved on the population of wild mosquitoes in town?
2. What effect do nonthermal insecticide applications have on non-target species in the area?
3. What effect do the applications have on blood cholinesterase levels in personnel directly involved in the application or in other individuals exposed to the aerosol?

OPERATIONAL PROCEDURES.– The three applications evaluated here were made on June 30, August 19, and September 9, 1975. Two nonthermal aerosol units constructed by the Colusa MAD and one from the Glenn County MAD were used in the tests. All were powered by four cylinder gasoline engines direct coupled to a positive displacement air pump. The insecticide tanks were pressurized to 4.5 psi with air bled from the blower. Commercially available air shear nozzles (Afa 6208-208A) were modified by reducing the liquid orifice to 1/32-inch with brass tubing inserts. The machines were mounted on ½-ton pickup trucks.

Immediately prior to each test a calibration check was run on each machine. On tests 1 and 2 output was adjusted to 13 fl.oz/min (384 ml/min). For these two treatments Dow Mosquito Fogging Concentrate® containing 6 lb/gal (719 gm/l) was diluted with Super 94® spray oil (Occidental Chemical Co.) to 0.5 lb/gal (60 gm/l). Vehicle speed during each of these applications was 8 mph (12.8 km/hr). For Test No. 3 the output of the machines was reduced to 1 fl.oz/min (29.5 ml/min), undiluted Dow Mosquito Fogging Concentrate® containing 6 lb/gal (719 gm/l) chlorpyrifos was used, vehicle speed was reduced to 5 mph (8 km/hr). Two machines were operated on parallel courses in the main portion of town. One traveled the alleys and the other the streets, giving an assumed swath of 165 feet (50.3 meters). The third vehicle covered the suburbs and detached areas. Applications were started simultaneously from each machine. Total area covered on each of the three applications was approximately 1800 acres (728 ha). Total output of the three aerosol units was 29.5 gallons (111.65 l) on each of the first and second tests and 3.2 gallons (12.1 l) on the third application.

Application was begun when data from the weather monitoring stations indicated that conditions of wind velocity, inversion strength, and relative humidity were within acceptable limits as described by Townzen & Whitesell (1975). This usually occurred between 2000 and 2100 hours.

EFFECTS UPON MOSQUITOES.– Caged Mosquito Bioassay – Caged *Culex pipiens quinquefasciatus* were exposed in standard disposable adult mosquito bioassay cages (Townzen and Natvig 1973) at six locations corresponding to light trap stations. Each station consisted of from four to fourteen cages placed 'high', 3 feet (91 cm) or 'low', 6 inches (15 cm) above ground level and either exposed or protected by vegetation or structures. The caged mosquitoes served to indicate whether or not insecticide reached all of the areas.

Table 1 gives the average mortality of caged *Culex pipiens quinquefasciatus* at 12 and 24 hours post treatment for each of the three tests. The Townzen and Ferriavolo locations comprise a complex that has been used over the past three years in a series of tests. Cage positions at these two sites represent a wide variety of degrees of exposure and protection. The high level of mortality at all stations indicates that dispersion of the aerosol throughout the area was good and that dosage rates were adequate.

Population Monitoring – Several methods of assessing the adult mosquito population pre- and post-application were used. American Model light traps and standard "Red Box ARU's" (Goodwin 1942, Loomis & Sherman 1950) were placed at eight stations in town plus a check site in the community of Meridian approximately seven miles (11.26 km) southeast of Colusa. These traps and resting stations were operated daily from June 10, 1975 through September 12, 1975. Data from these stations provided continuous information on the status of the mosquito population in the area.

Prior to each of the aerosol applications, two CDC type miniature traps baited with CO_2 were placed at each of the

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[4]University of California, Department of Agricultural Engineering, Davis, California 95616.

[5]Colusa Mosquito Abatement District, Post Office Box 405, Colusa, California 95932.

stations in Colusa and at Meridian. In addition, a second check site with two CDC-CO_2 traps was established in Meridian, and two CDC-CO_2 traps plus two Red Box ARU's were operated in the town of Williams ten miles (16.09 km) west of Colusa. Each trap was numbered to correspond with a specific station in order that the same trap would be used at each location on each night of operation. This helped to lessen variations due to individual trap differences. During the first test, the traps were operated two nights prior to application and two night post. On the second application, operation was for two nights prior and one night post application. On the third treatment they were operated for one night pre- and one night post.

Figures 1, 2, and 3 show average numbers of female *C. tarsalis* collected pre- and post-treatment at each of the stations in town and at the check locations. Data from these traps appear to substantiate a measurable reduction in the numbers of wild *C. tarsalis* following the first application (June 30) and the third application (September 9). The second application (August 19) resulted in no significant reduction in numbers of mosquitoes captured. Most stations showed a sizable increase in captures on the first night post-treatment.

The traps located at the check sites in Meridian and Williams reflect the same pattern of reductions in captures after the first and third applications and an increase after the second treatment. We attribute these results to variations in the weather which occurred during the pre- and post-treatment trapping periods on each of the tests. Data

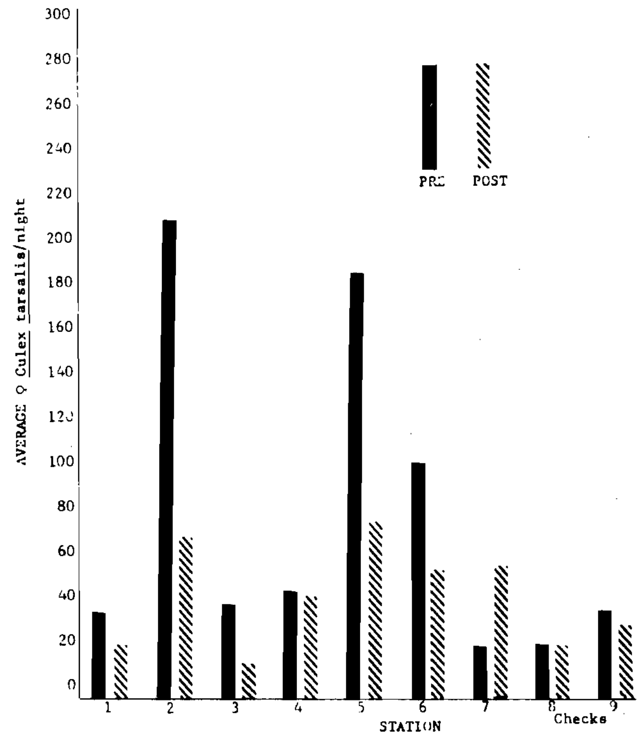

Figure 1.–Pre and post treatment *Culex tarsalis* captures. CDC-CO_2 traps. Colusa MAD. June 29 - July 2, 1975.

Table 1.–Average mortality of caged *Culex pipiens quinquefasciatus* following nonthermal aerosol applications of chlorpyrifos at Colusa, California, June 30, August 19, and September 9, 1975.

	Percent mortality					
	June 30		August 19		September 9	
Site	12 hour	24 hour	12 hour	24 hour	12 hour	24 hour
Townzen[1]	95	99	97	100	98	100
Ferriavolo[1]	95	98	95	100	99	99
T+F	95	99	96	100	99	100
Total cages in town[2]	96	99	92	97	97	100

[1]Consistent cage locations for all applications.

[2]Inconsistent cage locations and number of cages.

Table 2.–Average captures/trapnight and percentage change CDC traps. Pre and post treatment. Colusa MAD 1975.

		Average/trap night					
Treatment Date/Location		*Culex tarsalis*		All species		% change	
		Pre	Post	Pre	Post	*C. tarsalis*	All species
June 30, 1975	Inside	82.3	40.7	94.8	47.3	-50.6	-50.2
	Check	26.75	20.75	53.0	42.1	-22.5	-20.6
August 20, 1975	Inside	69.0	93.75	84.4	151.25	+ 35.8	+ 79.2
	Check	42.37	116.0	54.25	145.25	+174.2	+167.7
September 9, 1975	Inside	177.4	22.4	209.0	49.2	-87.4	-76.5
	Check	197.25	30.75	197.5	35.25	-83.6	-82.2

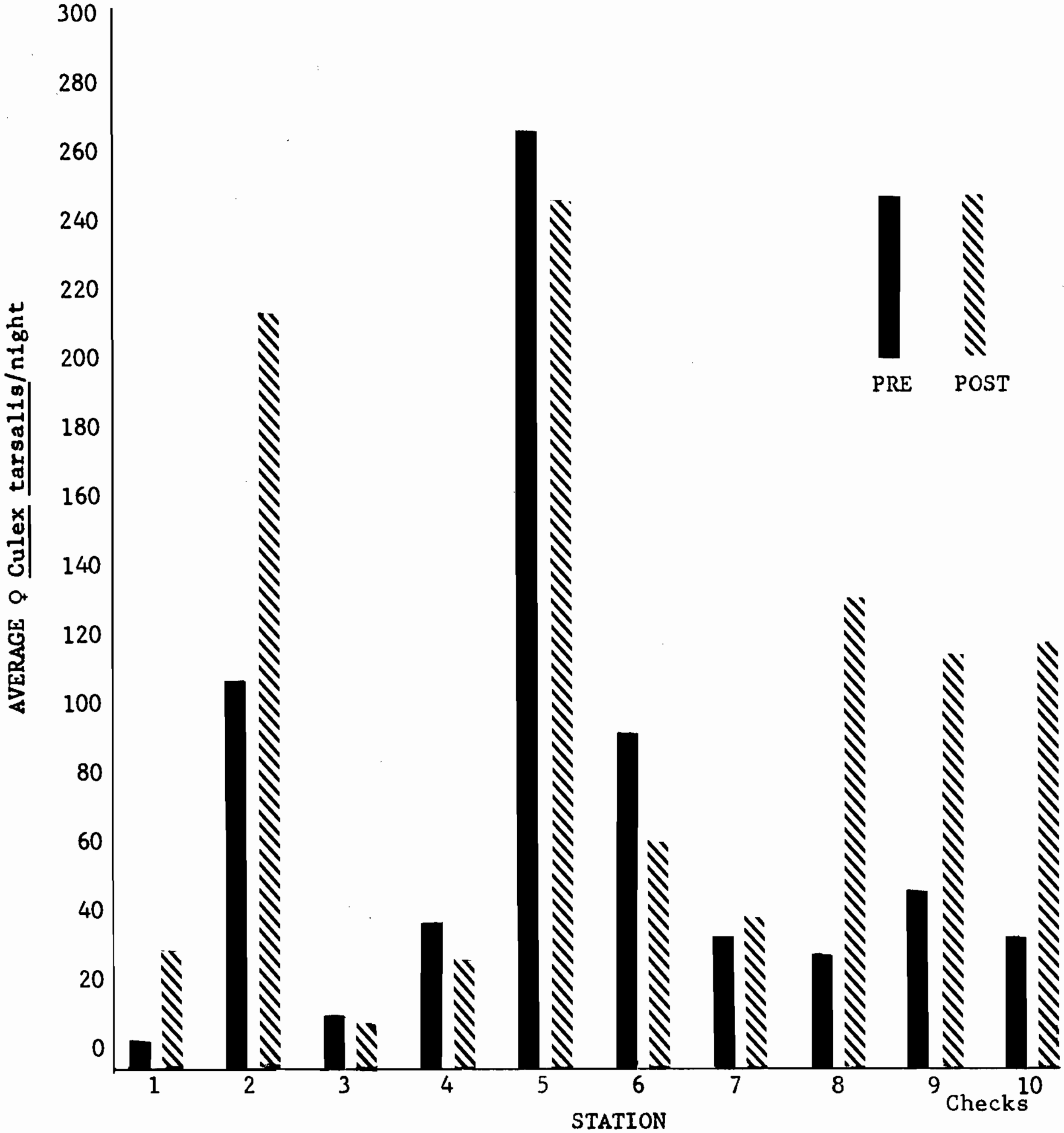

Figure 2.–Pre and post treatment *Culex tarsalis* captures. CDC-CO_2 traps. Colusa MAD. August 17 - 20, 1975.

from the American Model light traps and ARU's operated daily throughout the summer bear this out.

Table 2 shows the average capture/trap night and the percentage of changes in captures for all CDC traps inside the area treated and for the check traps outside the treated area. For the first treatment the percentage decrease was over twice as great inside the treated area as it was outside. On the second treatment, although the captures were much higher after treatment than before, the percentage increase outside the treated area was nearly six times as great for *C. tarsalis* and over twice as high for all species as it was inside the area treated. Captures were much higher pre-treatment than post-treatment on the third application both inside the test area and at the control sites. The effect of weather variations on adult mosquito activity obfuscated any measurement of population decline due to the third insecticide application.

Actual knockdown of mosquitoes and non-target organisms was measured on 16 ft^2 paper sheets in wood frames (Muirhead-Thomson 1968) placed at five of the light trap stations. These were not used during the first treatment, but were put in place on the evening of the second treatment and collections were made the following day and for one day post-treatment. On the third test they were put in place and operated for one night prior to treatment, the night of application, and one night post-treatment.

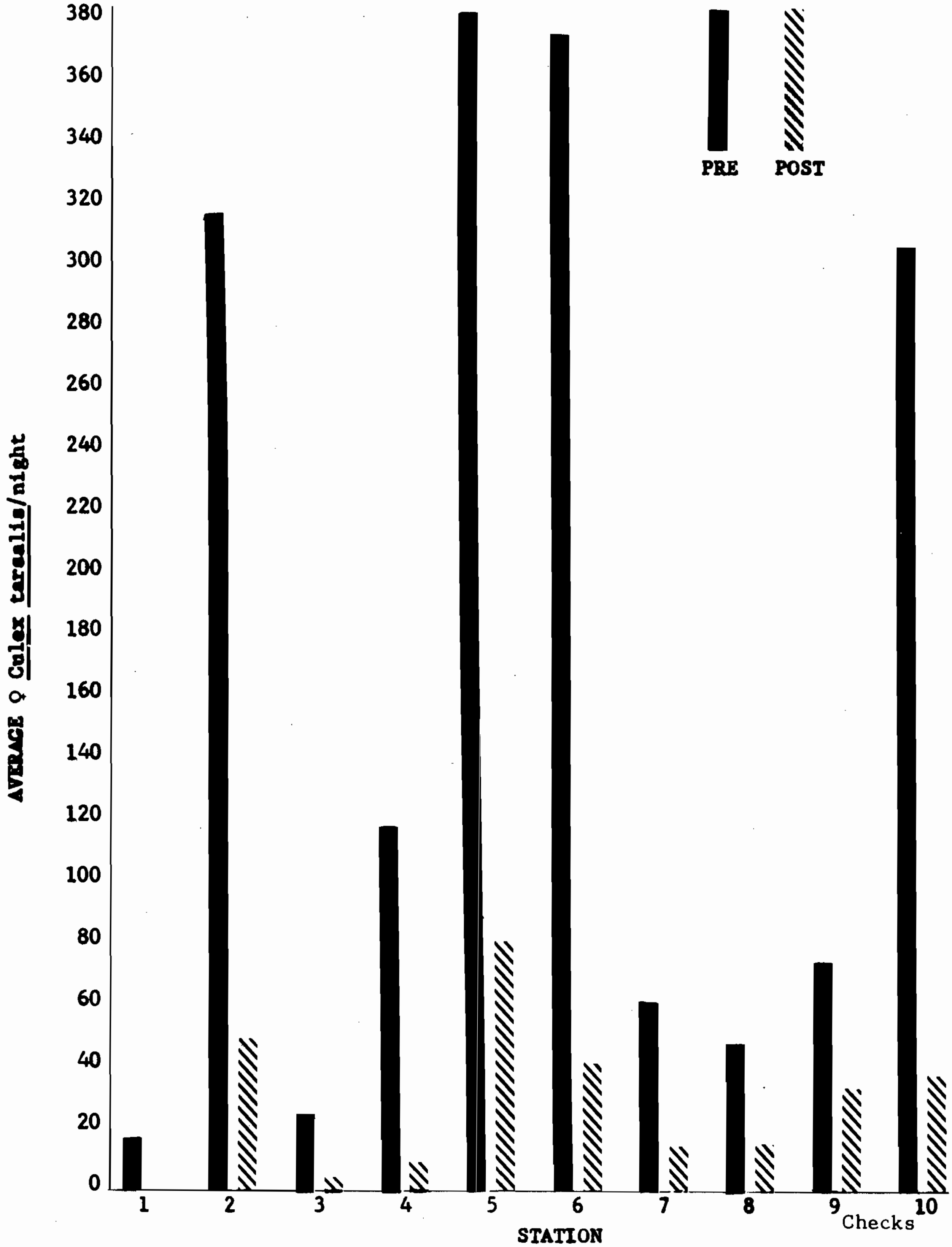

Figure 3.–Pre and post treatment *Culex tarsalis* captures. CDC-CO_2 traps. Colusa MAD. September 8 - 10, 1975.

de Zulueta 24-ft^2 net collections (de Zulueta 1950) to obtain actual total population data on mosquitoes and other insects were made at three of the light trap stations approximately four hours prior to the second application and at twelve and thirty-six hours post-treatment.

Table 3 shows the total number of mosquitoes of all species falling out on the 16-ft^2 paper sheets at five stations during and post-treatment for the second and third applications and during the 24 hour period prior to the third treatment. The species composition was 58% *Anopheles freeborni*, 31% *Culex tarsalis* and 11% *Aedes melanimon*. Mosquito mortality as measured by these knockdown sheets provides specific information on the effect of the aerosol application on the population of wild mosquitoes. By augmenting mortality data obtained by this technique with pre-treatment population counts from de Zulueta net collections it may be possible to obtain more definite information on the effect of an insecticide application.

Effects Upon Non-Target Arthropods – Pre- and post-treatment estimates of non-target arthropod populations were made with de Zulueta net traps at three locations for the second application. Table 4 shows the numbers of individuals of the 31 families collected by this method. The aerosol application resulted in some mortality to non-target arthropods, but the impact on the population was apparently minor since in more than 50% of the collections numbers of individuals present after treatment were higher than before treatment.

Table 5 shows the number of families and individuals for each of the 10 orders of non-target arthropods collected from the knockdown sheets during and post-treatment on Tests number two and three. Diptera and Hymenoptera made up 75% of the total collections. Chironomidae accounted for 37.5% of the Diptera. Two families (Formicidae and Chalcidae) constituted 97% of the Hymenoptera; 59.5% and 37.5% respectively.

Effects Upon Human Blood Cholinesterase – During the June run, volunteers were exposed in pairs to the chlorpyrifos aerosol. Maximal exposure was achieved by placing two people in the open back of a pickup chasing the aerosol machine. Other degrees of exposure involved drivers of the pickups transporting the foggers and passengers in the cabs (four people), riders in the rear of the fogger pickup (two people) and individuals standing in the yards of houses throughout the village (eleven people). In August, only drivers, passengers, and a person in the chase vehicle were monitored.

The District's medical adviser served as the supervising physician. Blood samples were taken about 10 days pre-treatment; 7 days pre-treatment; within a few hours pre-treatment; approximately one hour post-treatment and 1, 2, and 7 days post-treatment. Analyses were performed by the laboratory of the California Department of Food and Agriculture, using a Technicon Autoanalyzer II technique.

Air samplers were operated with each pair of subjects. "Personal" samplers, which draw air at the rate of 0.5 cfm

Table 3.– Mosquito mortality, all species, as measured by 16 ft^2 knockdown sheets, Colusa MAD, 1975.

Station		KD/16 ft^2		KD/16 ft^2		
		During treat. 8/19-8/20	Post-treat. 8/20-8/21	Pre-treat. 9/8-9/9	During treat. 9/9-9/10	Post-treat. 9/10-9/11
1	♂♂	0	0	0	3	2
Fairground	♀♀	7	0	0	3	0
2	♂♂	0	0	0	2	1
13th & A	♀♀	1	1	0	0	0
3	♂♂	0	0	0	3	0
Betty's	♀♀	7	1	0	7	2
4	♂♂	0	0	0	2	0
Fremont	♀♀	1	0	0	4	0
5	♂♂	0	Not	0	4	0
Disney	♀♀	3	recorded	0	1	0
Σ	♂♂	0	0	0	14	3
	♀♀	19	2	0	15	2
x	♂♂	0	0	0	2.8	0.6
	♀♀	3.8	0.5	0	3	0.4

Table 4.--Pre- and post-treatment estimation of nontarget arthropods as sampled by de Zulueta net collection, Colusa, California 1975.

Order/Family	Fairground			Betty's			Brown		
Arthropod	8/19	8/20	8/21	8/19	8/20	8/21	8/19	8/20	8/21
Ephemeroptera									
Baetidae	1	--	1	--	2	1	1	--	--
Odonata									
Coenagrionidae	1	--	1	1	2	1			
Hemiptera									
Lygaeidae	--	1	--				--	--	1
Miridae				1	--	2			
Homoptera				3	--	--			
Cicadellidae	1	--	-	3	5	3			
Lepidoptera									
Hesperiidae				--	2	--			
Lycaenidae				1	1	--			
Microlepidoptera*				1	--	1			
Noctuidae	--	1	1	--	2	--	--	3	2
Pyralidae									
Diptera									
Agromyzidae	2	1	3						
Anthomyiidae	3	--	-	2	--	--			
Calliphoridae				1	--	--			
Chironomidae	3	2	--	--	--	4			
Chlcropidae	1	-	7	1	--	--	--	--	1
Drosophilidae	2	--	--						
Ephydridae				1	--	--			
Lauxaniidae	--	--	3						
Muscidae	1	--	--				--	1	--
Psychodidae	2	--	--						
Syrphidae				--	1	--			
Tabanidae				1	--	--			
Hymenoptera									
Anthophoridae				--	2	--			
Braconidae				3	--	--			
Chalcidae				2	--	--			
Halictidae	1	--	--	2	2	1			
Ichneumonidae	1	1	1	1	--	--			
Megachilidae				--	--	1			
Vespidae				--	2	--			
Araneida									
Oxyopidae				--	1	--			

[1]Division

(approximating the inhalation rate of a man engaged in low activity) through a fiber glass filter and then through ethylene glycol, were placed at each station. These samples were also analyzed by the Department of Food and Agriculture.

Data selected from the cholinesterase test results are presented in Table 6. Results are listed for pairs of subjects in each of the four exposure situations. These pairs were chosen because the air sampler analyses show that these people received the highest exposure in each situation. Under the conditions of the test, the maximum exposure via inhalation for people in house yards was just under a total of 10 micrograms of chlorpyrifos for 90 minutes exposure as measured by a ½ cfm sampler, about 4 times that for people in and on the fogging truck, and was over 100 times that amount for people continually exposed to the fog.

Even with the maximum exposure, no significant cholinesterase depression occurred. The conclusion is that under the conditions of this study no hazard was demonstrated to people acting as casual residential area observers

Table 6.–Blood cholinesterase determinations for people exposed to chlorpyrifos aerosols, Colusa, Calif. June 30, 1975.

Subject Exposure situation and amount[1]	Date		Hematocrit	Plasma	W. B.	R. B. C.
KRT	6-17-75		44	9.11	19.30	32.26
Houseyard	6-23-75		45	9.56	19.81	32.34
9.35 micrograms/	6-30-75	Pre	45	8.4	17.1	27.70
0.5 cfm/90 min	6-30-75	Post	43	8.0	15.8	26.1
	7- 1-75		44	8.2	16.7	27.5
	7- 2-75		42	8.5	17.0	28.8
	7- 7-75		43	8.3	17.6	30.0
R. C.	6-20-75		44	8.53	20.16	34.96
Houseyard	6-23-75		45	9.11	21.27	36.13
9.35 micrograms/	6-30-75	Pre	46	7.2	17.7	30.1
0.5 cfm/90 min.	6-30-75	Post	45	7.1	18.3	32.0
	7- 1-75		42	6.5	16.1	29.4
	7 -2-75		44/44	7.9/7.8	18.7/17.7	30.2/30.3
	7 -7-75		44	8.29	18.4	31.26
RGR	6-19-75		45	5.38	18.80	34.20
Cab of fogger truck	6-23-75		50	10.50	21.77	33.05
(windows open)	6-30-75	Pre	47	7.5	19.9	33.9
37.2 micrograms/	6-30-75	Post	47	8.0	18.1	29.5
0.5 cfm/90 min.	7- 1-75		46	8.2	19.7	33.1
	7- 2-75		45	8.2	17.9	29.8
	7- 7-75		46	7.4	18.7	31.9
JFC	6-23-75		51	6.85	17.89	28.50
Cab of fogger truck	6- -75		–	–	–	–
(windows open)	6-30-75	Pre	47	5.8	14.5	24.3
37.2 micrograms/	6-30-75	Post	46	5.6	15.1	26.2
0.5 cfm/90 min.	7- 1-75		48	6.0	15.1	25.0
	7- 2-75		45	5.6	15.6	25.5
	7- 7-75		45	5.6	15.1	26.8
JRC	6-17-75		43	7.35	18.00	34.21
In truck, beside	6-23-75		44	7.26	17.39	30.28
fogger	6-30-75	Pre	43	6.4	14.6	25.5
38.9 micrograms/	6-30-75	Post	43	6.5	15.4	27.3
0.5 cfm/90 min	7- 1-75		45	6.3	16.1	28.0
	7- 2-75		46	7.9	17.7	29.3
	7- 7-75		46	7.1	17.9	30.5
SB	6-23-75		46	8.14	20.51	35.03
In truck, beside	6- -75					
fogger.	6-30-75	Pre	43	6.3	16.5	30.0
38.9 micrograms/	6-30-75	Post	44	6.9	16.8	29.4
0.5 cfm/90 min.	7- 1-75		42	5.8	16.8	32.1
	7- 2-75		45	7.3	17.7	30.4
NBA	6-20-75		45	9.94	21.42	35.45
Chase vehicle	6-23-75		48	10.73	22.73	35.73
1181 micrograms/	6-30-75	Pre	46	9.2	20.4	33.50
0.5 cfm/90 min	6-30-75	Post	48	9.7	19.8	30.6
	7- 1-75		46	8.8	18.9	30.7
	7- 2-75		48/48	10.1/10.0	20.6/20.1	32.0/31.0
	7- 7-75		46	9.56	20.66	33.71
DJW	6-10-75		46	7.09	17.39	29.48
Chase vehicle	6-23-75		49	9.41	19.56	30.12
1181 micrograms/	6-30-75	Pre	46	7.9	16.2	25.8
0.5 cfm/90 min	6-30-75	Post	48	9.5	16.8	24.8
	7- 1-75		48	8.0	17.1	27.0
	7- 2-75		48	8.4	17.3	27.0
	7- 7-75		48	8.4	17.4	27.2
Sigma	6-17-75					
Plasma standard	6-19-75	5.6, 5.9				
	6-23-75	5.8, 5.8				
	6-30-75	4.8, 4.8, 4.8				
	6-30-75	4.8, 4.8				
	7- 1-75	4.6, 4.7, 4.8, 4.8, 4.9				
	7- 2-75	5.8, 5.9, 5.9				
	7- 7-75	5.35				

[1]Results obtained from "personal samplers".

Table 5.—Nontarget arthropod families evaluated by KO Boxes, Colusa, California, 1975.

Order	No. Families	No. Individuals
Ephemeroptera	1	7
Odonata	1	7
Hemiptera	2	15
Homoptera	3	3
Coleoptera	3	8
Neuroptera	2	5
Lepidoptera	5	36
Diptera	13	96
Hymenoptera	4	141
Araneida	2	2

of the chlorpyrifos aerosol application, or to the workers themselves.

SUMMARY AND CONCLUSIONS.– The nonthermal chlorpyrifos applications in the village of Colusa resulted in a short-lived depression in the wild mosquito population within the treated area. Variations in weather conditions during the pre- and post-application periods caused major fluctuations in adult mosquito activity, severely hampering interpretation of data from the collection devices. The applications affected a wide variety of non-target organisms but the determination of the significance of this effect awaits further investigation. Measurement of pre- and post-exposure cholinesterase levels in twenty-two individuals exposed in a variety of situations indicated no significant depression of blood cholinesterase activity.

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EFFECTS OF DIMILIN ON NONTARGET ORGANISMS: REPEATED UTILIZATIONS ON THE SAME HABITATS AS A MOSQUITO LARVICIDE

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ABSTRACT

Although Dimilin® [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)-urea] has shown acute, short-term toxic effects on some planktonic organisms and mayfly nymphs when applied at rates for mosquito control in irrigated pastures, it is relatively safe to other nontarget organisms. No deleterious effects were observed against nontarget organisms after 8 consecutive treatments with Dimilin for a 2-year period.

During the past several years, this laboratory has been conducting laboratory and operational evaluations of insect-developmental inhibitors as mosquito control agents (Schaefer and Wilder 1972 and 1973, Schaefer et al. 1974, Schaefer et al. 1975) and also as to their effects on common nontarget organisms found in mosquito breeding habitats (Miura and Takahashi 1973, 1974, and 1975). Associated with the latter, this study reports the effects on nontarget organisms (in this instance mosquitoes are the targets) when Dimilin® [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)-urea] is applied repeatedly to waters breeding mosquitoes.

STUDY AREA.– Two mosquito breeding habitats were used; one in Tulare County, the other in Stanislaus County, California. The Tulare County site is located about 15 miles southwest of the city of Tulare and the Stanislaus County site is about 2 miles southeast of the city of Newman.

The Tulare Site – This is about 32 acres of irrigated pasture and is one of the most persistent mosquito-producing areas of its type. The water used to irrigate this pasture is supplied from a sump located on the north side of the parcel. Water is pumped from a wet well near the sump into a piped underground distribution system and then to the head of each check where outflow can be regulated with valves. Predominant vegetation consists of bermuda grass with a few dallis and fescue grasses, and ladino clovers. There are many large depressions in the tail end of the pasture. No vegetation in the deepest parts but shallow depressions are covered either with common spike rushes or bearded sprangletops. Water clovers are also abundant in the drainage ditches. This pasture was irrigated 10 or more times in a year.

The Newman Site – This is an unimproved irrigated pasture. Irrigation water here is taken from a swale where agricultural and industrial waste effluent collects. The water is pumped onto the field through primitive canals (laterals) and contour ditches and then drained into basins. Excess water from the last basin remains in the lower land or in the wasteway where it is often wet and covered by tall cattails and bulrushes. Predominant vegetation is bermuda grass in the basins and common spike rush in the depressions. This pasture is irrigated once a month, 6 or 7 times a year.

METHODS AND MATERIALS.– For all tests, a 25% WP formulation of Dimilin was applied by aircraft (Schaefer et al. 1975). The methods used for evaluating effects on nontarget organisms were the same as previously described (Miura and Takahashi 1975) except where noted otherwise.

Each test was assigned a Field Test Number (F. T. No.) and the compound, amount used, formulations, location, environmental conditions, and spray coverage were recorded.

RESULTS AND DISCUSSION.– A total of 35 taxa including planktonic organisms, insects, spiders, and fish were observed in the study sites. They are listed in Table 1.

Table 2 shows immediate effects of Dimilin on planktonic crustaceans and larval chironomids and shore flies. The water flea population, in the water (2,250 ml) collected immediately after application and held in the laboratory for observation, was completely exterminated. The copepod population was reduced. The water boatmen and shore fly populations were unchanged.

The results of repeated applications on the same habitats at the Tulare and Newman Study Sites are shown in Tables 3 and 4 respectively. Dimilin, at the rates used for mosquito control, is relatively safe to nontarget organisms. Although immediate toxic effects on water fleas and mayfly nymphs are substantial (Miura and Takahashi 1974, 1975), these populations did thrive at the end of 8 consecutive applications for a 2-year period (Table 3). Deleterious effects of Dimilin might be milder than that of raw untreated septic industrial wastewater (Table 4). On July 16, 1975, August 15, 1975, and September 12, 1975, the Newman Site was irrigated with septic industrial wastewater; very few planktonic organisms were observed in the water samples collected on those days. Particularly in the September 12 samples, when Dimilin was not used, only adult beetles, shore flies, mites, spiders, and mosquitofish were collected.

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Table 1.–A list of organisms observed in the study area.

Organism	Stage
Planaria	
Bothromesostoma spp.	Adults
Mesostoma spp.	Adults
Rotifers	
Asplanchna spp.	Juveniles & Adults
Water fleas	
Ceriodaphnia spp.	Juveniles & Adults
Daphnia spp.	Juveniles & Adults
Moina spp.	Juveniles & Adults
Simocephalus spp.	Juveniles & Adults
Seed shrimp	
Cyprois spp.	Juveniles & Adults
Copepods	
Cyclops spp.	Juveniles & Adults
Diaptomus spp.	Juveniles & Adults
Crayfish	
Astacus spp.	Juveniles
Mayflies	
Callibaetis spp.	Nymphs
Dragonflies	
Pantala spp.	Nymphs
Damselflies	
Enallagma spp.	Nymphs
Water boatmen	
Corisella spp.	Nymphs & Adults
Backswimmers	
Notonecta unifasciata	Nymphs & Adults
Giant water bugs	
Belostoma spp.	Nymphs & Adults
Dytiscid beetles	
Thermonectus basillaris	Larvae & Adults
Laccophilus spp.	Larvae & Adults
Cybister explanatus	Adults
Copelatus chevrolati renovatus	Adults
Hydrophilid beetles	
Hydrophilus triangularis	Larvae & Adults
Tropisternus lateralis	Larvae & Adults
Helophorus spp.	Adults
Enochrus hamiltoni pacificus	Adults
Chironomid midges	
Chironomus stigmaterus	Larvae
Chironomus spp.	Larvae
Goeldichironomus holoprasinus	Larvae
Tanypus spp.	Larvae
Rattailed maggots	
Xylota spp.	Larvae
Shore flies	
Brachydeutera argentata	Larvae
Oribatid mites	
Oribatei	Adults
Spiders	
Pardosa ramulosa	Adults
Tetragnatha spp.	Adults
Mosquitofish	
Gambusia affinis	Adults

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Table 2.- Effects of Dimilin (25% WP) on nontarget organisms: Applied at 0.02 lb AI/acre against pasture mosquitoes, May 16, 1975 (F. T. No. 75-1).

	May							
Organism	15	16	17	18	19	20	21	23
	No. of Organisms in the Pre-Treatment Water (2,250 ml)[1]							
Water fleas	175	226			1,321	1,119	1,136	
Copepods	27	53			102	119	139	
Mayflies N	1	1			1	1	1	
Midges L	20	20			20	20	20	
Shore flies L	4	5			3	3	3	
	No. of Organisms in Water (2,250 ml) Collected Immediately After Treatment[1]							
Water fleas		432	95	0	0	0	0	
Copepods		143	16	16	12	11	11	
Water boatmen		1	1	1	1	1	1	
Shore flies L		2	2	2	2	2	2	
	No. of Organisms in Water (2,250 ml) from Daily Field Collections							
Water fleas			333	1,545	838	754		516
Seed shrimp			2	0	3	0		5
Copepods			8	56	93	50		37
Mayflies N			0	1	3	2		0
Water boatmen			0	0	1	1		6
Backswimmers			0	0	1	0		0
Beetles L			2	2	4	1		2
Midges L			0	0	4	1		1

[1]Water samples (pre-treatment and immediately after treatment) were held in the laboratory, and organisms were observed daily.
N - nymphs.
L - larvae.

Table 3. Effects of Dimilin (25% WP) on nontarget organisms: Repeated applications on the same field against pasture mosquitoes, Tulare, California (F. T. Nos. 74-7, 18, 26, and 31; 75-4, 6, 9, and 14).

	1974				1975				
Organism	May 30	June 25	July 13	July 27	June 12	June 10	July 1	July 18	August 3[1]
Planaria	+	+	+	+	+	+	+	+	+
Rotifers	+	+	+	+	+	+	+	+	+
Water fleas	+	+	+	+	+	+	-	+	+
Seed shrimp	+	+	+	+	+	-	+	-	-
Copepods	+	+	+	-	+	+	-	+	+
Mayflies N	+	+	+	+	+	+	+	+	+
Dragonflies N	-	-	-	-	-	-	-	+	-
Water boatmen	+	+	+	+	-	-	+	+	+
Laccophilus spp. A, L	-	+	+	+	+	+	+	+	+
T. lateralis A, L	-	+	+	+	+	-	+	-	+
Helophorus A	-	-	-	+	-	-	+	-	+
Midges L	+	+	+	+	+	-	+	+	+
Rattailed maggots	-	-	-	-	-	-	+	+	-
Shore flies L, P	+	+	+	+	+	+	+	+	+
Oribatid mites	+	+	+	-	+	+	+	+	+

[1]Not treated on this day, but water samples were taken for examination of organisms.
+ - present. A - adults.
- - absent. P - pupae.
N - nymphs. L - larvae.

Table 4.- Effects of Dimilin (25% WP) on nontarget organisms: Repeated applications on the same field against pasture mosquitoes, Newman, California (F. T. Nos. 74-5, 13,28, and 35; 75-1, 5, 13, and 25).

	1974				1975					
Organism	May 17	June 14	July 17	Aug. 15	May 16	June 17	July 16[1]	Aug. 15[1]	Sept. 12[1,2]	Oct. 10[2]
Planaria	-	-	-	-	-	-	-	-	-	-
Rotifers	+	+	+	+	+	+	-	-	-	-
Water fleas	+	+	+	+	+	+	+	-	-	+
Seed shrimp	+	+	+	+	+	+	-	-	-	-
Copepods	+	+	+	+	+	+	+	-	-	+
Mayflies N	+	+	+	+	+	+	+	-	-	+
Water boatmen	+	+	+	+	+	+	+	+	-	+
Backswimmers	+	+	+	+	+	+	+	+	-	+
Giant water bugs	-	+	+	-	+	+	+	+	-	+
T. basillaris A, L	+	+	+	+	+	+	+	+	+	+
Laccophilus spp. A, L	+	+	+	+	+	+	+	+	+	+
Cybister spp. A	+	+	-	-	+	+	+	+	-	-
Copelatus spp. A	-	-	-	+	-	-	+	+	-	+
H. triangularis A, L	+	+	+	+	+	+	+	+	+	+
T. lateralis A, L	+	+	+	+	+	+	+	+	+	+
Enochrus spp. A	+	+	+	-	-	-	+	+	-	-
Helophorus spp. A	+	+	+	+	+	+	+	+	+	+
Midges L	+	+	+	+	+	+	+	+	-	+
Rattailed maggots	+	-	-	-	-	+	+	+	-	-
Shore flies L, P	+	+	+	+	+	+	+	+	+	+
Oribatid mites	+	+	+	+	+	+	+	+	+	+
Pardosa spp.	+	+	+	+	+	+	+	+	+	+
Mosquitofish	+	+	+	+	+	+	+	+	+	+

[1]Highly polluted water (fresh industrial wastewater).

[2]Not treated on this day, but water samples were taken for examination of organisms.

+ - present, - - absent.

N - nymphs, A - adults, L - larvae, P - pupae.

INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN CALIFORNIA: STATUS OF ORGANOPHOSPHORUS RESISTANCE IN LARVAL *AEDES NIGROMACULIS, CULEX TARSALIS* AND *CULEX PIPIENS* SUBSPP.

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ABSTRACT

Resistance to the organophosphorus larvicides has continued to increase in both area and severity. Multiple-resistance, extending to all the commonly used organophosphorus compounds, has now been documented for three species: *Aedes nigromaculis, Culex tarsalis,* and *C. pipiens* subspp.

Although not so severely affected, populations of *A. melanimon* and *C. peus* have demonstrated high levels of tolerence to one or two of the organophosphorus compounds, generally malathion and/or fenthion.

ORGANOPHOSPHORUS INSECTICIDE RESISTANCE IN *AEDES NIGROMACULIS* AND THE EFFECT OF TWO NEW SYNTHETIC PYRETHROIDS

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Aedes nigromaculis (Ludlow) is one of the species that causes serious control problems in California. Although the species is not known to transmit any endemic disease, it is a voracious biter and seriously affects man and animals.

The extensive use of several organophosphorus (OP) insecticides against *A. nigromaculis* has induced a high level of resistance against these compounds (Brown et al. 1963, Schaefer and Wilder 1970). The data reported here were obtained in the course of a study of the activity of two new pyrethroid insecticides on larvae and adults of various species of mosquitoes. Here we report data on the present status of resistance in *A. nigromaculis* larvae toward parathion, fenthion and malathion and on their susceptibility to the two pyrethroid compounds.

The populations studied were collected in Tehama and Tulare Counties. Eggs were obtained in the laboratory on wet sphagnum moss. After being kept for seven days at 85°F for embryonation, the eggs were stored at 60°F for up to five months before incubation. They were flooded with water at 85°F and the larvae were used for bioassay in the 4th instar. The test method used was as already described by Georghiou et al. (1966). The tests were replicated 3 or 4 times on different days.

Figure 1 shows the effect of parathion and fenthion on the two populations. The LC_{50}'s of Tulare larvae were found to be 50X and 27X higher than those of Tehama larvae toward parathion and fenthion, respectively. As expected, the low slope values of the regression lines reflect a relatively large degree of heterogeneity in susceptibility within each population.

Table 1 shows that the highest resistance ratios (RR) are in the Tulare larvae, the compounds being affected in the following order: parathion > fenthion > malathion. The relatively low resistance to malathion at Tehama may be of some interest from the practical control standpoint.

Searching for new insecticides for use against the resistant mosquitoes, we tested two new pyrethroid chemicals, NRDC 167 and NRDC 161, against both populations. The RR values of the two populations were 1.2 and 1.3 for NRDC 167 and NRDC 161, respectively. These data suggest that the mechanisms which are now responsible for the high level of resistance of Tulare larvae toward parathion, fenthion and malathion do not extend to the two new pyrethroids. It is also worth noting that the two pyrethroids are far more toxic in comparison to the other insecticides used in this study. For instance, the LC_{50} of parathion against Tulare larvae was 0.38 ppm, i.e. 704X and 500X higher than the LC_{50}'s of NRDC 161 and NRDC 167, respectively. The LC_{50} of parathion against Tehama larvae was only 0.0076 ppm, i.e. 18X and 12X higher than those of NRDC 161 and NRDC 167.

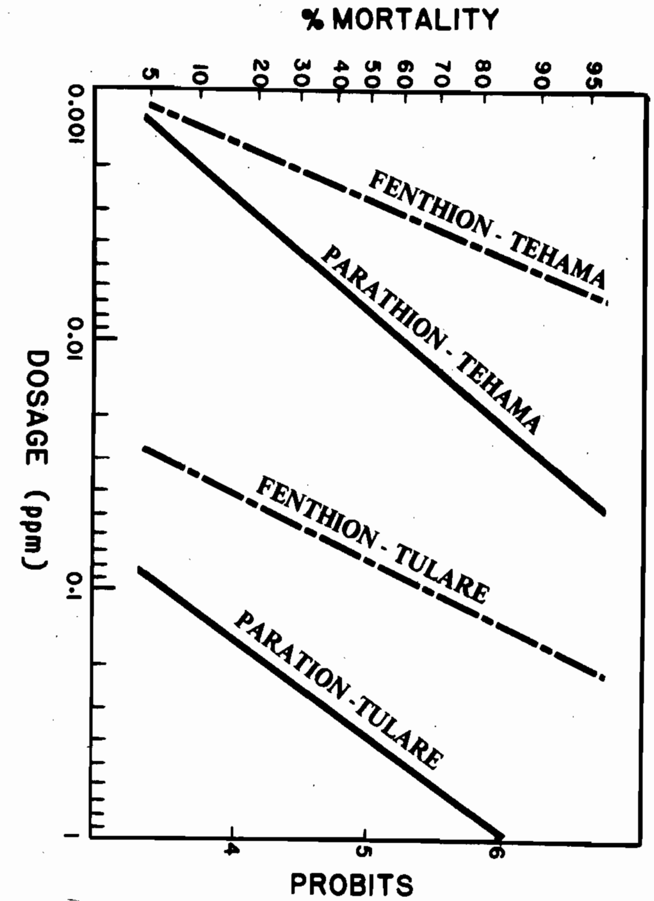

Figure 1.—Effect of parathion and fenthion on the larvae of Tehama and Tulare populations of *Aedes nigromaculis*.

Table 1.—Resistance ratios of two field populations of *Aedes nigromaculis*.

Compounds	Susceptible strain LC_{50} (ppm)	Resistance ratios[1] of field strains Tehama	Tulare
Parathion	.00004[2]	190	9500
Fenthion	.00011[3]	24.5	663
Malathion	.011[3]	4.7	16.4

[1]Resistance ratio = $\frac{LC_{50} \text{ of resistant strain}}{LC_{50} \text{ of susceptible strain}}$

[2]From Lewallen and Nicholson (1959).

[3]From Brown et al. (1963).

The high toxicity of the two pyrethroids to both Tehama and Tulare larvae is encouraging since it indicates that these compounds might be useful against resistant populations.

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AN ATTEMPT TO ISOLATE ORGANOPHOSPHORUS RESISTANCE MECHANISMS IN *CULEX PIPIENS QUINQUEFASCIATUS* BY USE OF SYNERGISTS

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Organophosphorus (OP) resistance in *Culex pipiens quinquefasciatus* is relatively new and very important, not only to California but to other areas of the world as well (Hamon and Mouchet 1967). In California very high and broad-spectrum resistance to OP's was reported by Georghiou et al. (1975) in a strain collected from Hanford (Camara strain) in 1974. Studies with synergists showed that this strain possesses an esterase mechanism for degradation of OP compounds. This was deduced from the observation of strong synergism by DEF, a known inhibitor of esterases. Piperonyl butoxide (p.b.), a microsomal mixed function oxidase (MFO) inhibitor, showed either no synergism or antagonism towards these compounds. OP resistance due to DEF-sensitive mechanisms has also been reported from other species including the horn beetle and silkworm by Shishido and Fukami (1963) and Fukami and Shishido (1966), in mosquitoes by Plapp et al. (1963) and in houseflies by Lewis (1969), Lewis and Sawicki (1971) and Motoyama and Daughterman (1972).

Since it is understood that no one has yet isolated this type of mechanism in mosquitoes, this study was undertaken with this objective in mind and also to relate the results obtained to field situations.

A study conducted before further selection of this strain in the laboratory showed that OP resistance in the population was very unstable, having regressed to very low levels after three generations under insecticide-free rearing. There was no apparent relationship between the new low resistance levels and the structures of the compounds tested or the amounts of these compounds used in the field.

SELECTION AND ISOLATION OF MECHANISMS.– Abate was chosen as the compound to use for selection. Selection with Abate was expected to enhance all possible resistance mechanisms available in the strain, thereby reaching high resistance levels. The figure shows this increase of resistance from a level of 7.7x (parent P) to 322.2x within a period of nine generations.

The same parent strain was selected with Abate + p. b. based on the assumption that this would suppress or eliminate individuals with MFO mechanisms, thereby isolating a strain possessing the major esterase mechanisms. Since this strain may be free of MFO contributions, resistance was expected to rise to a level lower than in the strains selected with Abate alone. This is shown in the figure where the resistance level increased from 7.7 x (P) to 122.2 x. This partial but still high increase in resistance indicates an isolation or concentration of the major resistance mechanism in the strain. Further studies are being carried out to confirm this isolation.

A selection with Abate + DEF was expected for similar reasons to eliminate individuals with the major esterase-sensitive mechanisms, thus concentrating individuals with MFO-mediated mechanisms. The latter were shown to be of only minor importance in this strain. The drop in resistance level from 7.7x (P) to 4.4x at F_9 as shown in the figure indicates that this probably occurred. However, here too further studies are necessary to confirm this.

Genetic studies with the strain selected with Abate alone showed that a single major gene is responsible for resistance. It is assumed that this is the DEF-sensitive mechanism referred to above.

CONCLUSION.–Selection with Abate + p.b. may have isolated a strain which is relatively pure for an esterase mechanism. Similarly, selection with Abate + DEF appears to have isolated a strain with an MFO-mediated mechanism. Studies are being conducted with both synergists, oxone analogues of OP compounds and Abate analogues to obtain more information to support this claim. The fact that Abate + DEF combination failed to induce high resistance levels could be developed into a useful field technique to avoid or delay the development of resistance in certain populations.

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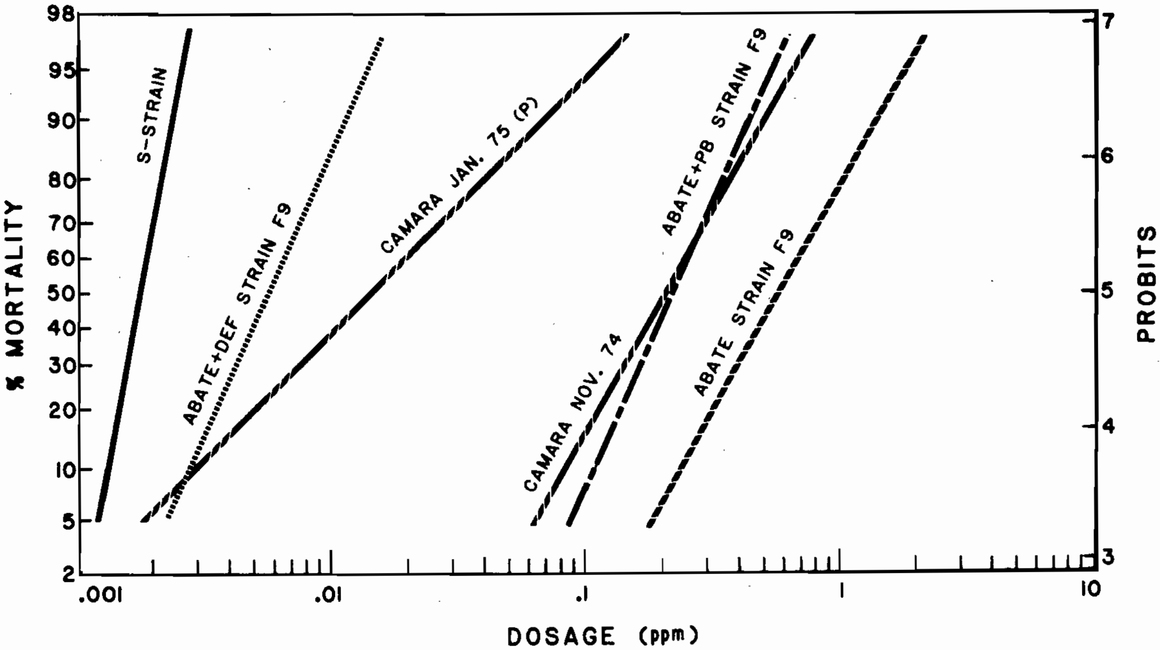

Figure 1.–Dosage-mortality relationships of susceptible and resistant strains of *Culex pipiens quinquefasciatus* to Abate.

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OVERCROWDING FACTORS OF MOSQUITO LARVAE – ACTIVE ANALOGS OBTAINED BY STRUCTURAL MODIFICATIONS

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Due to their relatively high activity and possible use in mosquito control, the overcrowding factors of mosquito larvae have now been intensively investigated from the standpoint of both chemical and biological aspects. Active compounds of the overcrowding factors are shown to be 2-ethyl-substituted carboxylic acids, 7-methyloctadecane, and 8-methylnonadecane (Ikeshoji and Mulla 1974a).

$$\begin{array}{c} C_2H_5 \\ | \\ CH_3(CH_2)nCHCO_2H \end{array}$$

2-Ethyl-substituted carboxylic acids

$$\begin{array}{c} CH_3 \\ | \\ CH_3(CH_2)_5CH(CH_2)_{10}CH_3 \end{array}$$

7-Methyloctadecane

$$\begin{array}{c} CH_3 \\ | \\ CH_3(CH_2)_6CH(CH_2)_{10}CH_3 \end{array}$$

8-Methylnonadecane

These compounds caused high mortality in larvae of *Culex pipiens quinquefasciatus* Say and other mosquitoes.

In seeking more active analogs of the overcrowding factors by structural modifications of the natural products, we found that 3-methyloctadecane and 3-, 4-, 5-, 7-, and 9-nonadecanes were more active than the naturally occurring 7-methyloctadecane and 8-methylnonadecane (Hwang and Mulla 1975, Hwang et al. 1976a). In addition to causing mortality, these alkanes showed growth retarding effects on mosquito larvae at sublethal concentrations.

Various homologs of 2-ethyl-substituted carboxylic acids were synthesized and evaluated for their biological activity against mosquito larvae (Hwang et al. 1974a, b; Hwang and Mulla 1976a). Esterification of these active acids yielded more active methyl esters (Hwang and Mulla 1975, Hwang et al. 1976b).

In view of these studies, we found that it was possible to obtain analogs of the overcrowding factors with increased activity. Consequently, further investigations have been carried out in synthesizing more active compounds by structural modifications of existing compounds. In this communication, we report the biological activity of 2-bromoalkanoic acids and 3-methylalkanoic acids and their esters.

2-BROMOALKANOIC ACIDS AND THEIR METHYL ESTERS. —Although 2-ethylalkanoic acids were active, 2-methylalkanoic acids did not show any activity (Ikeshoji and Mulla 1974b, Hwang et al. 1974a). The difference in activity was attributed to the fact that 2-ethyl-substituted carboxylic acids are more sterically hindered than 2-methyl-substituted carboxylic acids in enzymatic esterification (Ikeshoji and Mulla 1974b). The biological activity of 2-substituted aliphatic carboxylic acids is, therefore, dependent on substituents attached to the C-2 position of the acids. Consequently, it is interesting to know whether replacement of the methyl group in 2-methylalkanoic acids with a halogen atom will influence the activity of resulting 2-halo-alkanoic acids.

To determine the biological activity of 2-haloalkanoic acids, we first synthesized 2-bromoalkanoic acids and their methyl esters from C_{10} to C_{22} (Hwang and Mulla 1976b).

$$\begin{array}{c} Br \\ | \\ CH_3(CH_2)_nCHCO_2R \end{array}$$

2-Bromoalkanoic acids and methyl esters

$R = H, CH_3$

$n = 7, 9, 11, 13, 15, 17, 19$

2-Bromoalkanoic acids were prepared by reaction of carboxylic acids with bromine and phosphorus tribromide to give 2-bromoalkanoyl bromides and subsequent hydrolysis prepared by reaction of 2-bromoalkanoic acids with diazomethane in ether or by solvlysis of 2-bromoalkanoyl bromides in methanol.

These acids and esters were bioassayed against first instar larvae of *C. p. quinquefasciatus*. We found that 2-bromodecanoic acid (C_{10}, R=H, n=7) was inactive, but 2-bromododecanoic acid (C_{12}, R=H, n=9) showed moderate activity. The activity decreased slightly in 2-bromotetradecanoic acid (C_{14}, R=H, n=11) and increased in 2-bromohexadecanoic acid (C_{16}, R=H, n=13) and 2- bromooctadecanoic acid (C_{18}, R=H, n=15), the latter 2 showing LC_{50} below 1 ppm and LC_{90} about 3 ppm. 2-Bromoeicosanoic acid (C_{20}, R=H, n=17) and 2-bromodocosanoic acid (C_{22}, R=H, n=19) did not show good activity.

Methyl 2-bromodecanoate (C_{10}, R=CH_3, n=7) was an active compound. As the length of carbon chain increased, the activity steadily increased from methyl 2-bromododecanoate (C_{12}, R=CH_3, n=9) through methyl 2-bromotetradecanoate (C_{14}, R=CH_3, n=11) and methyl 2-bromohexadecanoate (C_{16}, R=CH_3, n=13) to methyl 2-bromooctadecanoate (C_{18}, R=CH_3, n=15). The last two highest homologs, methyl 2-bromoeicosanoate (C_{20}, R=CH_3, n=17) and methyl 2-bromoeicosanoate (C_{22}, R=CH_3, n=19) did not show good activity. Among these esters, methyl 2-bromooctadecanoate was the most active with LC_{50} and LC_{90}at 0.9 and 1.5 ppm, respectively.

By replacing the methyl group in inactive 2-methylalkanoic acids with a bromine atom, we obtained 2-bromoalkanoic acids, some of which showed good activity.

3-METHYLALKANOIC ACIDS AND THEIR ESTERS.—In investigating structure-activity relationship of the overcrowding factors of mosquito larvae, it was concluded that five types of carboxylic acids exhibited good activity. They are: (1) 3-methylalkanoic acids, (2) 2,3-dimethylalkanoic acids, (3) 2-ethylalkanoic acids, (4) 2,2-dimethylalkanoic acids and (5) 2-bromoalkanoic acids (Ikeshoji and Mulla 1974b, Hwang and Mulla 1976a). The larvicidal activity of 2-ethylalkanoic acids and 2-bromoalkanoic acids has already been reported (Hwang et al. 1974b, Hwang and Mulla 1976b). Some studies on 3-methylalkanoic acids is discussed here.

$$CH_3(CH_2)_nCH(CH_3)CH_2CO_2R$$

3-Methylalkanoic acids and esters

$R = H, CH_3, C_2H_5,$ *iso*-C_3H_7

$n = 13, 14, 15$

3-Methylalkanoic acids for biological studies were synthesized as follows. 1-Alkenes were oxymercurated with mercuric acetate in aqueous tetrahydrofuran to give oxymercurials which, without isolation, were reduced by sodium borohydride in a basic medium to yield 2-alkanols. Reaction of the secondary alcohols with *p*-toluenesulfonyl chloride in pyridine gave tosylates which, upon malonic ester condensation with diethyl malonate, yielded substituted malonic esters. Hydrolysis of the esters and subsequent thermal decarboxylation of the resulting substituted malonic acids produced the desired 3-methylalkanoic acids. Methyl, ethyl, and isopropyl esters of the acids were prepared by solvolysis of 3-methylalkanoyl chlorides in methanol, ethanol, or isopropyl alcohol.

Among 3-methylalkanoic acids prepared and bioassayed, 3-methylheptadecanoic acid (R=H, n=13), 3-methyloctadecanoic acid (R=H, n=14), and 3-methylnonadecanoic acid (R=H, n=15) were the most active against first instars of *C. p. quinquefasciatus*. The LC_{50} and LC_{90} of these acids were at or below 0.5 and 0.5-1.3 ppm, respectively.

Methyl esters of these acids, i.e., methyl 3-methylheptadecanoate ($R=CH_3$, n=13), methyl 3-methyloctadecanoate ($R=CH_3$, n=14), and methyl 3-methylnonadecanoate ($R=CH_3$, n=15) also exhibited a high level of activity with LC_{50} and LC_{90} at <0.5-0.7 and <0.5-1.4 ppm, respectively. Except for methyl 3-methyloctadecanoate, the activity was not increased by esterification process.

In comparing the activity of methyl, ethyl, and isopropyl 3-methyloctadecanoates, we found that the activity drastically decreased from the methyl ester ($R=CH_3$, n=14) through the ethyl ester ($R=C_2H_5$, n=14) to the isopropyl ester (R=*iso*-C_3H_7, n=14) (Figure 1). Obviously, the activity decreased as the size of the alcohol moiety of the esters increased. Methyl 3-methyloctadecanoate was by far the best compound among those synthesized and bioassayed.

In conclusion, it is quite possible that structural modifications of existing active or inactive compounds would likely lead to the findings of more active compounds. From the present studies, we have obtained new analogs of the overcrowding factors which show good biological activity against larvae of mosquitoes. Further laboratory and field studies are planned to determine the practical dosages of these compounds.

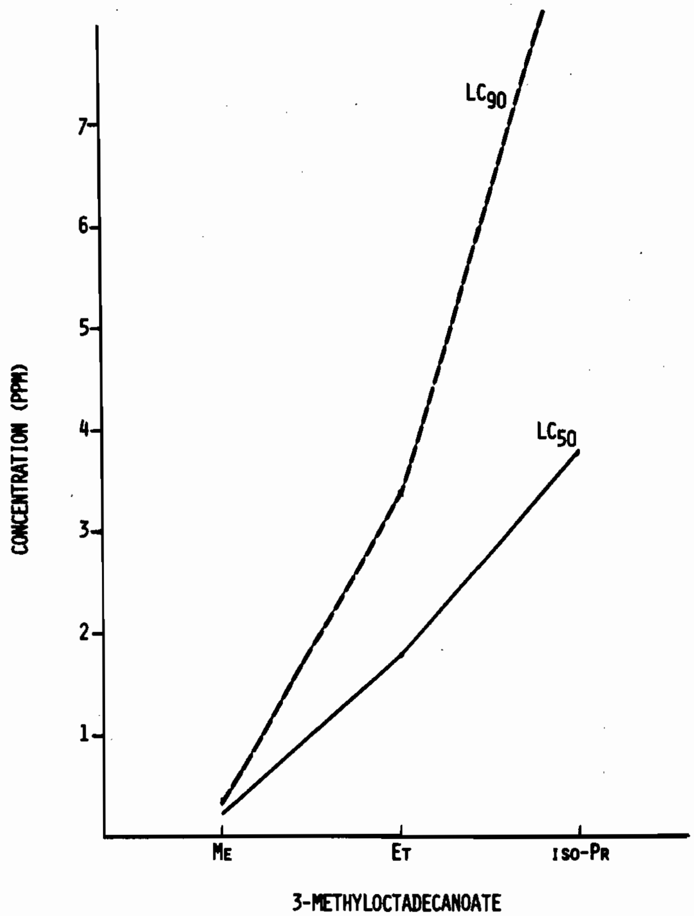

Figure 1.—Activity of methyl, ethyl, and isopropyl 3-methyloctadecanoates.

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PRELIMINARY EVALUATION OF SILICATE CONTROLLED-RELEASE CAPSULES FOR MOSQUITO CONTROL

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ABSTRACT

The effectiveness and diversity of environmentally compatible silicate capsule formulations for the sustained control of mosquito larvae in intermittent waters is described. Chlorpyrifos insecticide was employed as toxicant. Chlorpyrifos release rates from the capsule formulations were compared with a control treatment containing an equal quantity of unformulated toxicant. Bioassay analyses using *Daphnia magna* established that all capsule formulations evaluated release chlorpyrifos at least three times more slowly than the unencapsulated material.

INTRODUCTION.–Increasing interest in the economic and aesthetic values of outdoor recreation in the midst of rapidly increasing costs to control intermittent water mosquito breeding species, particularly *Aedes*, has focused attention on the financial and operational advantages a sustained release larval control formulation would offer. International Controlled Release Pesticide symposia held the past two years in Ohio, have placed into perspective the current technology on controlled release, including its broad application in industry and agriculture. In addition to offering sustained release, controlled release formulations should provide reduced toxicity during handling and protection of the toxicant from environmental degradation before release occurs.

Over the years, mosquito control workers have utilized various types of slow release devices in field control programs. Included in the list are oil-soaked sawdust, plaster of paris cubes, granules, drip applications of dilute and concentrated insecticides, charcoal briquets, resin strips and more recently, various rubber or other polymeric matrices. Each method has its merits, limitations and disadvantages.

If a controlled release device is to be used widely, it must be low in cost, easy to apply, compatible with the environment, and consistently provide effective activity for a prolonged period. Accordingly, the Metropolitan Mosquito Control District and the Biological Transport Laboratory at Washington University have initiated a joint program to develop a device that meets these specifications. The goal is to achieve extended control of flood water or rain pool mosquitoes, primarily *Aedes vexans*. This paper summarizes results obtained thus far.

MATERIALS AND METHODS.– Several silicate capsule samples were formulated. The capsules, 2-5 mm diameter, contained 19 wt. % chlorpyrifos (0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl phosphorothioate)) (Dow Chemical Company, Midland, Mich.). Ability of the capsules to disintegrate on storage in water was assessed by placing a few capsules in a water-filled vial. The vial was briefly shaken manually once every week or two and the degree of capsule disintegration visually estimated.

Release of chlorpyrifos from the capsules into water was monitored by a bioassay procedure in which *Daphnia magna* Straus were used as bioassay organisms. The bioassay procedure involved placing 3.5 liters of water into one gallon wide-mouth jars.

The water was tap water which had various specific ions added to it after purification by reverse osmosis (Muscatine & Lenhoff 1965). In all cases, replicate test samples of chlorpyrifos-containing silicate capsules or pure chlorpyrifos pearls fabricated by a melt technique were placed in stainless steel screen envelopes suspended at mid-depth in each jar with a stainless steel wire. Openings of the stainless steel screen were 139 microns in diameter. In order to obtain consistent release data, the water in each jar was agitated with a magnetic stir bar/stirrer combination for 20 min. each day. This agitation was done 6-8 hrs. before sampling. The weight of silicate capsules or chlorpyrifos pearls added to each jar was calculated to give a 1.0 ppm chlorpyrifos concentration if all chlorpyrifos in the capsule or pearls dissolved in 3.5 liters of water. Because this is about 2.5 times more than the saturation solubility of chlorpyrifos at the incubation temperature of 21°C, the water in each jar was changed every 24 hrs. Just before this was done, two samples were collected from each container. The first (150 ml) was taken one inch above the bottom of the jar; the second (150 ml) was taken one inch below the water surface. The two samples were combined to give a single sample of 300 ml. The undiluted mixture and two dilutions (10X and 100X) were used for bioassay. Each sample dilution was replicated three times.

Bioassays were performed in wax-treated paper cups (8 oz.) containing 10 *Daphnia magna* per cup. The *Daphnia* were preconditioned to the water 24 hrs. before use. The

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[2]Washington University, Biological Transport Laboratory, Department of Chemical Engineering, St. Louis, Missouri 63130.

undiluted or diluted water samples taken from jars containing chlorpyrifos capsules or pearls were added to the paper cups, and mortality counts were taken 22 hrs. after *Daphnia* introduction. All *Daphnia* which did not exhibit swimming or filtering appendage movement upon being disturbed were counted as dead. The data were corrected with Abbott's Formula.

RESULTS AND DISCUSSION.– Figure 1 shows how three silicate capsule formulations containing 19 wt. % chlorpyrifos behave when stored in water at 25°C for 15 weeks. As shown, the capsules disintegrate at varying rates in water. The BJ-9-30-75-1A capsules disintegrate nearly completely within a week; BJ-9-30-75-1C capsules disintegrate more slowly; BJ-9-23-75-2B capsules disintegrate the slowest. Not shown are data for other chlorpyrifos silicate capsules that experience <10% disintegration when stored more than 15 wks. in water. Significantly, Figure 1 indicates the silicate capsule fabrication process can be altered to yield capsules which differ greatly in their rate of break-up in water. The capsule coating material undergoes little, if any, dissolution during the disintegration process, but simply fragments into many small pieces. The ability to prepare capsules that undergo controlled disintegration in water is a significant asset. Capsules that rapidly undergo total disintegration release their payload quickly whereas capsules that remain intact retain their payload much longer. Blending silicate capsules that disintegrate at varying rates in water should enable one to tailor the release behavior of a capsule sample to suit a range of specific needs.

Figure 2 contains bioassay data for the three chlorpyrifos silicate capsule formulations used to obtain Figure 1. Included are bioassay data for unencapsulated chlorpyrifos. The bioassay data correlate well with the rate of capsule disintegration data. For example, Figure 2 shows that unencapsulated chlorpyrifos caused only 50% mortality about 2 days after immersion in water. During this period the water in the test jar was changed twice. In contrast, capsule samples BJ-9-30-75-1A and BJ-9-30-75-1C cause ≤50% mortality for 6-7 days (i.e., through 6 to 7 water changes). The BJ-9-30-75-1C capsules disintegrated more slowly than the BJ-9-30-75-1A capsules and hence, were effective for a longer period.

The data of Figure 2 demonstrate that the silicate capsules slow the release of chlorpyrifos into water and thereby extend the effectiveness of the chlorpyrifos. Note that the BJ-9-23-75-2B capsules gave erratic and usually low mortality at all test points. This is attributed to the slow rate of capsule disintegration in water and an accompanying slow rate of chlorpyrifos release. Daily agitation considerably longer than 20 min. may reduce the scatter points shown by ensuring good mixing of the water in the test jar. However, it appears that the BJ-9-23-75-2B capsules disintegrate too slowly in water to cause a desirable high mortality of *Daphnia magna* under the conditions used in this test. Accordingly, silicate capsules that disintegrate at a rate between that of BJ-9-30-75-1C and BJ-9-23-75-2B will be fabricated and evaluated.

Before concluding, it is relevant to mention that controlled-release formulations of chlorpyrifos may offer no distinct advantage due to the exceptional residual characteristic of chlorpyrifos under field conditions. Nevertheless,

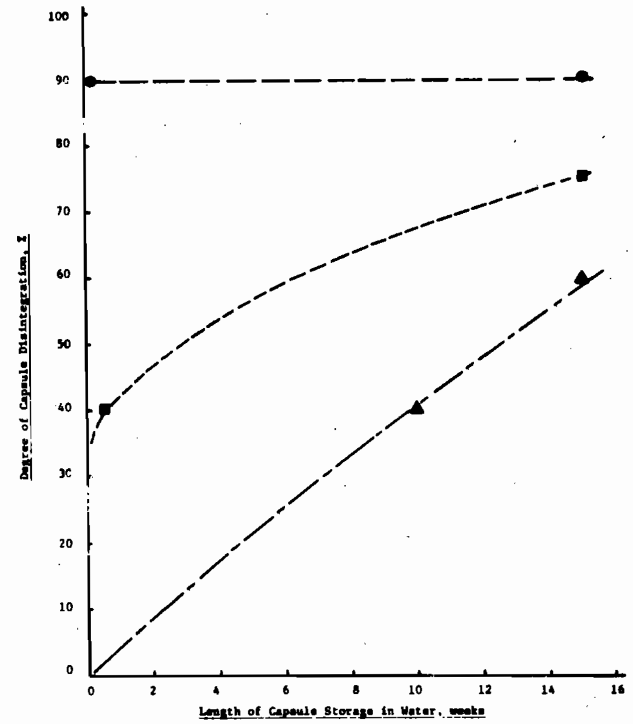

Figure 1.–Degree of silicate capsule disintegration as a function of storage time in distilled water at 25°C. All capsules initially contain 19 wt. % chlorpyrifos:
●, WJ-9-30-75-1A sample; ■, WJ-9-30-75-1C sample; ▲, WJ-9-23-75-2B sample.

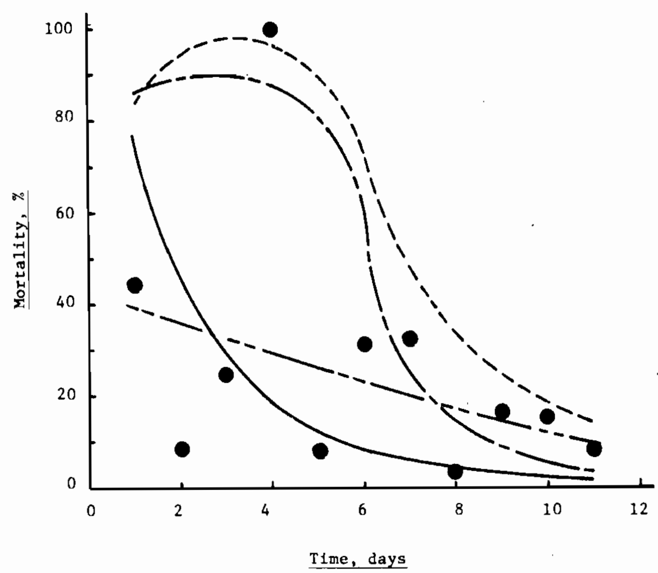

Figure 2.–Effect of equivalent amounts of encapsulated and unencapsulated chlorpyrifos on mortality of *Daphnia magna*: —, unencapsulated chlorpyrifos; – · – · –, WJ-9-30-75-1A silicate capusles; — — — —, WJ-9-30-75-1C silicate capsules; and ●, WJ-9-23-75-2B silicate capsules. All silicate capsules initially contained 19 wt. % chlorpyrifos.

it provides a useful model for examining the effectiveness of silicate capsules as controlled release devices for insecticides.

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EVALUATION OF DIMILIN™, BAY MEB 6046, SD41706 AND SD43775 AS MOSQUITO CONTROL AGENTS

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ABSTRACT

Studies in 1975 on Dimilin™ continued to prove its efficacy as a mosquito control agent. A new potential adulticide, BAY MEB 6046 showed good promise in laboratory tests but yielded relatively poor results in field evaluations. A new synthetic pyrethroid, SD-43775, shows excellent potential as a mosquito larvicide and pupicide.

Efforts were continued during 1975 to evaluate compounds having new or different structural-types in order to find effective means of controlling organophorus-resistant strains of mosquitoes.

DIMILIN™, or TH6040.–Studies in 1974 on Dimilin (Schaefer et al. 1975) were continued in order to obtain the vast amount of data required for registration. A summary of 1975 field trials with Dimilin is given in Table 1. A pasture (Monteiro) in Tulare County was sprayed four successive times at .04 lb AI/acre and one (Colosso) in Stanislaus County was sprayed simiearly at .02 lb AI/acre; samples of water, soil, and vegetation were collected immediately before and at 1 hr and at 1, 3, 5, and 7 days, after each treatment, and at 28 and 56 days after the 4th treatment of each field. These are currently being analyzed for residues, which will support a petition for the registration of Dimilin. The applications of .04 lb AI/acre gave complete control of mixed populations of *Aedes nigromaculis* and *Aedes melanimon* larvae but the .02 lb AI/acre rate showed varied results from 75 to 99% control.

An application of 0.1 lb AI/acre along the shoreline of Borax Lake in Lake County against larvae of the biting midge *Culicoides variipennis* was very ineffective, which largely may have been due to dilution of the active ingredient.

Applications of Dimilin to dairy drains gave good results when clear, open water occurred, and poor results where large amounts of organic debris were being circulated due to pumping. In cases where dense vegetation occurred along the bank (FT75-26), good results were obtained when the application was concentrated along the pond perimeter.

A detailed study on the persistence of Dimilin in water (Schaefer and Dupras 1976) showed that while Dimilin persists long enough to achieve larval control, it does not have long-term stability in water. Persistence of Dimilin in water appears to be limited due to hydrolysis and to adsorption onto organic matter; it is least stable when water temperature and pH are both relatively high. Its persistence is not greatly affected by sunlight or by microorganisms.

BAY MEB 6046.– [3, 4 dichloro-alpha-(trichloromethyl) benzenemethanol acetate]. This compound was carefully evaluated since it was reported to be adulticidal to mosquitoes and has a very different structure than other adulticides in use. The compound is not very toxic to larvae; our tests yield an LC_{50} of .16 ppm and an LC_{90} of .21 ppm to 4th-instar *Culex pipiens quinquefasciatus* larvae. In laboratory tests adults were exposed to filter paper disks treated with a range of concentrations (Georghiou and Metcalf 1961); the results are shown in Table 2. As Baygon® has an LC_{50} of .011 and LC_{90} of .019 against either the S or OP-R strains of *Culex tarsalis* and against the S strain of *Culex pipiens quinquefasciatus*, BAY MEB 6046 offered promising results in these laboratory tests.

Two trials using aerial applications of the 70 WP formulation of BAY MEB 6046 were made in the Tulare MAD. In the first test (7/25/75) a population of *A. nigromaculis* adults (14/leg count) over a 5-acre pasture was sprayed with 0.1 lb AI/gal/acre. There was an approximate reduction of 93%, while no direct, deleterious effects were observed on nontarget insects, including butterflies and bees, that were present. A second aerial application was made on 7/30/75. In this case a 20-acre pasture having a population of *A. nigromaculis* adults (30/leg count) was sprayed with 0.2 lb AI/acre. The final population reduction was estimated at 95%. These results were discouraging since (1) Baygon at rates of .05 to .07 lb AI/acre will completely eliminate such *A. nigromaculis* populations, and (2) there was little, if any, improvement in the results when the rate was doubled from 0.1 to 0.2 lb AI/acre. No further tests are planned.

SYNTHETIC PYRETHROIDS.– Another type of chemical structure that is currently receiving considerable attention as a potential tool for mosquito control is the synthetic pyrethroid. Mulla et al. (1975) have reported on the activity of NRDC-143 [cyclopropanecarboxylic acid, 2, 2-dimethyl, (±) *cis-trans* 3-(2, 2 dichlorovinyl), 3-phenoxyphenyl, methyl ester] against several species of mosquitoes. During 1975 we have evaluated SD41706 [cyclopropane-

Table 1.–Summary of Dimilin field tests, 1975.

Field Test No.	Date (1975)	MAD[1]	Location and Habitat	Acres Treated	Method of Application	Formulation	Rate (lb AI/acre)
75- 1	5/15	Turlock	Colosso-Pasture	40	Aircraft	25% WP[2]	0.02
75- 3	5/29	Lake County	Borax Lake-(pH 9.5)	6[3]	Power Sprayer	25% WP	0.1
75- 4	6/12	Tulare	Monteiro-Pasture	30	Aircraft	25% WP	0.04
75- 5	6/17	Turlock	Colosso-Pasture	40	Aircraft	25% WP	0.02
75- 6	6/20	Tulare	Monteiro-Pasture	30	Aircraft	25% WP	0.04
75- 9	7/ 1	Tulare	Monteiro-Pasture	30	Aircraft	25% WP	0.04
75-13	7-16	Turlock	Colosso-Pasture	40	Aircraft	25% WP	0.02
75-14	7-17	Tulare	Monteiro-Pasture	30	Aircraft	25% WP	0.04
75-23	8/13	Tulare	Machado-Dairy Drain	0.35	Power Sprayer	FL[4]	0.1
75-25	8/19	Turlock	Colosso-Pasture	40	Aircraft	25% WP	0.02
75-26	8/19	Kings	DeBrum-Dairy Drain	0.27	Hand Sprayer	FL	0.1
75-28	8/27	Tulare	Machado-Dairy Drain	0.35	Power Sprayer	FL	0.11
75-30	9/ 1	Consolidated	Roberts-Pasture	6.5	Aircraft	1% G[5]	0.032
75-31	9/ 6	Consolidated	Lone Oak Road-Pasture	10	Aircraft	0.5% G	0.0227

[1]Mosquito abatement districts.
[2]25% wettable powder.
[3]Shoreline.
[4]Flowable liquid (2.07 lb/gal).
[5]Granules.

Table 2.–The adulticidal activity of BAY MEB 6046 against mosquitoes.

Species	Strain	Average[1] LC_{50}[2]	Average[1] LC_{90}[2]
Culex pipiens quinquefasciatus	S	.016	.021
Culex tarsalis	S	.0037	.0055
Culex tarsalis	OP-R	.0044	.0071
Aedes taeniorhynchus	S	.011	.019
Aedes nigromaculis[3]	OP-R	.018	.037

[1]Combined average of three tests (two replicates per test).
[2]In % solution of active ingredient applied to filter paper disks.
[3]Field-collected larvae reared in the laboratory to adults.

carboxylic acid, 2, 2, 3, 3 tetramethyl-, alpha-cyano (3-phenoxyphenyl, benzyl ester] and SD43775 [benzeneacetic acid, 4-chloroalpha-(1-methylethyl)-, cyano (3-phenoxyphenyl) methyl ester]. SD41706, like NRDC-143 (Figure 1), has a cyclopropane moiety, which increases potential manufacturing costs.

Both SD41706 and SD43775 were highly active to mosquito larvae in the laboratory (Table 3). SD43775 is also very effective against mosquito pupae (Table 4). Field tests with SD43775 against mixed populations of *A. nigromaculis* and *A. melanimon* larvae gave excellent results at 0.1 lb AI/acre (Table 5).

At Fresno, 1 m^2 outside ponds containing 70 gal water each, initially, were treated with 0.1 and 0.2 lb AI/acre of SD43775; duplicate water samples were collected 2 hr and 1, 2, 3, and 4 days after treatment. These were bioassayed in the laboratory using 4th-instar *C. p. quinquefasciatus* larvae. The persistence of SD43775 in the outside pond water is shown in Table 6; this compound persists for several days but apparently does not have long-term residual.

Table 3. Toxicities of SD41706 and SD43775 against mosquito larvae in the laboratory (ppm).

Species and Strain	Average LC_{50}	Average LC_{90}	No. Tests[1]
SD41706			
Culex pipiens quinquefasciatus-S[2]	.0029	.0050	2
Culex pipiens quinquefasciatus-R[3]	.0081	.017	3
Culex tarsalis - Jessup-OP-R[3]	.0041	.010	3
Culex tarsalis-S[2]	.0045	.0085	3
Aedes taeniorhynchus-S[2]	.012	.017	4
Aedes nigromaculis-OP-R[4]	.0087	.014	2
SD43775			
Culex pipiens quinquefasciatus-S[2]	.0086	.020	2
Culex pipiens quinquefasciatus-R[3]	.0093	.021	3
Culex tarsalis - Jessup-OP-R[3]	.0060	.014	4
Culex tarsalis-S[2]	.0039	.009	3
Aedes taeniorhynchus-S[2]	.024	.090	4
Aedes nigromaculis-OP-R[4]	.009	.020	4

[1]Multiple concentrations run in duplicate in each test.
[2]Laboratory strain, OP-susceptible.
[3]Laboratory strain, OP-resistant.
[4]Field-collected, OP-resistant.

SD43775 was found to have broad toxicity to nontarget aquatic organisms (Miura and Takahashi 1976), but in

Table 4.–Toxicity[a] of SD43775 to mosquito pupae in the laboratory (ppm).

Species and Strain	LC_{50}	LC_{90}	No. Tests[b]
Culex pipiens quinquefasciatus-S[c]	.0057	.0075	2
Aedes nigromaculis-R[d]	.0015	.0025	1

[a]Includes mortality in pupae plus adults unable to successfully emerge.

[b]Multiple concentrations run in duplicate in each test.

[c]Laboratory strain, OP-susceptible.

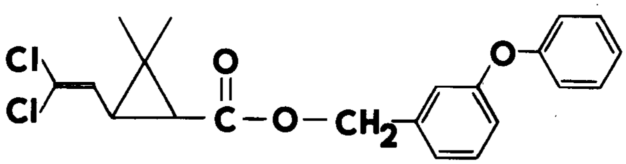
[d]Field collected, OP-resistant.

many habitats, e.g., irrigated pasures, nontarget organisms are of questionable importance. Also, in some cases, polluted waters used for irrigation devastate nontarget organisms while development of mosquito larvae is not impaired. Thus, there are many habitats where nonspecific compounds can provide mosquito control.

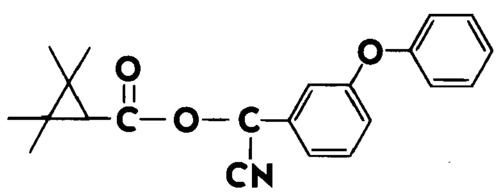
ACKNOWLEDGMENT.–The extensive support and cooperation from the Consolidated, Kern, Kings, Lake County, Tulare, and Turlock Mosquito Abatement Districts allowed for the successful completion of these studies.

Table 5.–Hand applications of SD43775 against mixed populations of *A. nigromaculis* and *A. melanimon* on .05, acre pasture plots in Kern County, California.

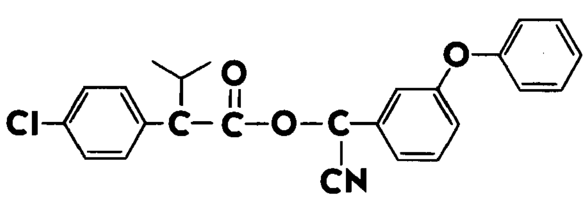
Field Test No.	Date (1975)	Dose AI/Acre	Larval Stages Present	% Mortality
75-10	7/1	0.1	4	100
75-12	7/15	0.05	4	∿94
75-15	7/22	0.025	2,3,4	∿80
75-18	7/29	0.075	4	90
75-21	8/5	0.1	3,4	100



NRDC 143



SD 41706



SD 43775

Figure 1.–Structures of pyrethroid-type compounds showing promising activity against mosquitoes.

Table 6.–Bioassay of water from 70 gal outside ponds treated with SD43775 on 8/3/75 (in % mortality of 4th-instar *Culex pipiens quinquefasciatus* larvae).

Dose	Sample Collection Time (days post-treat.)				
(lb AI/acre)	0[a]	1	2	3	4
.1	100	82	12	10	0
.2	100	100	64	20	0

[a]Sampled 2 hours after treatment.

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LABORATORY AND FIELD TESTS ON DEET REPELLENCY TO MOSQUITOES AS A MEANS OF PROTECTING SQUABS FROM FOWL POX

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Fowl pox (avian pox) is caused by a filterable virus that frequently causes commercial losses in the poultry and squab industry. Mosquitoes have been implicated in the transmission of pox. Research has demonstrated in the laboratory that *Culex tarsalis* Coquillett and *C. pipiens* L. are very efficient transmitters (Da Masa 1966). Birdpox viruses have also been recovered from mosquitoes and primarily from *C. fatigans* Wiedemann, taken in fowl runs in Australia (Lee et al. 1958).

In California the outbreaks of the disease seems to be seasonal in squabs and records by growers indicate they occur most frequently in the late summer. Commercial losses in fryers and squabs are due to skin blemishes or pox marks caused by the characteristic lesions. Egg production is occasionally affected.

At the request of the Turlock Mosquito Abatement District a preliminary study was conducted in 1974 to correlate the mosquito species populations in poultry ranches with the incidence of pox. The results were inconclusive. An additional study was also carried out to determine if a commercially available mosquito repellent such as Deet, could be used to protect squabs against a potential pox outbreak and to provide additional evidence confirming the importance of mosquitoes in the transmission of pox. A commercially available mosquito repellent was selected to reduce bites because of its availability for practical application in existing squab facilities.

MATERIALS AND METHODS.–Laboratory Tests: Newly emerged adult mosquitoes used in these tests were fed on raisins placed on top of a one cubic foot, screened cage. A piece of terry cloth was draped over the top of the cage and kept wet. The mosquitoes were held at room temperature, 10-21°C. Repellency tests were not conducted with mosquitoes until they were 3 to 4 days old.

A quantity of pine needles sufficient for a pigeon nest was dipped in a 50% ethanol solution of deet (N,N-diethyl-m-toluamide) and allowed to dry thoroughly before testing.

Young squabs 2 to 5 days old were placed in a cardboard pint ice cream carton with the cover punched out, and a piece of gauze netting held over the end by means of the band from the cardboard lid. Young squabs thus positioned were then introduced into the mosquito cage. Observations were made to determine that the female mosquitoes were seeking blood. This usually occurred within 2 to 5 minutes.

When it was determined that the mosquitoes were in a blood-seeking condition, the females probing on the gauze were disturbed and forced to leave and the pint carton was removed.

The clump of deet treated pine needles was placed on the floor of the mosquito cage on top of paper toweling. The young squab was reintroduced into the cage and placed in the middle of the pine needle clump, but without the protection of the gauze covered carton.

Observations were made at 10-15 minute intervals for one hour with the room darkened between observations.

To determine if untreated needles had any repellency effect under the experimental conditions, a clump of untreated pine needles was placed in the cage, and a young squab placed in the middle of the clump. Within a minute, three females had landed on the squab and were ready to begin feeding, indicating no repellent effect from untreated pine needles.

Weekly observations were made on the repellency of treated pine needles. After 14 weeks the repellent effect had diminished to the point where mosquitoes would feed on young squab.

In addition, treated pine needles from a nest where squabs of market age had just been removed were taken to the laboratory and tested for repellency. Five weeks had elapsed from time of placing treated pine needles in the nest to time of the test. The needles were heavily laced with feathers and pigeon manure, and failed to repel caged mosquitoes that had access to a young squab placed in the middle of the clump.

Field Tests: The critical period when squabs must be protected from mosquito bites is from hatching up to about 3 weeks of age. To test the efficacy of deet in the field, experiments were set up at the Warkentin Squab Ranch and the Snow Squab Ranch near Turlock. Pigeons are raised at these ranches in units containing 32 to 36 nest compartments each. The wood compartments measure approximately one foot in each dimension. Pine needles are used as nesting material rather than straw to reduce mite problems.

Nests with one to three day old squabs were selected for treatment. Pine needles were treated several hours prior to introduction into the nests to assure thorough drying of the repellent since wet pine needles were found to be toxic to day-old squabs. Solutions were prepared by diluting deet with an equal volume of ethanol or isopropyl alcohol. Pine needles were either dipped in this solution, or thoroughly saturated by spraying with a one pint household fly sprayer, or a one gallon air pressurized hand can with an adjustable cone nozzle. Nest compartments were thoroughly wet down with solution applied by sprayer. The landing board in front of the compartment, the top, back, and sides of the compartment were treated. Ten milliliters of solution were required to saturate pine needles, and 15 ml. were required to treat nest compartments.

FIELD TESTS IN 1974.– At the Warkentin Ranch, 6 compartments were sprayed with deet on the wooden surfaces only, 6 compartments contained only treated pine needles, and 6 had both treated wood surfaces and treated pine needles. All treatments at the Snow Ranch consisted of treated pine needles and wooden surfaces.

Table 1.—Incidence of fowl pox in squabs from deet treated and untreated nests, Warkentin Ranch 1974[1].

Number of squabs examined	Status	Number of Squabs with pox	Percent infected
12	pine needles only	3	25
12	wood surfaces only	3	25
12	pine needles & wood	2	16.6
20	untreated nests and compartments	17	85

[1]Nesting material and wooden compartments were treated on August 25, 1974. Squabs were examined for fowl pox on October 3, 1974.

Table 2.—Incidence of fowl pox in squabs from deet treated and untreated nests, Snow Ranch 1975[1].

Number of squabs examined	Status	Number of squabs with pox	Percent infected
320	untreated	10	3.12
256	treated	0	0.0

[1]Squabs were examined weekly between September 17, and November 17, 1975. Date of incidence 9/23, 2 cases; 9/30, 1 case; 10/7 2 cases; 10/14, 5 cases. No cases after 10/14. Peak mosquito activity 10/10.

Table 3.—Light trap counts of *Culex* spp. during period of fowl pox outbreak, Snow Ranch, south trap.

Date	Light trap counts	Date	Light trap counts	Date	Light trap counts
9/12	41	10/3	75	11/7	38
9/19	37	10/10	166	11/14	9
9/26	77	10/17	65	11/21	2
		10/24	45		
		10/31	61		

At the Warkentin Ranch, since most nests contain 2 squabs, 12 squabs were included in each category with a total of 36 squabs for all 3 categories.

A squab from each treatment category was slaughtered when it reached market size, and quick frozen for residue analysis of skin, breast muscle, and liver. The wood surface only and the combination of wood and pine needles were analyzed since funds were not available to have all categories analyzed.

An American Model Light Trap was operated at both ranches during the time the repellency experiment was being conducted.

Tissue Analysis: Squabs exposed to deet from sprayed wooden surfaces only, and from a combination of wooden surfaces and nesting material were analyzed by gas chromatography. The samples were extracted from minced tissue with acetonitrile in the presence of anhydrous sodium sulfate. Lipids were removed by partitioning with hexane and deet was partitioned into toluene. The extract was cleaned up through an activated florisil column. Detection of deet was by GLC (Gas Light Chromatography) using a nitrogen specific detector. No significant residue problems resulted from deet applications as evidenced by the data presented in Table 4.

Field Tests: The incidence of fowl pox in squabs at the Warkentin Ranch during 1974 are reported in Table 1, and the Snow Ranch during 1975 in Table 2. The results of light trap counts during 1975 are shown in Table 3. Fowlpox outbreaks occurred between September 9 and November 7 when 90 percent of the mosquitoes taken in the light trap were *C. pipiens*.

Additional Field Tests 1975: To supplement data from the Warkentin Ranch, additional tests were conducted during the 1975 mosquito breeding season. These tests were performed at the Snow Squab Ranch. The shed selected for treatment contained about 200 pairs of pigeons, some of which were not productive. During the test period, nests were examined weekly, and all pairs with 1 to 3 day old squabs had nesting material and wooden surfaces sprayed in the same manner as the preceeding year, except that isopropyl alcohol was used as the solvent instead of ethanol and the spray was applied by a one gallon hand can. Usually 15 to 30 nests were treated per week. In this series of tests

Table 4.—Deet residue analysis by gas chromatograph of squab tissue samples[1].

Samples identification	Part sampled	Residue (ppm)
wooden surface only	breast	0.1
wooden surface only	liver	n.d.[2]
wooden surface only	skin	0.4
wood & pine needles	breast	0.1
wood & pine needles	liver	n.d.
wood & pine needles	skin	n.d.

[1]Analyses performed by Stoner Laboratories, Inc., 409 Mathew St., Santa Clara, CA 95050.

[2]n.d. = none detected.

136 nests were treated, from which 256 squabs were marketed, none of which had fowl pox (Table 1).

Treatment Costs: The cost of materials to treat nests with ethanol solutions of deet was about $.10 per nest. This cost is reduced to about $.08 when isopropyl alcohol is used.

CONCLUSIONS.–Although the role of mosquitoes in transmission of this virus has not been unequivocally confirmed, the low incidence (16.6% to 25%) of fowl pox in squabs from deet treated nests in 1974, (compared to 85% in untreated nests) and the lack of fowl pox in treated nest in 1975 lend credence to the supposition that mosquitoes are an important vector.

ACKNOWLEDGMENTS.– Mosquitoes used in the laboratory tests were obtained from the University of California Mosquito Research Laboratory at Fresno. Light trap data were provided by the Turlock Mosquito Abatement District and Stephen M. Silviera, Manager, and Marc R. Pittman, Entomologist, assisted on various aspects of the study. Cooperation of the squab ranchers who participated in the study is greatly appreciated. Samples of deet were supplied by McLaughlin-Ghormley-King Co.

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FIELD TRIALS OF MOSQUITO REPELLENTS IN FLORIDA, ALASKA, AND CALIFORNIA

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ABSTRACT

Four repellents were tested under different climatic conditions found at Turtle Mound, Florida; Fort Wainwright, Alaska; and Colusa, California. Test repellents cyclo-hexamethylene-carbamide and n-butane-hexamethyleneimine-sulfonamide were found to protect as long as or longer than N,N-diethyl-m-toluamide, the standard military repellent, under all test conditions at equal concentrations. Trioxa-pentadecan-1-01 was statistically no different from deet. Mild temperatures in Alaska compared to Florida and California caused all repellents tested to be effective for 7-9 hours despite heavy mosquito populations.

INTRODUCTION. In areas where malaria, yellow fever, dengue, encephalitis, and other mosquito-borne diseases are endemic, mosquito repellents are important to the health of a mobile military force in situations where usual vector control methods are impractical. In view of increasing ecological restrictions on the use of pesticides and growth regulators in addition to mosquitoes developing resistance to these measures, an effective personal repellent can be an important part of individual protection in an overall program of mosquito control.

As a part of a program to find a longer-lasting mosquito repellent, field trials were conducted in North Carolina so repellent efficacy in laboratory testing could be compared to efficacy under field conditions (Shimmin et al 1974). It was felt that evaluation of candidate repellents under different climatic conditions and against different mosquito populations would be desirable for further comparison to laboratory results. Three different areas were selected: the hot, humid tropical-like summer climate in Florida where Gilbert et al (1957) have reported field testing, the arctic summer climate of Alaska where Gorham (1974) has reported heavy mosquito populations during repellent tests, and the hot, dry summer climate of California where heavy localized mosquito populations were found around irrigated fields. The current paper presents the results of those studies.

MATERIALS AND METHODS. Repellents: Repellent compounds tested were cyclohexamethylene-carbamide (carbamide), 99% pure (Dremova and Smirnova, 1970); n-butane-hexamethyleneimine-sulfonamide, 99% pure (Maslii and Razbegaeva 1966); 3,6,9-trioxapentadecan-1-01 (SRI-6), 96% pure (Skinner et al. 1974); N,N-diethyl-m-tolamide (deet), 95% pure (Eastman Chemicals, Practical Grade).

Test Design: A four-site field test method was employed in all three tests (Shimmin et al. 1974). Adhesive-backed foam strips about 2 cm wide were wrapped around the wrist, upper forearm, and mid-forearm of test participants. The foam strips served as boundaries and protective devices against abrasion for the sites. Foam strips outlined 2 sites on each forearm of every volunteer (4 sites per individual). One of the four test repellents was applied in ethanol solution to each site at a constant concentration per unit of skin area. The area of each site was determined by measuring the forearm circumference at points 3, 12, and 21 cm from the wrist. The surface area of each test site was calculated as the area of a frustrum of a cone: Area = $s \times (R_1 + R_2) \times \pi$ where s = 8 cm and R_1, R_2 are the radii at the upper and lower boundaries of the site.

The total area marked off by foam strips was covered with repellent solution for that site. Repellents were applied so no repellent appeared on a given elbow or wrist (right or left) site more than once in any group of four individuals. Moreover, all repellents were paired at least twice on the same forearm among 2 groups of 4. One site on each subject was a deet control. All results were analyzed using

Table 1.—Repellent protection times against mosquitoes, Turtle Mound, Florida[1].

Weather	Protection Time in Hours, Mean ± s_d			
	Deet	Carbamide[2]	SRI-6	Sulfonamide[3]
33°C, 80-85% RH, 0-5 mph wind	7.6 ± 1.0	9.8 +	4.5 ± 1.7	7.0 ± 2.5

[1]Repellent applied at 0.48 mg/cm^2; mosquito activity ranged from 1200 to 2000 body landings/hour.
[2]No carbamide-treated sites failed at termination of trial.
[3]One site failed in first exposure.

Table 2.—Repellent protection times against mosquitoes, Alaska. [1,2]

June	Protection Time in Hours, Mean ± s_d			
	Weather	Deet	Carbamide	SRI-6
25	16-26°C, Clear, 85-92% RH	7.4 ± 1.4	7.6 ± 1.0	7.2 ± 1.2
26	22-25°C, Clear, 39% RH	7.9 ± 0.7	7.8 ± 0.7	7.7 ± 1.2
27	14-16°C, Rainy, 88% RH	8.6 ± 1.6	9.2 ± 1.5	7.2 ± 1.3
Mean (24 total)		8.0 ± 1.3	8.2 ± 1.3	7.4 ± 1.2[3]
Ratio to deet			1.03	0.93

[1]Repellents applied at 0.40 mg/cm^2.
[2]Concentration of sulfonamide was lower than 0.40 mg/cm^2; hence, results are omitted.
[3]Significant at 5% level ($p < 0.05$).

a paired comparison with deet. Statistical significance was determined at the 95% level ($\alpha < 0.05$) by the student's t-distribution and Tukey's w-procedure (Ostle 1963).

After repellent applications, volunteers exercised or engaged in moderate physical activity as described below for each separate location. Head nets and gloves were worn whenever individuals entered mosquito-infested test areas. When a bite was received on any treated site, the individual recorded the site and the time in a notebook. A confirmed bite, one bite followed by a second bite within one-half hour, was the criterion for repellent failure.

Mosquito populations were determined by counting the number of mosquitoes landing on a single individual's body in a specified time interval. This number was then extrapolated to body-landings per hour. Alternately, the number of mosquitoes landing on an untreated control forearm were recorded for an individual who was not participating in the repellent testing. This number was recorded as forearm landings per hour. Mosquitoes were collected for identification from the same individual who was employed in population counts.

Turtle Mound State Park, Florida, was characterized by coastal brackish water and coniferous wooded areas with marsh vegetation. Repellents were applied at a dose of 0.48 mg/cm^2 to 4 subjects at 0700 hrs. Initial exposure to mosquitoes was from 1000 to 1230 hrs in the test area. Participants moved about in the test area to stir up mosquitoes. At 1330 hrs the group ran for 3-minutes to induce sweating and reentered the mosquito-infested area for 1 hour and 15 minutes. Since some repellents were still effective, volunteers hiked on the beach for 1 hour under the hot sun. At 1545 hrs, exposure to mosquitoes began again and continued until termination of testing at 1645 hrs, when one repellent-treated site on each individual was still effectively protected from mosquitoes. Mosquito activity ranged from 1200 to 2000 body-landings per hour.

Ft. Wainwright, Alaska test areas were typically taiga regions in low lying wooded areas between rivers and tundra. Each day repellents were applied at a dose of 0.40 mg/cm^2 to 8 subjects in 2 groups of 4. Sulfonamide had precipitated from solution, and the applied dose was somewhat lower. Exposure to mosquitoes began between 1200 and 1300 hrs after application at 0900 hrs. Continuous heavy populations of mosquitoes were experienced as volunteers hiked, forded streams and fished to simulate moderate field activity. Test areas were located north and east of Ft. Wainwright, Alaska, as follows: 25 June, N 64° 54'W 147° 15' on the Little Chena River; 26 June, N 65° 10' W 145° 10' on the East Fork Chena River; and 27 June, N 64° 18' W 146° 25' on the Chena River. If a repellent still afforded protection from mosquitoes at the termination of testing between 1700 and 1900 each evening, the time of termination was recorded as the repellent protection time. During June in Alaska, sunset occurred approximately 2300 hrs and sunrise about 0130 hrs, although there was enough light to read a book during the short night.

Colusa, California test areas were located along the banks of irrigation or drainage ditches where mosquito activity was apparent. Repellents were applied at a dose of 0.40 mg/cm^2 to 8 subjects in 2 groups of 4 at 0900 hours. Three replicates were run on 25, 26, and 27 September. Mosquito exposure was accomplished periodically during the day when participants entered mosquito infested areas; however, peak biting activity was observed from 1 hour before to one hour after sunset when repellent failures occurred. Participants recorded numbers of bites received on each site as well as the time. During the day volunteers engaged in hiking. Locations of mosquito exposure included a ricefield, the bank of a creek and a ditch next to the highway near the Colusa airport.

RESULTS.–Protection times against mosquitoes afforded by the candidate repellents in Florida, Alaska, and California are indicated in Tables 1, 2 and 4, respectively. Analysis of the results from Turtle Mound (Table 1) indicated that the site treated with carbamide protected for the duration of the trial on all four individuals. Hence, the protection time is recorded as 9.8 plus.

In Alaska constant mosquito pressure was experienced throughout the afternoon. Testing was terminated between 1700 and 1900 hrs each day although the sun had not set. Little or no sweating was observed among the participants because of mild temperatures; consequently, all repellents persisted for a significant period (Table 2). Intermittent light rain on the third day apparently had little effect on repellent protection. Mosquito populations are described in Table 3.

At Colusa no repellent-treated sites received two bites during the daylight testing; hence, the length of repellent protection was from application in the morning until the two-hour period of exposure around sunset. Consequently, all protection periods were restricted to the range 10 to 12 hours. A better means of comparing repellents under these conditions is the use of relative protection from mosquitoes compared to deet (Shimmin et al. 1974). The number of bites received on deet-treated sites was considered as the baseline for protection and candidate repellents were evaluated in terms of percentage more protection (or percentage fewer bites) than were received on deet-treated sites (Table 4). Mosquito activity and populations are described in Table 5.

DISCUSSION.– In Florida under hot, humid tropical-like conditions, test participants exercised sufficiently to induce profuse sweating. Under these test conditions against a heavy population of *Aedes taeniorhynchus*, carbamide was superior to deet (Table 1), while SRI-6 was somewhat less effective and sulfonamide was approximately equal to deet. This finding is in agreement with two laboratory observations. Higher temperatures favor low volatility repellents like carbamide, sulfonamide, and SRI-6 by raising their evaporation rate and increasing the amount of repellent vapor (Gabel et al. 1976). In addition to the temperature stress, heavy sweating also introduces a water washing

Table 3.–Mosquito populations for Alaska field trials.

Species	Collected From Whole Body June 25	June 26	June 27
Aedes communis	30	30	19
Aedes nigripes	-	3	-
Aedes cinereus	-	1	1
Aedes excrucians	-	-	3
Aedes pienips	-	-	1
Ophyra leucostoma[1]	-	1	-
Leptoconops sp.[1]	-	-	1
Mosquito Activity (Body landings/hr)	980	1200	2400

[1]Collected with mosquitoes.

Table 4.–Percent repellent protection, Colusa, Calif.[1].

Sept.	Weather	Percent Protection from Biting Compared to Deet: Deet [2]	Carbamide	SRI-6	Sulfonamide
25	22°C, 52% RH 5-9 mph wind	0	100	38	77
26	24°C, 50% RH 3-5 mph wind	0	38	48	24
27	29°C, 17% RH no wind	0	76[3]	76[3]	57

[1]Repellents applied at 0.40 mg/cm^2 to 8 subjects, testing took place 10 hours after application in the period one hour before to one hour after sunset; bites on each site were recorded.

[2]Mean number of bites received on the deet-treated site for each individual was 1.3, 2.1, and 2.1 bites/site for 25, 26, and 27 September, respectively.

[3]Statistically different from deet ($p < 0.05$).

Table 5.–Mosquito populations for California field trials.[1]

September	Collected from Forearms 25	26	27	Collected from Whole Body 25	26	2
Aedes dorsalis	25	28	2	14	20	2
Aedes vexans	1	6	-	1	4	7
Anopheles freeborni	2	1	1	-	1	2
Culex tarsalis	1	1	-	1	2	4
Mosquito activity[2]	116	144	12	68	108	72

[1]Mosquitoes collected in 15 min. from sites indicated.

[2]Landings/hr, a different control was used on forearm collections of 27 Sept; all whole body collections were from the same individual.

stress. Resistance to water is greater for carbamide than for deet (T. Spencer, unpublished results), whereas SRI-6 has somewhat less wash resistance than deet (A. Khan, unpublished results). Hence, one might expect carbamide to persist longer and SRI-6 shorter than deet under a sweating stress (Table 1).

In Alaska the mosquito populations were boreal species, principally *Aedes communis* (Table 3). Despite heavy mosquito activity, deet, carbamide and SRI-6 all afforded protection from mosquitoes for more than 7 hours. Sulfonamide results in this test are not comparable because the repellent had precipitated from the application solution and the applied dose was much less than 0.40 mg/cm^2. When testing was terminated each afternoon, some sites treated with carbamide, deet, and SRI-6 were still protected; therefore, the protection times are underestimated in the Alaska trials (Table 2). Although repellents were applied at 15% lower concentrations in Alaska than in Florida, both deet and SRI-6 exhibited an increased protection period in Alaska compared to Florida. This is attributed to cooler temperatures and the absence of a sweating stress on the treated sites in Alaska; hence, climatic conditions might influence repellent efficacy in the field.

In California the mosquito population was principally *A. dorsalis* and *A. vexans* (Table 5). One species, *Culex tarsalis*, is a known vector of encephalitis, while another species, *Anopheles freeborni*, is a potential malaria-vector. Although mosquito activity was somewhat lower than in Florida or Alaska (Table 4), the presence of these potential vectors was significant. Relative repellent efficacy in these tests was reported in terms of percent protection from mosquito bites compared to deet for the period one hour before until one hour after sunset. Warm, dry weather over the three days of testing (Table 4) introduced a high temperature factor more favorable to the low volatility repellents; moreover, test participants did not sweat significantly in the dry weather in contrast to the sweating stress observed in Florida. Consequently, SRI-6 as well as carbamide and sulfonamide afforded more protection than deet.

In these field trials where abrasion is not a major factor, carbamide provided as much or slightly more protection time from mosquitoes than deet under all three climatic conditions. SRI-6 afforded comparable protection to deet under conditions which did not include heavy sweating stress. Finally, sulfonamide was as effective as deet in Florida and California.

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THE ROLE OF WATER QUALITY DETERMINATIONS IN A VECTOR CONTROL PROGRAM

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The use of water quality determinations in various aspects of aquatic nuisance control is far from new, with several quality factors being critical to past ecology studies on vector species, while dissolved oxygen levels and bacterial counts have long been utilitized for pollution control and secondarily for mosquito and midge control.

However, a more extensive program of water quality measurement and monitoring may be significantly more effective today for control agencies than it was 20 years ago. Several factors account for this.

First, much of our mosquito, midge, and aquatic nuisance problems are a direct by-product of poor water quality, in different degrees, type, and stages, usually attributable to man's usage.

Second, the very awareness and concern by the public over environmental pollution can be utilized as a pressure, augmented by other regulatory agencies, to remedy these nuisance causes where the district may provide the factual data and documented correlation with specific vector or nuisance production.

Third, techniques and equipment for making such quality determinations have been greatly advanced, making reliability with reduced time consumption a practicality.

Fourth, advances in species determination of both vectors and dominant index organisms of the aquatic habitat, together with a proliferation of studies and evaluative findings concerning influence of various water quality factors on the aquatic biota, permit greater reliability in predicting nuisance development on a measureable basis.

Data as to specific water quality factors alone without correlated information on other environmental and ecological conditions and influences, is of little benefit to the District's objectives, although often helpful to other interests. The complexity of ways in which water quality influences vector production, prevention, or varied control aspects is too extensive to pursue in detail here. Interpretive use of such data is very dependent upon the analytical literature available. Although a listing of general references utilized follows, a great quantity of new findings pertinent to vector ecology is continually being published and periodic summaries of current literature, such as that on Eutrophication by the Water Resources Information Program of the University of Wisconsin, should be reviewed.

An elementary diagram is shown in Figure 1, listing some primary physical, chemical and biological factors interacting to govern productivity in the aquatic habitat which in turn provide the requisites for potential vector populations at nuisance levels. In more detailed consideration, nearly every factor serves as a limiting or enhancing influence, in accord with limiting ranges, on the growth and population potential for each species of organisms in the aquatic habitat. Preventive approaches or control measures may be directed toward even one of these factors to alter the nuisance potential, although some changes may serve to enhance different problems.

The use of selected species as biological indices and multifactor parameters from water quality data will permit greater predictability and reliability in establishing recommendations for water management and preventive measures for individual vector species as our knowledge of such species requirements are better developed with study and documentation. Obviously, the aquatic vector species which may be readily influenced by water quality management are those associated with higher nutrients levels, vegetative growth, and human usage and pollution of water sources. These include many species of midges, certain *Culex* mosquitoes and a variety of other aquatic Diptera.

A primary question is how can such water data and associated findings be put to use -- what are the benefits? Some of these are:

Promotion of awareness and concern by the public and vested interests in the nuisance potentials inherent with poor water quality in given sites,

Documentation of problems associated with water pollution and quality indices for aid through regulatory agencies or vested interests,

Documentation of aquatic conditions prior to corrective action as a justifying and protective measure against charges by regulatory agencies claiming negligent environmental harm,

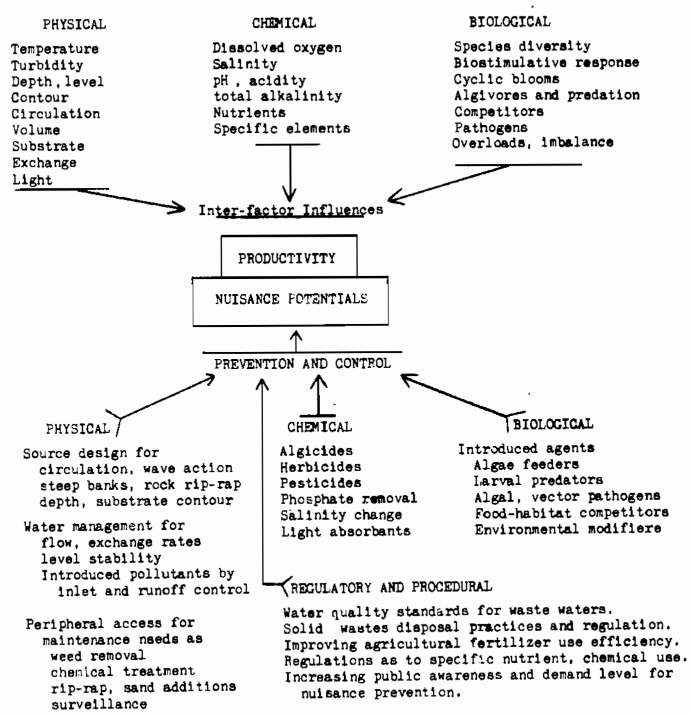

Figure 1.--Factors influencing development and reduction of aquatic nuisance potentials.

Establishment of yardsticks for predictability and reliability as to vector production potentials for use in water management recommendations,

Development of data input and recognized competence for participation in community and project planning of water usage, as well as guiding district program planning and input for environmental impact reports,

Provision of evaluative knowledge relative to environmental compatability for employment of an increasing array of biological control agents.

There are many subaspects of the above uses aiding in preventive and corrective actions by the control agencies. However, it is recognized that the benefits are dependent upon the types of water sources and problems within each agency's domain and their evolutionary stages. It is also recognized that use is dependent upon management policy and inclination, along with the requisite of technical personnel with interpretive capabilities in aquatic biology.

DISTRICT PROGRAM.– The Northern Salinas Valley Mosquito Abatement District initiated a multi-year program of water quality measurement and monitoring in 1975. Justification for the program entailed increasing problems of control in extensive empondments of agricultural and domestic wastewaters, many miles of agricultural drainage ditches emptying into the Salinas River channel, eutrophic, sediment-filled natural lakes, and potential extensive development of sewage treatment waters to be held in percolation lakes within the Salinas River Basin as proposed by the Corps of Engineers. Secondarily, the inhibition of the District's control functions of drainage and source modification created a need for measureable data and problem documentation to cope with the demands of multiple regulatory and planning agencies under a system of permits, project exemptions, and environmental impact concerns.

Although admittedly limited in terms of meeting the total objectives at desired levels, program facets were developed as follows:

I. Water source monitoring:
   A. Provision of data on a monthly basis for 10 water quality factors with pertinent notes as to problem and major modifying influences for mailing to pertinent interests as owners, local government, regulatory agencies, planning bodies, engineers as associated, and study groups upon request.
   B. Monitoring data in conjunction with supplementary observations for use in establishing trends and baselines on a multi-year basis.

II. Individual source analysis:
   A. Measurement and documentation of vector species production in study sources as feasible.
   B. Observation and quantitative documentation as to specific populations of dominant organisms, especially cyclic factors and blooms, potentially associated with vector species development.
   C. Correlation of water quality monitoring data and supplementary input on other environmental factors with nuisance productivity levels on a volumetric basis.
   D. Interpetive evaluation on problem potentials, causes, and evolution as a basis for corrective, preventive, or other recommendations.

III. Application:
   Multiple approaches affected by management and Vector-Ecologist toward the benefits previously listed by means of direct consultation, mailing of monthly data and annual summary evaluations, interagency communications and meetings, water use planning and management recommendations, and serving as a community resource of such quality data and nuisance potentials for other interests who commonly share similar objectives.

PROCEDURES.– The selection of equipment and methods, as well as limitations on the specific quality factors to be measured, are determined by the program objectives and feasibility levels affordable by agency resources, since both the number of latently significant quality factors and the kinds of sophisticated water quality determination instruments and methods are overwhelming. Although the demands of universities and research institutes certainly exceed the precision levels and specificity proposed for a district program, a relatively modest equipment outlay of four to five thousand dollars (at current costs) will provide for the projected needs of a basic program. This includes a spectrometric analyzer (primarily for nutrient factors), a portable dissolved oxygen meter with adjusting factors and probes, a good pH meter, nephelometer, and specialized glassware, samplers, reagents and minor items. These are independent of standard laboratory equipment and supply as incubator, refrigerator, deionizer, and microscopes.

The methods for determinations selected in this program have been in accord with those listed by the American Public Health Association, in collaboration with others, on Standard Methods for the Examination of Water and Wastewater, 1971 ed. The EPA also provides for standard methods which could be equally serviceable. Multiple methods are described for each different quality factor with equipment and reagent specifications, along with sampling procedures and an evaluative summary on method selection.

The basic determinations routinely provided by this District include:

Water Temperature	Filterable Orthophosphates
Specific Gravity	Ammonia-Nitrogen
Turbidity	Albuminoid Nitrogen
pH	Nitrate-Nitrogen
Dissolved Oxygen	Microplankton Cell Counts
5-Day B.O.D.	Dominant Organisms Noted

For phosphate determination, the ascorbic acid method (with spectrometry) is utilized (up to 0.25 ppm P read at 880 mu with infra-red tube & filter. Ammonia-nitrogen, recovered by alkaline distillation of 250 ml sample, is determined by nesslerization with spectrometry, as is also the case for albuminoid nitrogen after digestion with alkaline potassium permanganate. Nitrate tests utilize the brucine method with transmission measured at 410 mu.

The significance of albuminoid nitrogen, turbidity, ammonia, BOD, and other quality relationships is very much dependent upon the forms of organisms present in the sample, quantitatively and qualitatively. A compound microscope, utilizing a Whipple micrometer and Sedgewick-Rafter counting cell are used for this purpose.

It should be noted that the developments in instrumentation for a wide variety of quality factors permit rapid and accurate, as well as continuous, determinations with com-

Table 1.–Water quality data for sources in Northern Salinas Valley Mosquito Abatement District, February, 1976.

	February 25 Reclamation Ditch no. 1665 at Airport Boulevard	February 18 Salinas Oxidation Pond no. 1	February 18 Salinas Oxidation Pond no. 2	February 19 Salinas River at Monterey Highway	February 29 Salinas River at Blanco Road	February 18 Spreckels Holding Pond no. Old 1-E	February 18 Spreckels Holding Pond no. 5-6	February 19 Sherwood Lake Outlet Cove
Temperature, °C.	16.0	11.0	11.5	13.0	13.4	12.0	14.0	12.0
Specific Gravity (60°F.)	1.0009	1.0013	1.0012	1.0009	1.0012	1.0005	1.0006	1.0002
Turbidity, ppm Formazin	160.0	74.0	53.0	18.0	25.0	138.0	145.0	6.0
pH	8.6	7.9	8.5	7.9	7.45	7.9	8.2	8.2
Dissolved Oxygen, ppm O_2	9.8	10.2	9.4	10.4	5.0	5.2	2.0	10.6
5-Day BOD, ppm O_2	46.0	36.0	26.0	11.2	11.0	58.0	144.0	4.8
Orthophosphate-P, ppm P	0.09	0.125	0.13	0.10	0.14	0.00	0.03	0.02
Ammonia-Nitrogen, ppm N	1.51	3.35	0.93	4.05	5.30	5.64	4.52	0.00
Albuminoid Nitrogen, ppm-N	0.80	3.37	2.34	0.68	1.35	3.90	5.86	0.19
Nitrate-Nitrogen, ppm N	7.+	0.06	0.83	2.12	1.33	0.09	0.07	0.07
Microplankton, Cells/ml^3						80,000		

Notes: Spreckels Pond Old 1-E was only source of midge larvae (30/sq. ft.). Other eastern ponds with good growth of sulfur bacteria (*Thiophysa*) showed no larvae.
BOD levels were up in most sources with high bacterial action and NH_3 production.
Sherwood Lake BOD rise was due to wave action agitating bottom algae.

puter readouts employing sets of electronic probes and selective membranes. Full implementation by these methods, however, may entail greater cost outlay than most districts might care to undertake.

All water samples for monitoring purposes reflect surface levels, since most sites and problem sources are of relatively shallow nature – 6 feet or less in depth – with depth gradient changes minimal. For special problem studies, substrate level samples are taken, utilizing a Kemmerer sampler, or equivalent. Water samples (1 liter) are brought back under refrigeration in an ice chest to prevent changes where testing delay will be more than ½ hour.

Procedures throughout are designed to provide reliability, by approved methods, within that level of precision of significance to the interpretive values sought. More elaborate testing procedures and other determinations would admittedly provide for greater accuracy and precision levels, as would be required for many research projects, yet these also exceed program feasibility and justification.

Monthly monitoring data are mailed out as in the form illustrated in Table 1 for fifteen different locations representing water systems and comparative water conditions as well as specific nuisance sources. Annual summaries for the different sources are also provided, including visual graphs of quality factors for each site, as shown in figure 3, representing an almost chronic midge source.

DISTRICT BENEFITS.–Although many program objectives entail analysis and findings through time-trend documentation, this first six months of released data and report input has produced varied benefits. Among these are the following:

Use of our EIR water quality data input and summary to aid in appropriation of $90,000 for in depth study and initial modification of two lakes in Seaside currently causing severe midge problems,

Endorsement by Fish and Game for District action in algiciding lakes for midge control followed by fish die-off, with justification based on monitoring data prior to treatment,

Requests by State and Regional Water Quality Control Board for data leading to corrective actions in pollutant discharge into prime midge sources,

Cooperative effort by a recreational lake manager in maintenance and algae control (as well as earlier in design) for effective problem avoidance,

Cooperative efforts by management in major industrial waste ponds (Spreckels) for biological management through cultivation of specific bacteria and exchange rates to minimize nuisance potentials,

Aid in providing input and district considerations into a feasibility report affecting establishing of a major natural preserve in the Elkhorn Slough,

Provision of evaluative understandings as to the actions of algicides and insecticides in midge control treatment.

SUMMARY.– The use of water quality data and monitoring program can constitute another valuable tool for local agencies in coping with the increasing problems of intragovernmental regulation, conservationists concern and the need for new control approaches. Such a program facet is primarily directed towards preventive planning, water management recommendations, documented justification for control actions taken in regard to environmental impact, and development of improved predictability on a measurable basis for aquatic vector and nuisance production. The feasibility and extent of such a program is necessarily dependent upon management approaches and the types of water sources, nuisance problems and stage of water management awareness in the agency locality.

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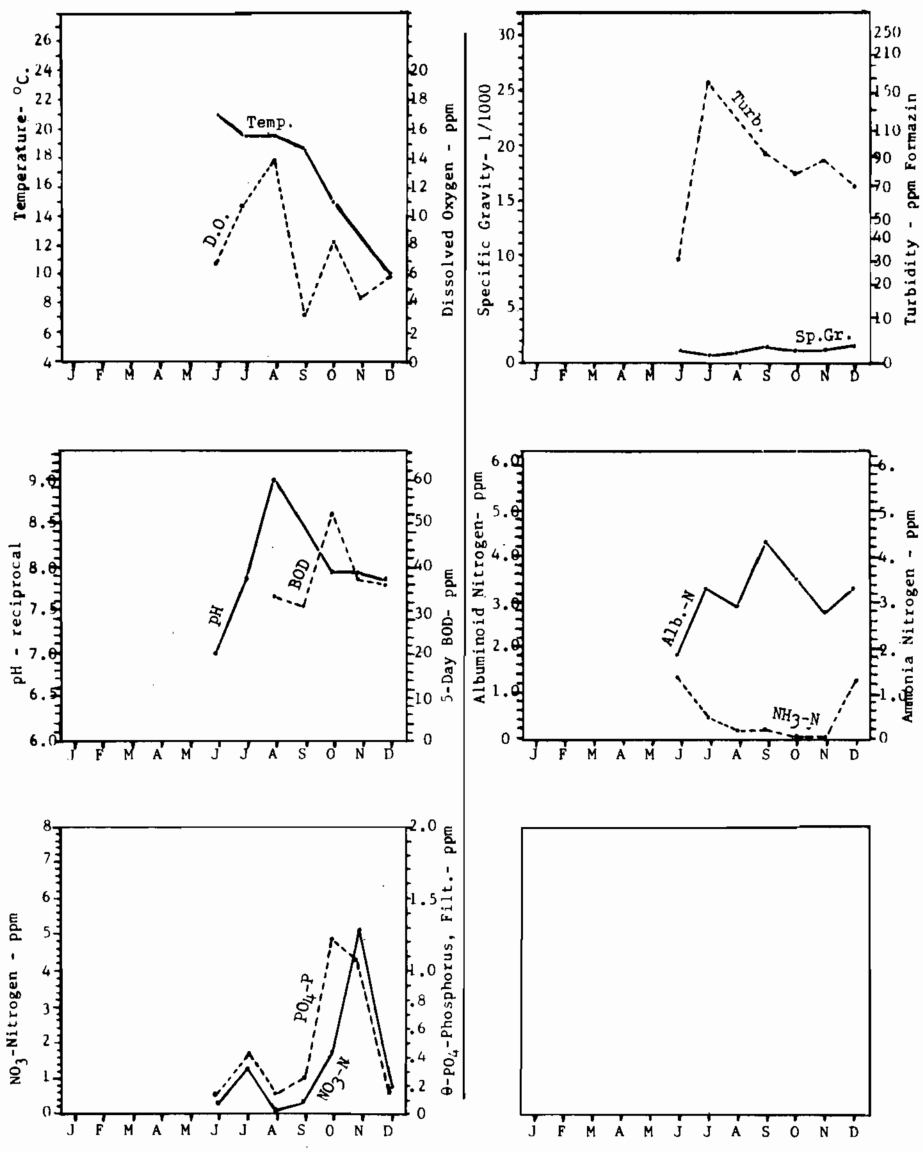

Figure 3.—Salinas oxidation pond no. 2 – 1975.

PROCEDURES FOR THE MASS REARING OF *CULEX TARSALIS* IN ARTIFICIAL OUTDOOR PONDS[1]

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INTRODUCTION.–Different strains of *Culex tarsalis* have been found to differ in their ability to serve as vectors of western equine encephalitis virus, and the differences appear to have a genetic basis (Hardy et al. 1976). Efforts are under way in the Arbovirus Research Unit, University of California, Berkeley, to develop a laboratory strain of incompetent vectors. Moreover, it is hoped that this trait can be linked with a chromosomal translocation so that mortality will occur in a significant proportion of the offspring. Once a *C. tarsalis* of this type has been engineered, pilot studies will be made to determine the success of releases of large numbers of the modified mosquitoes and their impact on native *C. tarsalis* populations. The pilot studies will be done first in large outdoor cages and later in small, geographically isolated field areas. The purpose of this presentation is to describe equipment and procedures developed for mass rearing and field releases of *C. tarsalis*.

We believe that a most efficient and economical approach for the release of genetically modified mosquitoes will be to produce large numbers of egg rafts in the laboratory and to transport them to standardized artificial ponds in the field. Larvae, pupae, and adults will then be reared under controlled but semi-natural conditions and known numbers of adults released. Accordingly, we have designed and constructed a number of identical rearing units (Figure 1).

POND DESIGN.– A pond is made by draping clear 6-mil polyethylene sheeting over a wooden frame. The frame, which rests on the ground, is made of 2 x 6-inch boards and forms two 45 x 69-inch compartments. These are filled with water to a depth of 4 inches. This method of making ponds is a modification of that used by Edman and Lea (1972) for mass rearing *Culex* mosquitoes in Florida.

A pyramid-shaped cover, made with light-weight wood frame-work, fits snugly onto the upper edges of the lower supporting frame. The cover is hinged to one of the edges, so it is firmly held in place and can be raised to give easy access to the water. The tapered upper sides are made with opaque polyfiber sheeting and are painted white on the outer surfaces, and the vertical lower sides are made with cellulose mosquito screen. At the apex is a 1-ft^2 piece of plywood with a hole 3-½ inches in diameter at the center. The hole is either plugged to retain mosquitoes or opened to permit their capture or release. This cover differs in size and shape from a similar structure described by Edman and Lea (1972), but in many respects follows their example. The cover suppresses light intensity and, thus, algal growth; prevents excessive heating and evaporation of water; excludes unwanted insects, including mosquito predators; deters birds and other vertebrates from using or damaging the ponds; and restrains and shelters emerged adults and allows their capture or release. The screened areas provide ventilation that prevents water condensation on the inner surfaces.

To measure adult production, a light trap is mounted over the opening at the top of the cover (Figure 1). The trap is of the type described by Chaniotis and Anderson (1968), but is modified to operate from a 6-v motorcycle battery. The trap is operated through each night of the period of adult emergence until a collection equals less than 5% of the largest earlier collection.

REARING PROCEDURES.– The rearing units were used experimentally in 1975 to rear *C. tarsalis* from a laboratory colony derived from specimens collected in 1971 at Knight's Landing, Yolo County, California. Objectives were to test the equipment and methods for handling mosquitoes and to evaluate various larval densities and feeding regimes.

Egg rafts were placed in ponds the morning after a night's oviposition in the laboratory. A sample of 50 to 100 rafts from each oviposition was tested to determine the proportion with viable eggs, which ranged between 75 and 95%. Usually 150 rafts were used per pond, although 225 or 300 were used in some trials. In a sample of 100 rafts, the mean number of eggs per raft was 227. Thus, if 85% of the rafts contained viable eggs, and if all of the eggs in fertile rafts hatched, 150 rafts would yield 29,325 1st instar larvae. This equals 9.4 larvae/inch^2 of water surface.

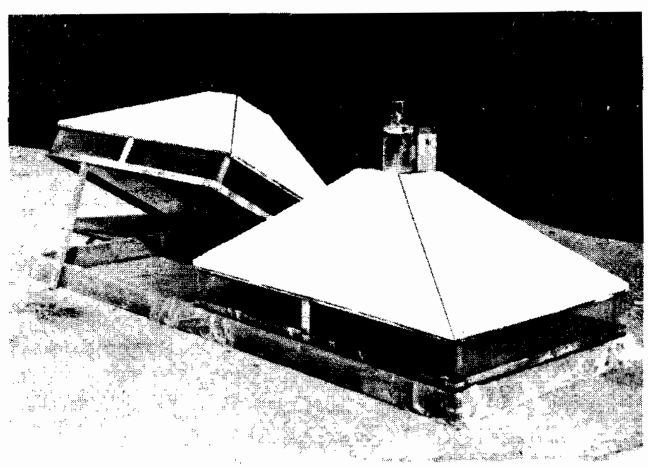

Figure 1.—Outdoor rearing units for *Culex tarsalis*.

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More than 3,000 rafts were obtained in one night's oviposition, in four 18 x 18 x 18-inch cages, by females reared in the laboratory from 150 rafts. The latter were placed in larval pans about 3 weeks before the mass production of rafts was desired. The females were blood-fed on chickens 4 nights prior to oviposition and were 3 to 7 days old when fed. The number of rafts, and the proportion with viable eggs, were both greater on the first than on the second or third night of oviposition.

Larvae were fed a mixture of 2 parts (by volume) TetraMin® (a commercial fish food), 2 parts Albers® Rabbit Family Ration, and 1 part brewer's yeast. The efficacy of TetraMin® in food preparations for *C. tarsalis* larvae was noted by Hayes et al. (1974). Each ingredient was autoclaved at 250°F for one hour and finely ground in a blender, whereupon all ingredients were thoroughly mixed. When 150 egg rafts were used, 12.5 g of food were scattered over the pond the day it was seeded with rafts and every other day thereafter through day 14. The amount of food sometimes was proportionately increased when a larger number of rafts was used. With the above quantities of food, water remained clear and scum-free without aeration or replacement of water lost by evaporation.

The length of time from egg placement to first adult emergence closely correlated with water temperature and ranged from 10 days in summer to 25 in spring. Adult trapping periods lasted from 8 to 13 days. In 9 trials from mid-April to mid-September, the mean number of adults per pond seeded with 150 rafts was 9,030. Males comprised 55% of the overall total adults, but collection of males probably was favored by the trapping schedule. The maximum number of adults from 150 rafts was 12,272. However, the greatest production, 15,147 (52% males), was from a pond seeded with 225 rafts. Comparison ponds with 150 and 300 rafts had 9,259 and 9,366 adults, respectively. Although the amount of larval food was adjusted according to the number of rafts, the pond with 300 rafts produced fewer and noticeably smaller adults than did the one with 225.

DISCUSSION AND CONCLUSIONS.– The equipment and procedures described were satisfactory for the mass rearing and field releases of *C. tarsalis*. But the numbers of adults collected were well below the estimated numbers of 1st-instar larvae. Possibly the hatch rate of eggs in fertile rafts was significantly less than assumed. However, the differences observed probably also were related to unobserved mortality in larval and/or later stages. To reduce mortality and increase adult production, we are testing other larval densities and feeding regimes, so the final procedures may differ from those described.

ACKNOWLEDGMENTS.– The described equipment and procedures were developed in collaboration with numerous individuals, including in particular Drs. W. C. Reeves, C. J. Mitchell, and R. K. Washino, all of the University of California.

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THE EFFECT OF MOSQUITO AND HOST SPECIES ON THE SUCCESS OF HEMOGLOBIN CRYSTALLIZATION METHOD FOR BLOOD MEAL IDENTIFICATION

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ABSTRACT

The hemoglobin (Hb) crystallization technique to identify mosquito hosts was evaluated in the laboratory. Reference mammalian and avian Hb crystals were prepared for comparison with crystals from mosquito blood meals. Mammalian Hb crystallised more readily than birds with the present reagent. Crystallization success was primarily determined by host blood solubility regardless of mosquito species and time in the midgut. The technique was most effective with insoluble Hb. Digestive processess or the midgut environment in *Aedes nigromaculis* appeared to differ from other mosquitoes, adversely affecting the more soluble Hb.

The main challenge in developing this technique for use with Californian mosquitoes, particulary *Anopheles freeborni*, was the inhibition of cow Hb crystal formation from mosquito blood meals, even though they formed readily from cow whole blood samples. Isoelectric focusing indicated that 6 hours after ingestion, 50% of the native Hb had been modified. This loss in Hb concentration should not have prevented Hb crystallization. Modification of buffer molarity and pH produced a reagent that increased cow Hb identification success rate from 0-30% to 50-90%. Individual meals were split longitudinally which allowed each meal to be tested by the two reagents.

MOSQUITO BREEDING AND PREVENTION IN IRRIGATION DRAINS AND BACKWATERS IN PALO VERDE VALLEY, CALIFORNIA

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ABSTRACT

The prevalence of mosquito larvae in irrigation drains and backwaters of the Colorado River was studied from March - November, 1975, in the Palo Verde Valley of California. The drains which drain and carry agricultural excess water back to the Colorado River, anastomose the valley and amount to some 150 miles.

To study the effects of mechanical clearing, drains recently cleared on one side or both, and drains uncleared were selected. Anywhere from 2-8 sites were established where larval sampling was carried out. At each site, 10 dips were taken, concentrated and the larvae and predators were preserved in 95% ethyl alcohol. The sample was analyzed and counted in the laboratory. Water velocity in the drains was 0-2 ft/sec, water temperature 63-78°F (March - October).

About 666 samples (10 dips each) were taken. A few of these were positive for mosquito larvae. Most of the positive samples contained 1-2 stage larvae, very few had 3-4 stage, and no pupae were recovered. Only the upper vegetated ends of the drains where water was stagnant were positive for mosquito larvae. Drains cleared on one or both sides, supported no larvae. Most of the samples from the drains contained *Gambusia affinis* and other predators.

Three expanses of backwater were sampled throughout the study period, where 209 samples (10 dips each) were taken. Only one of the backwaters showed significant numbers of larvae, including 3-4 stages. Most of the samples also contained mosquito fish and other predators.

From these studies, it can be concluded that irrigation drains (amounting to 150 miles) supported little, if any, larval breeding. Clearing on one or both sides provide a good control measure when necessary. The backwaters probably produce more larvae than the drains, but even this production is far less than that in the irrigated fields such as pastures, truck crop and vegetable crop fields, irrigation ditches and other surface water situations in the Palo Verde Valley.

INTRODUCTION.–During 1973 and 1974, mosquito light trap collections, carried out by the Riverside County Health Department, showed high numbers of *Culex tarsalis*, *C. p. quinquefasciatus* and *Psorophora confinnis* to prevail in the Palo Verde Valley. Detection of WEE virus in mosquito pools by the State Department of Health laboratory, signaled the danger of possible encephalitis epidemic in this valley. An emergency mosquito control program consisting of adulticiding was instituted in 1973 (Stains 1974). This program, on a limited scale, was continued into 1975 around and in the city of Blythe.

In order to organize and institute an effective mosquito control program, it became necessary to assess and determine the potential breeding capacity of the various biotopes producing mosquitoes. Irrigation drains, which amount to some 150 miles total length, anastomose Palo Verde Valley. At the request and support of the Palo Verde Irrigation District (Blythe, California), the current studies were initiated in 1975 to assess the role of irrigation drains, as well as backwaters of the Colorado River, in the production of pest and vector mosquitoes. The effectiveness of mechanical clearing of vegetation in the drains by PVID on mosquito production was also assessed. The contribution of spills carrying mosquitoes from agricultural fields into the drains was also investigated.

SAMPLING PROCEDURES.–Irrigation Drains - The Palo Verde Valley in southeastern California takes about 1 million acre-feet of water from the Colorado River annually for irrigation purposes. About 50% of this water, after being spread over crop land, is collected, drained and discharged back into the river at the lower end of the Valley. An impressive system of drains, amounting to 150 miles, is established throughout the Valley to collect and drain waste agricultural water.

Some of the drains are heavily vegetated at times (Figure 1) and it is not possible to keep them clear of vegetation during mosquito breeding season. It was postulated that these drains clogged with vegetation are an important source of mosquitoes. Therefore, the current studies were initiated to determine this potential.

For sampling, those drains or portions that were considered to be likely mosquito producers were selected. To determine the effect of clearing of vegetation, drains cleared on one side or both were also selected (Figure 2). The drains selected for sampling are listed in Table 1, along with the number of sampling sites and the physical condition. The sampling sites were located 0.25-0.5 m downstream from each other.

At each site, 10 dips were taken weekly (March - November 1975), the contents transferred to a concentrator cup (Mulla et al. 1975), excess water removed and transferred to vials to which 95% ethyl alcohol was added. The samples were brought into the laboratory at University of California, Riverside, where mosquito larvae and some nontarget organisms were counted and identified. Additionally, water velocity and temperature were measured each time.

Backwaters - Backwaters of the Colorado River at Goose Flats, 24th Avenue and 10th Avenue were selected. These recreational areas are located adjacent to residential areas along the river. Six sampling sites were established in locations deemed productive for mosquitoes. Weekly (March - November 1975) samples (10 dips each) were taken in the manner described above. Water temperature was measured each time the sites were sampled.

Irrigation Spills - Since the irrigation drains are as much as 5-20 ft below the surface ground, the excess water from the irrigated fields is drained through pipes into the drains. It was suspected that mosquito larvae breeding in the fields

Table 1.—Locations and number of sampling stations for mosquitoes in irrigation drains, Palo Verde Valley, California (1975).

Drain	Vegetation status[a]	Sampling sites	Nearest intersection[b]
Westside	Cleared	3	14th - Neighbors
Westside	1 side cleared	8[c]	18th - Neighbors
Lovekin	Heavy, clogged	5	16th - Broadway
Goodman	Heavy, clogged	5	14th - Intake
Fisher	Heavy, clogged	3	20th - Neighbors
Central	Heavy	4	16th - Arrowhead
11th Avenue	Heavy	3	11th - Intake

[a]Vegetation consisted mostly of tules growing along the sides and the bed of the channel where not cleared.

[b]Closest intersection of the start of sampling sites which were designated 0.25-0.5 m downstream from each other.

[c]4 sites on each of cleared and uncleared sides opposite each other.

Figure 1.—Lovekin drain clogged with tules. Most habitats of this type, where water flowed at 0.5-1 ft/sec and contained good populations of *G. affinis*, were free of mosquito larvae.

are carried with the excess water into the drains where they probably develop and turn into adults.

Plankton tow nets, with collection vial fitted with screen, were held in front of the spill pipes carrying water from fields supporting mosquito larvae. Larvae collected in the collection vial, if any, were preserved in alcohol for counting and identification.

RESULTS AND DISCUSSION.—Water velocity in all the drains and at all stations, ranged between 0.0-2 ft/sec. Water temperature from March - October, 1975, ranged from 63 - 78°F, the lower temperature prevailing in March and April while the high temperature prevailed during the rest of the season. Most of the samples from all drains contained *Gambusia affinis* and other predators. The status of mosquito breeding detected was as follows:

1.- Cleared Drains - Of the 96 samples collected, only 3 samples taken in July from Westside drain showed any larval presence. The larval density was very low (0.01 1st and 2nd stage per 10 dips). These larvae probably were washed into the drains from spills. Since the larvae were young,

Figure 2.—Goodman drain, dredged and cleared during 1975. No mosquito breeding detected in these types of drains.

Figure 3.—Outfall drain at the lower end of Palo Verde Valley, collecting and carrying water from other drains. Drains of this type have good water flow at all times, full of mosquito fish and other predators, and no potential for mosquito breeding exists here.

their survival to older stages in the presence of many predators (*Gambusia affinis* and others) was doubtful. The water current here was swift, ranging 1-2 ft/sec. This portion of the drain was heavily populated with *G. affinis* and other predators.

2.- Uncleared Drains - 5 drains with heavy tule growth and other weeds which were considered likely sources of mosquito breeding were selected. In the Lovekin drain (water flow 0.5-2 ft/sec), 143 samples (10 dips each) were taken. *Culex tarsalis* breeding (0.25-0.3/10 dips) were detected in March and June samples. These larvae were 1st and 2nd instars, possibly coming from spills or local oviposition. This drain was heavily populated with *G. affinis* and other predators, as these were recovered in every sample. It is unlikely that such a small infestation of larvae will survive the fast current and predators.

Fisher drain, which was also clogged with vegetation, showed little or no breeding of larvae. Of the 93 samples taken, only 1 showed a density of 1/10 dips of *C. tarsalis* in March. The larvae again belonged to 1st and 2nd stage, indicating absence of significant vector maturation in this drain. Water in this drain was quite stagnant and velocity ranged 0.3-1 ft/sec. This drain also supported heavy populations of *G. affinis*, as they were recovered in every sample.

In portions of the Goodman drain, clogged with tules and other weeds, water was quite stagnant, flowing at 0.0-1 ft/sec. Of the 99 samples (10 dips each), only 2 samples contained larvae of *Culiseta inornata*, *C. tarsalis* and *C. erythrothorax* during the period March - October, 1975. The larval density was low most of the time, but on one occasion, it reached 27/10 dips. All larvae recovered were either 1st or 2nd instars. This drain was full of *G. affinis* as they were recovered in every sample. Other predators were also prevalent in this drain. Since 3rd and 4th instar larvae were not recovered, it is logical to conclude that predation suppressed the larval populations.

Central drain was also heavily vegetated, water was stagnant, flowing at 0-1 ft/sec, and here 126 samples were taken. Of these samples, 21 were positive for *C. tarsalis* and *C. erythrothorax* larvae. The larval density in these samples was relatively high, ranging from 11-100 larvae per sample (10 dips). Third and 4th stage larvae were recovered in only 4 of these samples. The younger larvae outnumbered the other larvae in all the positive samples. In those samples where larvae were recovered in large numbers, few or no *G. affinis* were recovered. The larvae recovered were: *C. inornata*, *C. tarsalis* and *C. erythrothorax.*

About 50 samples were taken from 11th Avenue drain which was clogged with tules and other weeds. None of these samples showed any larval populations. Mosquito fish were recovered in each and every sample taken from this drain.

3.- Other Drains - Most of the drains in the lower end of the Valley are large and deep, carrying substantial amounts of water (Figure 3). These drains support good populations of *G. affinis* and other predators. Water current is swift. Repeated sampling of these drains turned up no mosquito breeding. In some situations, fluctuating water may inundate and flood shallow shore lines, and here some breeding may occur occasionally. No data were obtained on these situations.

4.- Backwaters - Water temperature in these waters was 63-78°F. Of the 136 samples taken here, several showed presence of significant numbers of larvae. Mosquito fish were recovered from most of the samples.

The 24th Avenue backwaters showed a very low level of mosquito breeding. Of the 88 samples taken, 3 samples showed 1-2 larvae (*C. tarsalis*) per sample. These larvae were 3rd and 4th stages. Most of the samples contained mosquito fish and other predators.

The 10th Avenue backwaters supported fair number of *C. inornata* and *C. tarsalis* with the latter predominating. Seventy-three samples were taken here. There were 8 positive samples on 5 occasions, containing 0.7-80 larvae/10 dip sample during March - June, 1975, and 1 positive in August. Most of the larvae were 3rd and 4th stage. Most, but not all of the samples, contained mosquito fish and other predators.

5.- Spills - Anywhere from 12-16 samples were taken monthly for assessment of mosquito larvae finding their way into the spills from irrigated fields. From March - July, 1975, only 2 samples were found containing larvae. These larvae were young *Aedes* or *Psorophora* larvae transported into the flowing water through the spill pipes into the drains. On account of the fast flow of water and pressure of predaceous organisms, it is very unlikely that these washed-in larvae will survive to maturity. Therefore, the contribution of larvae coming through the spills seems to be minor. Further studies will be needed to assess this phenomenon further.

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CONTINUING STUDIES ON THE NATURAL MORTALITY OF MOSQUITOES IN RICE FIELD HABITATS

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The survivorship of larval *Culex tarsalis* was measured in 36 rice fields in Sutter, Yuba and Sacramento Counties differing markedly in their fauna, flora, and physical attributes. Gallon-size plastic buckets with organdy screen windows on the sides and bottoms excluding all macroscopic predators were placed four to a field. Twenty-five third instar *Culex tarsalis* larvae were placed inside each bucket and each bucket was capped with a screen top. On a daily basis larvae which had died and adults which emerged in each bucket were counted and removed. Additionally, for each field we assessed: 1) the aquatic fauna by sweeping on biweekly basis with aquatic nets; 2) the relative abundance of ten of the dominant macroscopic algae and vascular plants; 3) the qualitative ocurrence of microscopic species of algae; 4) the abundance and types of zooplankton; 5) total aerobic bacteria counts in field water samples; 6) the content of chlorophyll and other pigments in water samples; 7) the fish fauna and; 8) the concentration of Ca^{++}, NO_3^-, NH_3^+, PO_4^-, Ca^{++} and hardness, alkalinity, and pH in water samples.

Mosquito-rich fields displayed high larval survival (greater than 80% per week) in the field emergence cages. Fields with few mosquitoes at the time, often displayed poor larval survivorship (less than 25% per week) even though all macroscopic predators were excluded from these cages. Yet, a number of fields with very few mosquitoes, nevertheless, displayed high larval survival. These latter fields, inevitably fell into two groups; either they had been stocked with *Gambusia* or they were relatively cold having an average summer mid-day temperature below 75°F.

An attempt was made to identify the sources of mortality in these fields by selectively altering the conditions within the emergence cages. In 26 rice fields we repeated these experiments, except that now two of the buckets received supplemental food (tetramin) while the two remaining buckets in each field did not. No significant increase in larval survival was detected. In two fields with particularly low survival rates other additives were tested, namely: 1) an algaecide, 2) vitamin supplements, 3) a fungicide, 4) a plant nutrient solution, 5) *Elodea*, 6) calcium carbonate, and 7) aeration. In no case was a significant increase in survival detected.

Since larval mortality is extremely rapid in these particular fields (above 50% in one day), food limitation was not indicated. Subsequent experiments testing the toxicity of field algae samples and potential disease transmission also proved negative. Upon close examination of the contents of these field buckets a clue was gained to the possible source of mortality. Tiny microscopic (under 1.0mm in length) flatworms were present within the cage. In the laboratory, two of these individuals were introduced into a 300 ml cup with 20 second instar *C. tarsalis* and observed under the microscope. Complete larval mortality occurred within 2 hours. Watching this, we noted that an immediate "paralysis" of the larvae resulted when they contacted the worms. More extensive field sampling revealed that at least two types (species?) of flatworms are involved.

New emergence buckets were constructed with a very fine mesh nylon fabric (50μ openings) through which the worms could not pass. These were placed, as before, in those fields which previously yielded poor larval survivorship. The older coarse-mesh buckets were also used as controls. In all cases there was a significant enhancement in survivorship with the fine-mesh buckets. Yet, survival rates were still below levels obtained in some rice fields supporting large mosquito numbers. Flatworms were never found in these lattter fields. Hence, we suspect that some additional mortality agent is associated with these worms which can pass through the fine-mesh fabric.

A COMPARISON OF NORTHERN CALIFORNIA POPULATIONS OF *CULISETA INORNATA* (WILLISTON): A PROGRESS REPORT

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A two year study was initiated during the fall of 1975 to assess population characteristics of both larval and adult *Culiseta inornata* associated with Suisun (coastal) Marsh, Central Valley, and Sierra foothill habitats of Northern California. These habitats represent a transect of Northern California at a latitude of 38°L. A tabulation of principle larval and adult stations surveyed on a biweekly schedule is presented in Table 1. Light trap locations have been designated as either rural or urban. All larval sampling stations were located in rural areas.

Assessment of larval populations included the determination of relative density (i.e., number of larvae-pupae per dip) recovered in three twenty dip samples taken from each station. In addition measurements were recorded for pH, temperature (14 day maximum-minimum), conductivity (ppm NaCl), and primary productivity (mg/M^3 total chlorophyll). Climatological data on atmospheric temperature and precipitation was obtained from U. S. Weather Bureau Stations at Fairfield, Davis, Yuba City, Folsom Dam, and Rocklin.

Adult activity and relative abundance were assessed by 5 lb. dry ice-baited CDC miniative light traps and standard New Jersey light traps operated one night every two weeks at each location. Physiological-age of adult female *C. inornata* collected by CDC light traps was determined by the Polovadova method (Detinova 1962). Autogeny rates were determined in females reared from field-collected 4th instar larvae and pupae. Mated females were held in one-gallon cannisters, given access to 10% sucrose, and held for ten days at 21°C under a 14-10 LD photoperiod. Individuals with primary oocytes beyond Christophers' stage II were considered autogenous.

RURAL LIGHT TRAPS.– Dry ice baited CDC miniature light traps operated in rural areas collected the greatest numbers of female *C. inornata* (Table 2). The combined collections from CDC traps located on Suisun Marsh (i.e., Joice and Grizzly Islands) yielded early and mid-seasonal collections substantually higher than collections obtained from an equitable number of traps operated concurrently in valley and foothill areas. Combined collections from CDC trap operated in the Central Valley (i.e., Dixon and Sutter) showed levels of female activity well above that observed in the foothills, but decidedly lower than at Suisun Marsh. Lowest levels of activity were observed in the Sierra foothills with a maximum of 5 females collected in the CDC traps operated at Folsom and Roseville during mid-October, 1975. Warmer evening ambient air temperatures occurred during the period of maximum female activity on Suisun Marsh. This condition perhaps, selected for prolonged biting activity, resulting in substantually higher CDC light trap recoveries on Joice and Grizzly Islands. Temperature regimes experienced in valley and foothill areas were typically colder.

The greatest number of females collected by standard New Jersey light traps was taken on Grizzly Island. Combined New Jersey light trap collections for Dixon and Sutter yielded numbers in the same magnitude, but individual station traps collected significantly fewer females. Due to logistical problems, no New Jersey light traps were operated in conjunction with CDC light traps at any rural foothill locations.

URBAN LIGHT TRAPS.– Results from urban light trap collections indicated significantly lower levels of *C. inornata* activity than observed in rural areas (Table 3). Female activity in Fairfield was not recorded until October which was approximately 7 weeks after the first collections were taken on Joice Island. Initial recoveries for foothill and valley stations, however, occurred in late September and early October respectively. The New Jersey light trap operated in the Sierra foothills (i.e., Fair Oaks) failed to collect a single female in nine nights of trap operation. The largest urban collections were taken in Fairfield. The number of females collected in Sutter light traps represented levels of activity somewhat intermediate to Fairfield and Fair Oaks observations.

PHYSIOLOGICAL AGE.– Physiological age was determined for 415 female *C. inornata* collected in dry ice-baited CDC miniature light traps operated on Joice and Grizzly Islands in Suisun Marsh. A subsample of 50 females was selected at random from the total when collection size prohibited aging each individual female. Aging results for females in foothill and valley collections with CDC light traps will be reported on when more data is accumulated.

Most female *C. inornata* collected in CDC traps operated on Suisun Marsh during September had completed at least one gonotrophic cycle (i.e., 1-parous, Table 4). The high parous rate can probably be attributed to the predominance of estivating females which presumably survived the summer and which deposited eggs during the late summer into newly available larval breeding sources. Alternative explanations are being considered but appear less plausible at this time. The occurance of nullipars in early October was coincidental to the period in which pupae were recovered in a majority of dipping samples. The collection of predominantly young females during this period suggests the emergence of the first major fall brood. Subsequent collections produced an increasing portion of parous females. Adverse weather conditions in December resulted in a substantial decline in evening temperature and corresponding adult activity. Prior to this period, the oldest female collected was determined to be 4-parous. From the same samples, 16 females were determined to have completed at least 2 gonotrophic cylces (i.e., 2-parous).

AUTOGENY.– The rates of autogeny determined for 10 day old female *C. inornata* reared from field-collected late

Table 1.–Number and location of adult and larval sampling stations surveyed at biweekly intervals.

Location	CDC LT + dry ice	New Jersey LT	Dipping
Suisun Marsh (Coastal)			
Joice Island (rural)	1	0	2
Grizzly Island (rural)	1	1	0
Fairfield (urban)	1	1	0
Sierra Foothill			
Folsom (rural)	1	0	1
Deer Creek (rural)	0	0	1
Roseville (rural)	1	0	2
Fair Oaks (urban)	1	1	0
Central Valley			
Dixon (rural)	1	1	1
Sutter (1 rural- 1 urban)	2	2	2

4th instar larvae and pupae were substantially higher in the Suisun Marsh (Joice Island) population (Table 5). For the period between October 10, to December 9, 1975, a dramatic increase in autogeny for the Suisun population was observed concurrently with a decline in females in CDC light trap collection. Maximum autogeny (i.e., 42.0%) was exhibited by females reared from samples collected on January 6, 1976. The increase in the expression of autogeny perhaps functions as a homeostatic mechanism to compensate for reduced ability of anautogenous females to become active and obtain blood meals during seasons of adverse conditions. Additional investigations are planned to further consider this possibility. The continued low rate of autogeny in the foothill population is not fully understood at this time. Nutritional aspects of principle breeding sources, low primary productivity, and other such considerations

Table 2.–Comparison of female *Culiseta inornata* collected by dry ice-baited CDC miniature light trap and New Jersey light trap in rural stations.

Suisun Marsh traps	date of collection								
	9-17	9-29	10-13	10-29	11-11	11-25	12-8	12-24	1-6
CDC (2)	11	53	100	325	189	322	3	10	7
NJ (1)	0	3	3	14	3	35	0	1	1
Sierra Foothill traps	date of collection								
	9-18	9-30	10-14	10-28	11-10	11-24	12-7	12-23	1-5
CDC (2)	0	1	5	2	1	1	1	1	1
Central Valley traps	date of collection								
	-	10-2	1020	11-5	11-19	12-2	12-15	12-29	1-12
CDC (2)	-	5	8	39	56	-	35	26	1
NJ (2)	-	6	3	16	8	-	5	47	0

Table 3.–Comparison of female *Culiseta inornata* collected by dry ice-baited CDC miniature light trap and New Jersey light trap in urban stations.

Fairfield trap	date of collection								
	9-17	9-29	10-13	10-29	11-11	11-25	12-8	12-24	1-6
CDC (1)	-	0	0	10	2	9	0	0	1
NJ (1)	-	0	0	1	3	33	3	1	1
Sierra Foothill trap	date of collection								
	9-18	9-30	10-14	10-28	11-10	11-24	12-7	12-23	1-5
CDC (1)	0	1	1	0	0	0	0	0	0
NJ (1)	0	0	0	0	0	0	0	0	0
Central Valley trap	date of collection								
	-	10-2	10-20	11-5	11-19	12-2	12-15	12-29	1-12
CDC (1)	-	1	2	0	1	-	2	0	0
NJ (1)	-	0	0	0	1	-	1	0	0

Table 4.–Physiological-age of female *Culiseta inornata* collected from Suisun Marsh in dry ice-baited CDC miniature light traps.

Physiological-age category	date of collection 9-17	9-29	10-14	10-29	11-12	11-24	12-8	12-23	1-6
Nulliparous	1	20	61	63	28	34	2	5	5
1-parous	10	30	4	33	39	48	-	5	2
2-parous	-	1	-	3	1	16	1	-	-
3-parous	-	-	-	1	-	1	-	-	-
4-parous	-	-	-	-	-	1	-	-	-
Total	11	51	65	100	68	100	3	10	7

Table 5.–Comparison of the autogeny rates in female *Culiseta inornata* reared from field-collected late 4th instar larvae and pupae.

	date of collection 10-9	10-23	11-10	11-25	12-9	12-23	1-6	1-20
Joice Island								
No. Dissected	56	76	50	50	50	80	50	64
% Autogenous	3.5	1.3	4.0	16.0	20.0	16.0	42.0	29.7
Roseville								
No. Dissected	-	-	-	-	50	60	50	77
% Autogenous	-	-	-	-	8.0	15.6	6.0	7.8

Table 6. Larval-pupal density and primary productivity in Suisun Marsh and Sierra Foothill stations.

Suisun Marsh (120 dips)	Date of Collection 9-17	9-29	10-13	10-29	11-11	11-25	12-8	12-24	1-6
Number larvae-pupae per dip	0	.02	.25	2.68	4.04	4.62	5.38	4.29	3.17
Total Chlorophyll (mg/M^3)	3312	1993	903	2252	1897	2124	2155	617	549
Sierra Foothill (180 dips)	**Date of Collection 9-18**	**9-30**	**10-14**	**10-28**	**11-10**	**11-24**	**12-7**	**12-23**	**1-5**
Number of larvae-pupae per dip	-	-	0	2.94	1.23	2.12	2.32	1.86	0.63
Total Chlorophyll (mg/M^3)	-	-	115	339	93	67	39	42	47

(see next section) may preclude the ability of larvae to build up ample dietary reserves capable of being utilized by autogenous females in maturation of eggs during the first gonotrophic cycle.

LARVAE AND PUPAE.– Preliminary observations on *C. inornata* restrained in cages placed in both salt marsh and foothill larval habitats indicated a causal relationship between observed larval density and primary productivity. Results on 4 replicates showed that a mean of 29 days is required for development from egg to pupa in cages placed in larval habitats on Joice Island. Caged individuals sequestered in foothill larval habitats beginning October 11, failed to progress beyond 2nd stage larvae as of January 20, 1975. Higher levels of primary productivity associated with salt marsh breeding sources were consistent with the highest observed levels of larval and pupal density (Table 6). Conversely, significantly lower densities were noted in foothill larval habitats having characteristically lower levels of primary productivity.

ACKNOWLEDGMENTS.– Tne authors wish to extend their appreciation to the following persons and respective organizations for their logistical support, equipment utilization, and assistance which has greatly facilitated the realization of this project: Embree G. Mezger, Manager, Solano Mos-

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INEXPENSIVE AERIAL PHOTOGRAPHY FOR MAP-MAKING

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The Tulare Mosquito Abatement District contains great areas of agricultural land which undergoes frequent changes from year to year, necessitating frequent redrawing of the District's section maps (8 inches = 1 mile) to keep them current, but map-making by ground is expensive because it requires a two-man team 1½ to 3 hours to traverse each section, record data, and draw a map for any section which requires complex detail. High time-labor costs suggested that aerial cartography might be more efficient.

We decided to use a 35 mm camera which had a built-in, automatic "F"-stop and shutter-speed adjustment changing mechanism. The camera was attached to the belly of the District's Piper Pawnee spray airplane with the mounting arranged so that, in level flight, the angle of vision of the camera would be perpendicular to the earth.

The camera was protected inside a 50-caliber ammunition box bolted to the airplane's insecticide-pump bracket. The camera was fixed in the box by expanded polystyrene blocks which occupied all excess space. An optically clear window was placed in the bottom of the box below the camera lens.

Because the pilot had to operate the camera by remote control, the film-advance thumb-trigger had to be redesigned. From quarter-inch aluminum stock, we fabricated a four-inch wheel and cut a deep groove into its circumference. From the "spokes area" of the wheel, a half-inch-wide crescent of about 290° arc was removed – making, in effect, a one-spoked wheel. (This crescent-shaped hole was necessary in order to allow the semi-rotating wheel to miss the camera's shutter trigger.)

This pulley-wheel acted as a "winding-drum" for the film-advance when activated by the pilot pulling a string in the cockpit. A rubber band, wound opposite to the string on the pulley-wheel, maintained tension and returned the camera's film-advance spindle to the "cocked" position. The shutter was clicked by means of a pneumatic remote-control trigger with a tube long enough to reach into the cockpit.

As an item of note, to avoid a frozen shutter which had been lubricated with tropical-grade oil, we made a heating-collar for the camera by enmeshing three 12-volt series-wired bulbs in a plaster-of-Paris block which was cast to fit closely around the barrel of the camera. This was wired to the cockpit heater switch so that whenever the pilot required heat, the camera was also warmed. (This heater would not be needed with a "winterized" camera; moreover, excessive heat should be avoided because some film may be damaged by temperatures in excess of 95°F.)

Minolta does not make a wide-angle lens to fit the camera we used, but Yashika makes an auxiliary lens which increases the width of field by 20%, an increase which we found did not cause undue parallax. However, geometric tests showed that the camera would still have required an altitude of 8,000 feet to completely "see" a one-mile sector. Because of this, we oriented the camera so that the long axis of the film lay parallel to the aircraft's wings. It requires two exposures in order to encompass one mile on the short axis of the film but, as a safety measure, we make three sequential exposures of each section.

Using a 15-DIN, 36-exposure roll of color-slide film, the camera is loaded, focused on "infinity", its exposure meter is set on "automatic", the heating-collar is plugged in, and the film advance string and pneumatic trigger are connected. Before take-off, two or three frames are snapped to test if all is functioning well.

Once aloft, the pilot climbs to 8000 feet. Then, on a heading of due north (to avoid glare as well as to retain orientation), and striving for a ground speed of 90 MPH, he adheres to the following flight plan:

1. Center airplane down the middle of the section. (Watch for drift, because you only have 1000 feet lateral deviation allowable.)
2. Ten seconds **after** you cross the section line, take frame No. 1.
3. Holding that course down the middle, ten seconds later take frame No. 2.
4. Still holding the course down the middle of the section, ten seconds later take the third (and last) frame of that section.

This pattern will result in photographs taken at about 1,300, 2,600, and 4,000 feet into each respective section with adequate overlap.

When the roll is exhausted (after mapping 10 sections) the film is processed into slides which are then projected into a drawing board for tracing. The drawing board is simply a long box with a mirror (tilted at 45°) placed directly under the level clear-glass tracing surface. The beam from the projector is directed at the mirror and the projector is manipulated until the picture is in sharp focus and the size of a section image measures exactly eight inches wide.

Regular 8½ x 11 inch bond paper is used to trace the maps. The paper has the necessary heading, as well as an eight-inch-square outline, printed on it. This paper form is moved about on the tracing table until the bottom portion of the 8 inch outline exactly matches the projected image. Then the paper is taped to the glass tracing surface and the image is traced according to the following instructions:

MAP DRAWING:

1. In loading the projector, the date on the slide should touch the projectionist's right thumb. In that way, the projected image will be correct. (i.e., top of screen will be north.)
2. In sequence, remember that the first slide will be the southermost, next slide will be middle of section, and third slide will be northernmost of that section. (You may not need to use all three: the 3rd is a fail-safe.)
3. Line up your projector so that the right-to-left boundaries (of the section; not the complete slide) just exactly touch your 8-inch map boundaries on the southerly (bottom) of your map-to-be.
4. Draw in all you can see clearly, then go to the next sequence in your three-slide group.
5. Realign your paper with the projected image so that you can draw in the middle part of your map.
6. Go on to the third slide and line up the paper to match the image so that you can do the top portion of your map and thereby complete tht section survey.

The use of aerial photography as an integral part of this District's map-making allowed us to achieve superior cartographic accuracy and has given us a better knowledge of irrigation patterns, water misuse, the location of backyard urban mosquito sources and permits a more accurate acreage determination of individual fields.

From the following, it can be seen that our ground mapping cost 5½ times more than this newer method and, since we mapped 115 sections by air in 1975 at a cost of $442, our theoretical savings in one year were $2,064. (In actuality, however, we would not have mapped 115 square miles by ground because there was not enough time to do so.)

The initial costs were:

1. 35 mm Minolta M-9 camera (purchase price: $300.00) owned by the author and loaned to the District without cost	$000.00
2. Wide-angle lens (Focal length: 37.7 mm combined with camera lens)	74.20
3. Surplus 50 caliber ammunition box	16.00
4. Window and polystyrene packing for box	2.00
5. Aluminum stock for film-advance pulley-wheel	1.25
6. Pneumatic remote-control shutter trigger	6.00
7. Labor costs for fabrication and assembly of the device	68.00
TOTAL INITIAL COSTS	$167.45 to $467.45

The operational costs are:

1. Pilot's flight time to photograph 10 sections @3 pictures each	$ 5.00
2. Cost of aviation gasoline for 50 minutes of flight	6.55
3. One roll of 36 exposure color film	2.75
4. Cost of processing and transporting film	2.90
5. One-half man-hour (each picture) to trace ten film pictures onto paper forms	21.20
OPERATIONAL COSTS FOR 10 FINISHED MAPS	$38.40

$3.84 per section map represents a substantial saving over our older method of ground-mapping each section because the costs used to be about $21.80 per map:

1. Two men in a vehicle to drive over the section (one driving, one recording data) took 1½ to 3 hours for an average cost of	$19.08
2. Gasoline consumed during survey	.60
3. One-half man-hour to draw map	2.12
TOTAL	$21.80

A PERFORMANCE EVALUATION OF WOMEN GNAT CONTROL OPERATORS

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ABSTRACT

Commencing April 1 through October 31, 1975, a seven month period, the Coachella Valley Mosquito Abatement District employed a total of 11 women gnat control operators varying from 18 to 35 years in age. They were both married and single. Five were the largest number employed at any one time.

Working hours were from 6:00 a.m. to 12:00 noon or 6 hours per day, 6 days a week. Wages were two dollars and a half per hour. Working conditions were the same for both male and female operators. Women and men operators were provided separate rest room faciltities equipped with showers and lockers.

Their work assignment involved distributing gnat bait formulation by means of a military-type jeep, without canopy, often over rough-type terrain. Temperatures varied from 100 to 115 degrees (F). The operator was required to get in and out of the vehicle to set up and replenish bait stations at intervals of about 300 feet. All operators were required to wear a District supplied short sleeve shirt with emblem and name badge.

The following conclusions are offered:

1. There was less tardiness and absenteeism among women operators than men.
2. Women were as agile and equal in stamina as were men operators.
3. The women operators were observed to be much less abusive to vehicles and had fewer breakdowns.
4. The women operators were in general more reliable and capable of following instructions.
5. The women operators did less complaining and were less critical of their work schedules than men operators.
6. Daily work report forms submitted by women operators were more complete and more legible.

To summarize, the women operators overall performance was considered superior to that of the male operators.

As a result of these observations the District is planning on increasing the number of women gnat operators for the 1976 season.

TILAPIA FISH FOR MIDGE[1] CONTROL IN A SEWAGE TREATMENT POND

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The city of Dinuba expanded its sewage facilities in 1973 with construction of a pond of about 10 acres in size for storage of effluent. Early in 1974 the Delta Vector Control District (DVCD) deposited the mosquito fish, *Gambusia affinis*, in this pond. These fish multiplied rapidly and by mid-summer were abundant over the entire pond. At no time were any mosquito larvae observed in this pond. In September, 1974, a report was received of heavy adult midge populations in the area. An inspection indicated that the midges were emanating from the new pond, and it was now observed that this pond had many shallow areas around the edges and around several small islands which had been constructed in hopes of attracting nesting water birds. Midge larvae were abundant on the bottom in shallow areas. Additional complaints were received from the neighborhood, up to a distance of about one mile from the pond. Indications were that the midges had first reached severe numbers about July, 1974.

Gambusia were observed feeding on swimming midge larvae, however they were ineffective in maintaining an acceptable population of the midges. The Dinuba City Council and Staff reviewed the problem closely with the DVCD staff and agreed to take whatever steps were necessary to resolve the problem. The midge species involved was *Glyptotendipes barbipes* Staeger in apparently pure culture, a species which in the Delta VCD occurred in shallow areas of sewage lagoons but not in water 7 or more feet deep. At the Visalia sewage disposal plant it was not present in the three large ponds which were uniformly 7 feet deep, but it was abundant in a sinking area which had shallow water. At the Dinuba plant the midges were found only in the shallow areas of the large pond, not in three deep ponds immediately adjacent to the treatment plant. Three possible remedies were suggested:

1. Chemical treatment. This was rejected because it could destroy the *Gambusia* so essential for control of mosquitoes.
2. Rebuild the pond and make certain that all areas would be at least 7 feet deep. This would be very expensive.
3. Biological control. Captain George S. Stains, Manager of the Coachella Valley Mosquito Abatement District, had had experience with the imported mouth brooder fish, *Tilapia mossambica*, and expressed optimism that this fish alone might solve the problem. This fish from tropical Africa had been distributed in various ponds and ditches in the Coachella Valley, and appeared promising in midge control. As a tropical fish it cannot withstand cold temperatures, and is reported to die at about 10°C (50°F).

The California Department of Fish and Game was approached on the possiblity of obtaining a permit to introduce this fish into the Dinuba sewage effluent holding pond. Maps were provided to the Department which demonstrated that these fish could not escape into natural waterways, inasmuch as all nearby canals were at a higher elevation. The sewage pond was in a sink. Further, there was a 6 foot fence around the sewage treatment plant and gates were locked except during normal working hours, when plant personnel were working at the facility, so the general public was effectively excluded. The permit was received promptly, first to transport the fish from the Coachella Valley and second to deposit them in the pond at the Dinuba facility.

On May 8, 1975, about 350 specimens, a few up to 6 inches in size but most about 3 inches, were collected from ponds adjacent to the Eisenhower Medical Center at Palm Springs. Personnel of the Coachella Valley MAD collected the fish in a large seine and deposited them in three 30 gallon plastic garbage cans supplied by the Delta VCD. Battery activated aerators were installed and turned on. It was stated that the flashlight batteries which operated these aerators would last about 8 hours.

The truck carrying the fish arrived at the Dinuba sewage facility at just 8 hours. One of the aerators had quit, apparently very recently, but the larger fish were in stress. The smaller fish were in good condition, and all the fish in the other 2 barrels were in excellent shape when deposited in the pond. Several days later, about 50 dead fish were observed washed up on the banks, mostly large ones, probably the fish observed in stress at the time of deposit. The plant manager reported that schools of the fish had covered the entire pond within a couple of days of their deposition.

On May 15, 1975, one week after the fish were deposited, an interested neighbor advised that the midge population had been drastically reduced. Several days later he phoned again to report that he had been premature, that the population of adult midges was again at an unacceptable level. Two weeks later he phoned again to report that the midges had disappeared. Continued inspection by the District staff verified that the midges had indeed disappeared, and they never returned! Biological control had essentially eradicated the midge from this pond.

In the meantime, it was noted tht the sewage effluent in the pond was clean, algal growth was no problem, and *Gambusia* were present in sufficient numbers to prevent mosquito production. What was happening to the *Tilapia* population was not readily apparent, since these fish usually were completely out of sight in the deeper areas. The question was asked many times – "did the fish actually control the midges?" As the water cooled, the answer was provided. In mid-November the air temperatures dropped into the 30's, and on November 16 the surface of the large pond reached 48°F. The *Tilapia* descended to deep areas at this time, but

[1] *Glyptotendipes barbipes* Staeger. Determined by Gail Grodhaus, VCS, California Department of Health.

as the water temperatures continued to cool, the fish lost control of their movements and swam erratically at the surface near the banks. On December 2 a survey was made and all fish were dead. On the basis of sample counts at various points along the banks, it was estimated that the original 300 plus fish had multipied to possibly 500,000. Many of the dead fish were carried up onto the banks by the abundant Coots and eaten. There was no significant odor, nor was the pond harmed biologically.

Prior to the demise of all the fish, the plant manager, with the encouragement of the District, collected several hundred fish and deposited them in the small but deep first pond adjacent to the treatment facility. At no time during the winter has this pond recorded lower than about 18°C. and it is possible that as of the time of this presentation some of these fish may be alive, however none has been observed. The oxygen content in this pond is extremely low and may not be adequate for *Tilapia*. In case there is no survival, Captain Stains has assured that the ponds at the Eisenhower Center have remained at a sufficiently warm temperature during the winter that the fish survival is good and new fish can be obtained to deposit into the Dinuba pond and thereby continue to provide **complete** control of the nuisance midges.

A DISEASE IN *CHIRONOMUS FROMMERI* CAUSED BY A RICKETTSIELLA-LIKE ORGANISM

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ABSTRACT

A rickettsiella-like organism, previously unreported in the Western Hemisphere, was found causing disease in larvae of *Chironomus frommeri* collected from an artificial lake at Westlake, California. The major sign of infection in patently infected larvae was a white discoloration distributed evenly throughout the fat body. Smears of infected fat body demonstrated the pathogen multiplied in vacuoles in the cytoplasm of infected cells. In examinations with an electron microscope of ultrathin sections of infected fat body cells, three major forms of the pathogen were delineated: (1) large developmental cells (up to 5μm in diameter); (2) smaller budding cells (1.4μm); and (3) small mature disc-shaped cells, approximately 60nm in width by 600nm in diameter (Figures 1 and 2). The small disc-shaped cells are probably the infectious agent of the disease.

This type of pathogen is rare and previously has only been reported from larvae of *Chironomus tentans* and *Chironomus th. thummi* collected in Germany. Our preliminary studies indicate the pathogen of *C. frommeri* is a close relative of the pathogens described from the above chironomids, and furthermore that these organisms are related to the rickettsiellae, but probably should not be placed in the genus *Rickettsiella*.

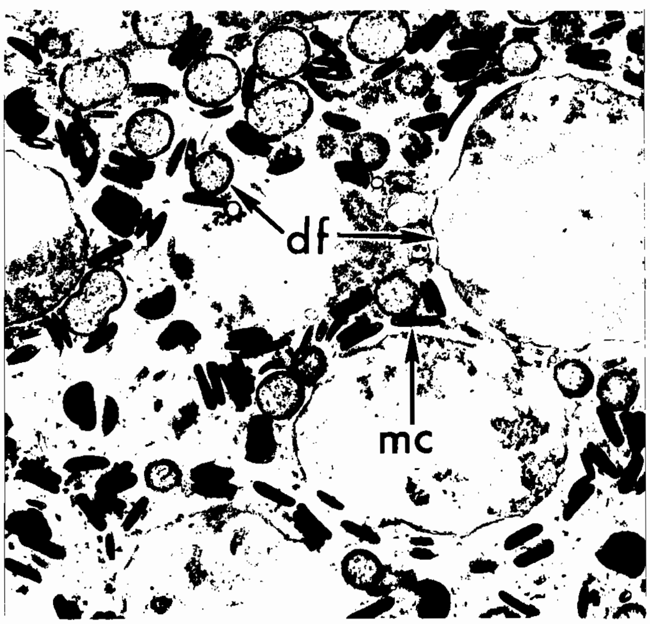

Figure 1.--Electron micrograph of developmental forms (df) and mature cells (mc) of the rickettsiella-like organism in a fat body cell of *Chironomus frommeri*. X10,000

Figure 2.--Electron micrograph of a cross section through a group of mature cells. X70,000.

DENSONUCLEOSIS VIRUS AND CYTOPLASMIC POLYHEDROSIS VIRUS DISEASES IN LARVAE OF THE BLACKFLY, *SIMULIUM VITTATUM*

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ABSTRACT

A densonucleosis virus (DV) and cytoplasmic polyhedrosis virus (CPV) were found causing disease in larvae of the blackfly, *Simulium vittatum*, collected from the Colorado River in the vicinity of Needles, California. The major site of infection for both viruses was the midgut epithelium, although the DV also infected fat body cells. The rates of infection for both viruses in field populations were less than 1%.

In patently DV infected larvae, the cuticle was translucent and melanized cells were observed distributed irregularly throughout the body. Additionally, the midgut epithelium was hypertrophied. Examinations of fresh smears and stained sections of infected larvae demonstrated that the virus replicated in the nuclei of midgut and fat body tissues. In the midgut infected nuclei were greatly hypertrophied, frequently measuring four times the diameter of healthy nuclei. Infected fat body nuclei hypertrophied to a lesser extent. All infected nuclei contained a large virus inclusion which was sponge-like in appearance (Figure 1). In ultrathin sections examined with an electron microscope, the inclusion was found to consist of an aggregation of small virus particles measuring approximately 18nm in diameter. These preliminary observations indicate this virus probably belongs to the parvovirus group, the first such virus reported from blackflies.

The gross signs of infection for the CPV were typical of those produced by this type of virus in other aquatic diptera, namely, hypertrophy of the midgut epithelium accompanied by an opaque white discoloration of this tissue. The virus replicated in the cytoplasm. The mature virus particles, measuring approximately 65nm in diameter, were occluded in tetrahedral proteinaceous occlusion bodies (Figure 2).

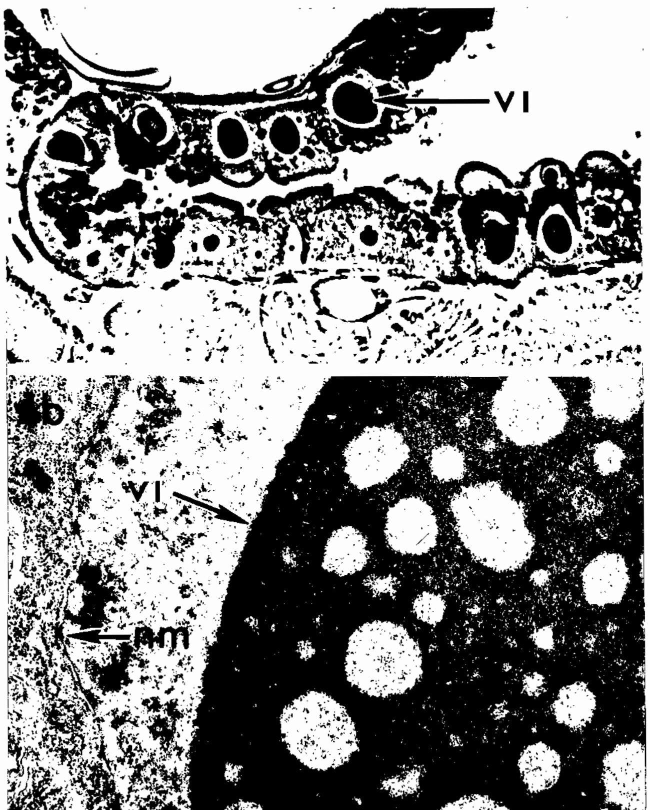

Figure 1.–Densonucleosis virus infected midgut epithelium of *Simulium vittatum*. (a) A section through the midgut demonstrating virus inclusions (VI) in hypertophied nuclei. (b) An electron micrograph of a portion of an intranuclear virus inclusion. nm, nuclear membrane.

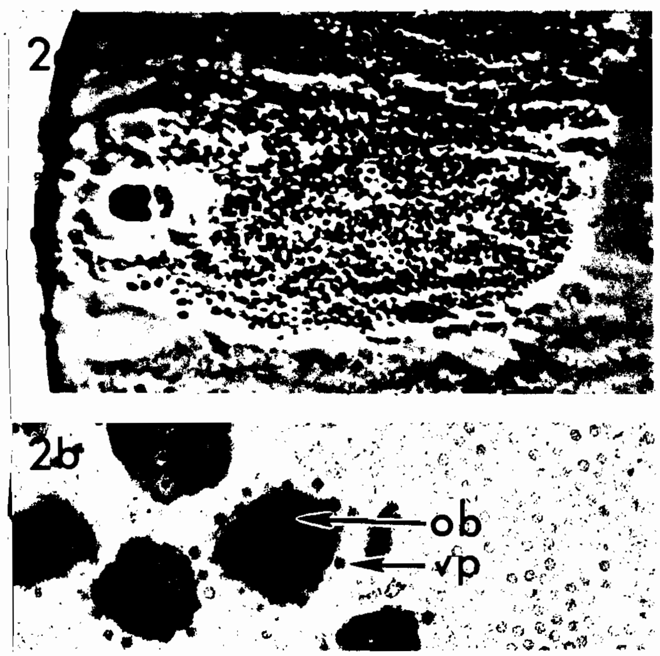

Figure 2.–Cytoplasmic polyhedrosis virus infected midgut epithelium of *Simulium vittatum*. (a) A single hypertrophied cell containing numerous polyhedral occlusion bodies within the cytoplasm. (b) An electron micrograph demonstrating occlusion bodies (OB) and virus particles (VP) in the cytoplasm of an infected cell.

SEASONAL EMERGENCE OF *LEPTOCONOPS CARTERI* HOFFMAN IN CONCORD, CALIFORNIA

Charles Beesley[1], Richard L. Tassan[2], Gary Riddle[3], and Hans Munck[4]

ABSTRACT

The seasonal emergence of an isolated population of *Leptoconops carteri* Hoffman was studied in June of 1974 and 1975. Populations were monitored within a 360 acre field by adult female counts on CO_2 attractant sticky traps distributed throughout the field and placed 1′ and 4′ above ground. Traps were placed in the morning and afternoon, three times a week for six weeks in 1974 and four in 1975. Seasonal emergence and distribution of adult activity was found to be relatively consistent for both years. Adults were collected in considerably higher numbers at the lower trap heights and when placed in the afternoon.

INTRODUCTION.–An isolated population of *Leptoconops carteri* Hoffman[5] was observed during the early summer of 1974 and 1975. The phenology of this species entails early summer emergence (June) which results in considerable biting activity to residents in the vicinity and persons involved in recreational activity at the emergence site.

Although several publications have reported on the phenology of *Leptoconops* species and related Ceratopogonidae, only a few have utilized techniques for relative population surveys. Basically, there are five methods for estimating adult gnat populations: (1) Ground emergence traps (Smith & Lowe 1948, Rees 1952, Foulk 1968). (2) Sticky cylinder traps using CO_2 and tanglefoot to bait and entangle flying adults (Schoeppner & Whitsel 1967, Kline & Axtell 1975). (3) A D-Vac portable vacuum (Foulk & Sjogren 1968, Whitsel & Schoeppner 1971) for resting and flying adults. (4) Light traps, for crepuscular and nocturnally active species (Kline & Axtell 1975) and (5) biting counts (Kettle & Linley 1967) for adults seeking a blood meal. For a thorough review of the biology and taxonomy of *Leptoconops* sp. the following publications are recommended: Smith and Lowe 1948 and Wirth and Atchley 1973.

Of the five potential sampling methods, CO_2 attractant sticky traps (Schoeppner & Whitsel 1967) were selected because they were inexpensive and easily transported over the large study site. Ground emergence traps could not be used because of grazing cattle and the large deep cracks that develop as the soil dries.

The main objective of this study was to determine which height of the trap would be most reliable and whether either morning or afternoon sampling would be adequate in establishing the adult activity of *L. carteri*. Determining this pattern of adult activity would allow the application of more efficient control measures than simply attempting to treat the entire population.

METHODS.–The study site was a large field approximately 360 acres (Figure 1), comprised of Montezuma adobe clay (Carpenter & Colby 1939). As spring progresses the soil dries and forms cracks which enables the indigenous population of gnats to emerge. The field is somewhat dome-shaped with slight North - South inclines at both ends. There was a small wet area or marsh located in the northern portion by trap no. 2 (Figure 1). Vegetation is characterized by the annuals as rye (*Lolium* sp.), wild oats (*Avena fatua*) and star thistle (*Centaurea* sp.) in the dry area and sedge (*Scirpus* sp.) in the wet area. They provided resting sites for adult gnats as well as food for grazing cattle.

Approximately 2½ - 3 lbs. of dry ice were placed in each trap and left for one hour at which time a reading was taken of the number of females trapped. Two trap heights were used: 1′ (low) and 4′ (high) measured from ground surface to the top of the trap. Air temperature (°F) and relative humidity (RH) were recorded with a sling psychrometer (Bacharach no. 12-7011). Wind velocity was averaged for one minute with a portable anemometer (Bacharach no.

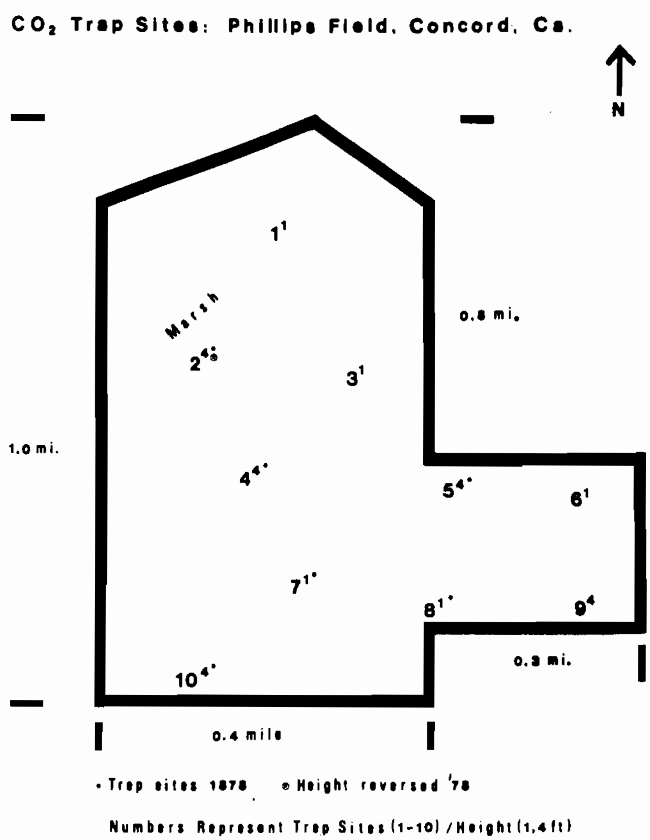

Figure 1.–Schematic illustration of black gnat location and trap sites for both years.

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Table 1.–ANOVA Table for numbers of adult *L. carteri* collected on CO_2 sticky traps: 1974.

Source	df	SS	MS	F-ratio	Explained Variance $\hat{\omega}^2$
Between Heights[a]	1	3,767.52	3,767.52	1.82	
Within Heights	318	658,937.37	2,072.13		
Within Height 1 (1′)	159	483,259.60			
Between Traps[b]	4	71,681.60	17,920.40		.11
Within Traps	155	411,578.00	2,655.34		
Time[c]	1	5,152.90	5,152.90	2.38	
Date[d]	15	69,821.00	4,654.73	2.15*	.11
DXT[d]	15	67,923.70	4,528.25	2.09*	.10
Residual I	124	268,680.40	2,166.78		
Within Height 2 (4′)	159	175,677.78			
Between Traps[b]	4	11,346.02	2,836.51		
Within Traps	155	164,331.75	1,060.10		
Time[c]	1	2,528.10	2,528.10	3.41	
Date[d]	15	31,869.58	2,124.64	2.87*	.05
DXT[d]	15	38,011.70	2,534.11	3.42*	.06
Residual II	124	91,922.38	741.31		
TOTAL	319	662,704.89			

[a] $F_{1,318}$: .05 = 3.86
[b] See Table 2
[c] $F_{1,124}$: .05 = 3.92
[d] $F_{15,124}$: .05 = 1.54
* = significant at α = .05

3035). Ambient conditions were recorded at each trap location at the height of the trap, each time the dry ice was placed in a unit. Traps were placed in the field Monday, Wednesday, and Friday and operated from 9-10 a.m. and 2-3 p.m.

In 1974 ten trap sites were selected with five at each height. Collections were made on 16 days during the period June 7 - July 19. However, due to limited availability of dry ice, the number of sites was reduced to six in 1975 with three at each height. Sampling was conducted 11 times during the period June 10 - July 2. Sites selected in 1975 were based on the six most productive ones of 1974.

The statistical analysis was carried out as repeated measures (split-plot) analysis of variance (ANOVA) within each trap height with the interaction model applied to the sampling date and sampling time (a.m./p.m.) factors (Kirk 1968).

RESULTS.–The complete ANOVA table for the 1974 catches are shown in Table 1. The trap count means (X) for the morning and afternoon, on each sampling date and for the low and high traps are presented in Tables 2 and 3 respectively.

Adult activity in 1974 commenced in early June, peaked the third week of that month (June 21), then quickly diminished by the end of the month with a few adults collected in July. Activity then covered approximately six weeks with a distinct peak during the third week (June 14-21).

In spite of the large difference in the total numbers of adults collected throughout the season in the low traps (2592) compared with the high traps (1494) this was not statistically significant at the 5% level (between height variation in Table 1). Within the low trap height, one trap (no. 7) caught significantly more gnats than the other four (Table 2). This accounted for 66% of all gnats caught at the low level over the entire sampling period. The other four low traps were not significantly different from one another and there was no difference between morning and afternoon catches. Significant differences within the five low traps were found to be due to the effects of sampling over different dates and the time-by-date interaction (Table 1). These two effects accounted for 11% and 10% respectively, of the total variation in the experiment ($\hat{\omega}^2$). The 1974 sampling date means (Table 2) for the low traps showed that in the morning counts, 69% (580/842) of the gnats were trapped on three dates (6/19, 6/21, 6/24). In the afternoon 84% (1466/1750) were collected on three earlier dates (6/14, 6/17, 6/19). This difference in the dates on which peak numbers of adults were trapped in the morning or afternoon was significant as indicated by the time-by-date interaction (Table 1).

There were no significant differences in the numbers of adults collected within the high traps (Table 2). However, throughout the season 39% and 33% of those captured in the high traps were in traps 2 and 10 respectively. Again, there was no difference between the morning and afternoon captures, and again there was a difference in the between-date capture and a time-by-date interaction (Table 1) which accounted for 5 and 6% respectively, of the total variation in the experiment ($\hat{\omega}^2$). In the morning, 49% of all gnats collected in the high traps were collected on one date (June

Table 2.—Mean values ($\bar{X}$) of adults collected ambient conditions: 1974.

Trap Sites	7	8	6	1	3
Season Trap Means[a]	106.9	37.4	7.06	6.0	4.6

	a.m.				p.m.			
1′ Trap Sites	Adults	°F	Wind MPH	% RH	Adults	°F	Wind MPH	% RH
6/ 7	2.0	82	5.0	28	0.8	91	7.2	20
6/10	1.0	73	4.1	55	2.4	89	9.8	30
6/12	0.4	66	5.4	58	6.0	76	7.0	48
6/14	2.6	68	6.4	65	88.8	79	4.2	46
6/17	0.6	65	3.5	62	111.8	71	4.2	51
6/19	30.6	67	2.3	58	92.6	74	3.8	51
6/21	74.0	75	3.3	54	3.6	90	7.5	37
6/24	11.4	70	5.1	51	25.6	84	8.4	28
6/26	11.0	71	6.1	49	9.4	84	8.1	30
6/28	10.4	82	2.4	43	0.8	98	4.4	23
7/ 1	10.2	75	3.4	54	5.2	89	8.4	27
7/ 3	1.4	74	5.6	50	1.8	84	11.2	35
7/ 5	2.2	79	7.7	41	1.0	90	9.3	32
7/12	0.8	74	4.1	51	0.2	85	7.3	31
7/15	0.0	84	10.2	29	0.0	82	6.0	43
7/19	0.2	77	6.4	58	0.0	91	6.4	38

Trap Sites	2	10	4	5	9
Season Trap Means[a]	36.1	30.8	15.8	6.6	4.3

	a.m.				p.m.			
4′ Trap Sites	Adults	°F	Wind MPH	% RH	Adults	°F	Wind MPH	% RH
6/ 7	2.4	83	7.4	21	0.2	91	7.0	21
6/10	1.6	74	4.5	50	2.8	87	11.0	31
6/12	1.4	65	4.7	59	1.8	76	6.2	47
6/14	0.8	68	6.5	54	21.0	80	4.9	43
6/17	0.0	66	3.5	60	92.6	72	5.4	51
6/19	4.0	68	1.4	58	77.4	75	2.7	46
6/21	41.8	74	3.2	69	2.0	91	8.3	36
6/24	9.2	69	5.1	55	9.8	82	8.8	29
6/26	3.2	70	5.5	51	2.2	85	9.3	31
6/28	17.4	82	1.7	43	1.6	96	4.1	24
7/ 1	2.2	74	6.3	55	1.0	89	8.2	31
7/ 3	0.6	73	6.1	53	1.6	84	11.9	38
7/ 5	0.8	79	7.0	40	0.8	92	9.5	33
7/12	0.4	73	4.0	52	0.2	85	8.0	35
7/15	0.0	85	9.8	30	0.0	81	7.0	46
7/19	0.0	76	7.7	59	0.0	93	7.3	36

[a]Means underlined by the same line are not significantly different from each other at $\alpha = .05$, using Tukey's method of pairwise separation.

21). In the afternoon the peak collection was four days earlier (June 17) and amounted to 43% of the total afternoon catches.

The ANOVA table for 1975 catches are presented in Table 3. The trap count means (X) for the morning and afternoon, on each sampling date, for the two trap heights are presented in Table 4.

Overall activity as reflected by the numbers of adults collected in the traps was considerably lower in 1975 when compared with 1974. The low populations experienced in 1975 had a peak during the third week of June (10-20) and adults were only active for four weeks.

The low traps collected more adults than the high traps over the entire season, 295: 45 respectively. This difference was found to be significant (between height variation in Table 3) and explains 5% of the total variation ($\hat{\omega}^2$) in the experiment. Within the low traps it was found that trap no. 7 collected 70% of the total season catch, significantly more adult *L. carteri* than either of the other low traps (Table 4). It should be noted that this trap (no. 7) was also

Table 3.–ANOVA Table for numbers of adult *L. carteri* collected on CO_2 sticky traps: 1975.

Source	df	SS	MS	F-ratio	Explained Variance ω^2
Between Heights[a]	1	477.28	477.28	6.94*	.05
Within Heights	130	8940.80	68.78		
Within Height 1 (1′)	65	8830.48			
Between Traps[b]	2	824.12	412.06		.09
Within Traps	63	8006.36	127.08		
Time[c]	1	218.18	218.18	2.09	
Date[d]	10	1976.36	197.68	1.89	
DXT[d]	10	1424.82	142.48	1.36	
Residual I	42	4386.59	104.44		
Within Height 2 (4′)	65	110.32			
Between Traps[b]	2	0.69	0.32		
Within Traps	63	109.68	1.74		
Time[c]	1	0.74	0.74		1
Date[d]	10	28.82	2.88		1.76
DXT[d]	10	11.4	1.42	1	
Residual II	42	68.70	1.64		
TOTAL	131	9418.08			

[a] $F_{1,130}$: .05 = 3.92
[b] See Table 4
[c] $F_{1,42}$: .05 = 4.07
[d] $F_{10,42}$: .05 = 2.06
* = significant at α - .05

in the area of highest adult activity in 1974. Nine percent of the total experimental variation in 1975 is explained ($\hat{\omega}^2$) by the variation in trap catches for the low traps. Even though almost 2.5 as many adults (108: 88) were collected in the afternoon in comparison to the morning, this difference was not significant (Table 3). There was no significant difference in the adult catch between sampling dates or in the time-by-date interaction as evidenced in same number of adults (Table 4). As with the low traps, there was no difference in adult catch between sampling dates or in the time-by-date interaction in 1975 (Table3).

In general the afternoon temperatures were about 10°F higher than morning temperatures for both years (Tables 2, 4). The temperatures in 1974 were considerably higher than 1975, especially during periods of peak activity. Also, the wind velocities were higher in the afternoon than in the mornings, with 1975 winds being slightly higher except during the afternoons of the latter part of the 1974 season. Relative humidity decreased in the afternoons but there was no apparent difference between years.

DISCUSSION.– Smith and Lowe (1948) reported the flight period of adult *L. carteri* ranged from four to six weeks, beginning usually in mid-May. Seasonal variations in their emergence occurred, with shifts up to two weeks relative to the prevailing climate. Emergence of gnats from any one spot did not usually exceed three weeks, and in fact most adults were seen to emerge within a ten day period. Our findings were in accord with these as adult activity went from six weeks in 1974 to four in 1975 corresponding to the lower temperatures in 1975. Within each season, adult activity was primarily within a week period with distinct high peaks over a seven to ten day period. When adults emerged there was a noticeable four to five day lag in counts between those taken in the morning and in the afternoon, which corresponded to the onset of warm afternoon temperatures earlier in the season. Specifically, a comparison of trap counts vs. temperature indicated a flight threshold of 67°F with upper limits of activity around 90°F. Periods of high adult activity were associated with temperatures between 70 - 80°F.

High numbers of gnats were collected at both trap heights, consistent with their known habitats of biting the upper and lower part of the body (Whitsel & Schoeppner 1966). However, the prevalence of gnats collected at the lower trap height, significant to the 95% level in 1975, suggests a behavioral preference for low heights as previously demonstrated for *L. bequarteri* by Kettle and Linley (1973). This would further correlate with the limited flight capacity of *L. carteri* and adult habits of resting near the ground surface.

Based on our data it seems that in order to time a control measure for maximal effect, sampling of adult activity would be best in the afternoon and at the one foot height. This would give the earliest seasonal indication of adult emergence, suitable for a control approach. Distribution of traps throughout a field would delineate areas of high activity, and as peak adult emergence lasts only about ten days an intensive control treatment could be applied for a limited time to those sites showing high counts. The adult habit of resting at or near the ground surface in the morning

Table 4.—Mean values ($\bar{X}$) of adults collected and ambient conditions: 1975.

1′Trap Sites		7	8	2				
Season Trap Means[a]		18.9	4.9	3.1				
		a.m.				p.m.		
Trap Date	Adults	°F	Wind MPH	% RH	Adults	°F	Wind MPH	% RH
6/10	15.6	87	7.5	43	4.7	90	7.1	30
6/11	1.0	66	7.0	65	2.0	75	6.2	46
6/13	4.7	76	5.3	60	10.3	90	8.5	39
6/16	0.3	67	8.7	67	1.7	73	8.7	51
6/18	1.0	67	4.8	57	16.7	76	6.5	31
6/20	4.3	68	4.3	65	30.7	77	6.0	42
6/23	0.0	59	8.9	78	0.0	69	7.4	56
6/25	1.7	62	1.2	--	1.7	76	4.4	--
6/27	0.7	68	3.1	--	1.0	84	3.8	--
6/30	0.0	62	6.2	--	0.7	72	5.6	--
7/ 2	0.0	62	6.8	--	0.0	73	5.1	--

4′ Trap Sites		4	5	10				
Season Trap Means[a]		1.3	1.6	1.2				
		a.m.				p.m.		
Trap Date	Adults	°F	Wind MPH	% RH	Adults	°F	Wind MPH	% RH
6/10	2.3	81	6.2	43	0.3	90	8.6	32
6/11	0.3	65	9.4	64	0.0	75	9.9	49
6/13	2.7	74	7.9	62	1.3	89	9.6	39
6/16	0.3	67	12.5	67	0.7	73	11.1	52
6/18	1.3	66	4.8	57	1.7	76	8.0	31
6/20	0.7	68	4.8	65	1.7	77	8.6	43
6/23	0.0	60	11.7	75	0.7	68	8.7	57
6/25	0.0	62	3.2	--	0.0	77	6.6	--
6/27	0.7	68	3.8	--	0.0	84	4.1	--
6/30	0.3	61	9.9	--	0.0	72	9.6	--
7/ 2	0.0	62	9.2	--	0.0	72	7.8	--

[a]Means underlined by the same line are not significantly different from each other at $\alpha = .05$, using Tukey's method of pairwise mean separation.

could be capitalized on for treatment application, i.e., early morning while temperatures are below 70°F. Thus, monitoring adult female counts on CO_2 traps when placed in a gnat producing area would allow for selective control applications to emerging populations of *L. carteri*.

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BITING FLIES IN THE LOWER COLORADO RIVER BASIN: ECONOMIC AND PUBLIC HEALTH IMPLICATIONS OF *SIMULIUM* (DIPTERA-SIMULIIDAE)[1]

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ABSTRACT

The biting gnat problem in the Lower Colorado River Basin, especially in the Mohave Valley area of Arizona, California and Nevada, has important economic and public health implications, affecting the health and well-being of most of the residents, tourists and visitors seeking to enjoy the clean air, outdoor leisure and a variety of water-related sporting activities. The main species of the biting gnats responsible for the deterioration of the human environment, leading to the misery and discomfort of thousands of people is *Simulium vittatum* Zetterstedt, which breeds profusely in several stretches of rapids below Davis Dam.

Influenced by climatic and weather factors, the fly populations expand reaching communities 20-30 miles distant from the river, during the cooler months in the fall and spring. During the summer months, their populations contract and are confined to the water course, seldom appearing in large numbers 0.5-1.0 miles away from the river. In general, the flies are highly pestiferous for 9-10 months of the year, biting a certain proportion of human hosts which manifest allergic reactions to their bites. Domestic animals such as dogs, horses and livestock are severely attacked by *S. vittatum*, being highly annoyed at times due to the hovering and bites of the flies, and sometimes these bites result in secondary infections.

The Colorado River is the only large river providing a major source of recreation, hydroelectric energy, domestic and irrigation water for the states of Arizona, California, and Nevada. To conserve its water and to implement flood control schemes, several major dams have been constructed along the length of this river bordering the 3 states, creating several large freshwater lakes. As a result of these water conservation schemes, hydroelectric power plants generate electric energy to be consumed by various users in the 3 states and elsewhere.

In addition to providing water and energy for industry, agriculture and millions of inhabitants in metropolitan areas, the river and its numerous lakes of pristine water serve as some of the most highly used recreational areas in the west. Urbanites travel hundreds of miles to reach this water course for fishing, water skiing and outdoor leisure throughout the year. It is a source of livelihood for thousands of inhabitants which live and pursue business ventures along both banks (Figure 1) of the river in the 3 states.

During the mid and late sixties, human population growth was quite rapid. Data obtained from utility companies in the area just below Davis Dam indicate considerable increase in utility connections indicating significant growth in the numbers of permanent residents. Similar increases are observed along the other stretches of the lower basin such as in Havasu and Parker Dam areas. Stabilization of water flow in the river by construction of a series of dams and the increased utilization of land and water resources along the river has created a serious biting gnat problem along some stretches of the river. The problem became so serious during the early 1970's that civic groups and county agencies in the 3 states pressed for an analysıs of the nature and scope of the problem with a veiw toward finding a practical solution. We, at the University of California, Riverside, were asked to initiate studies on this problem in late 1973.

This paper is first in a series of research papers analyzing the nature and scope of the biting gnat problem caused by *Simulium* species in the Lower Colorado River Basin area below Davis Dam. It focuses attention on the economic and public health significance of biting gnats in the area and alludes to the intensity and duration of biting fly problems in the basin.

Simulium Species

The Lower Colorado River Basin through which the Colorado River flows, contains several man-made lakes created by dams. The dams are part of a major national water conservation scheme, implemented in the basin over the past 4-5 decades. These schemes have drastically changed the flow characteristics and water quality of the river from that of the pre-dam era for the better. Water discharge from the lakes is now quite stable, varied only in response to power needs and agricultural demands on the water. The stable water flow in the river has created conditions which are highly suitable for the production of *Simulium* species in swift-flowing water stretches of the river.

At the present time, 2 important species of *Simulium* breed in the basin and annoy or seek blood from man and domestic animals and wildlife. The most abundant and highly pestiferous species breeding in rapids with suitable substrates is *S. vittatum* Zetterstedt. This widely distributed species breeds in rapids in several areas below Hoover Dam, Davis Dam, and Parker Dam. It is not highly anthropophilic and primarily feeds on large bovines, equines and possibly birds. The prevalence of very high populations of adult flies from Davis Dam to a distance of 50 miles downstream and lack of any large number of large mammals to provide a source of blood meals, indicate that *S. vittatum* is mostly autogenous in the area. Literally hundreds of females land on the human body but seldom feed except occasionally on certain individuals.

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Figure 1.—Colorado River below Davis Dam, traversing through Mohave Valley of Arizona, California and Nevada, with residential, recreational and business establishments located on both banks. Larval infestations are situated 1-20 ft. under water in these as well as other swift-flowing stretches.

S. tescorum Stone and Boreham, a recently described species, breeds in small streams and creeks emanating from springs or seeps below reservoirs. It inhabits only limited habitats in the basin and therefore has limited distribution and impact in the lower basin. This species, however, is anthropophilic, feeding readily on human hosts (Figure 2) and they induce severe allergic responses in certain individuals.

PUBLIC HEALTH AND ECONOMIC IMPACT.—*Simulium* species, in general, are hematophagous insects obtaining blood from humans and other mammalian and avian hosts. Several species are vectors of pathogenic organisms to man and animals. In Africa and Central America, certain species of *Simulium* vector *Onchocerca* worms which produce river blindness and other complex syndromes in humans (Dalmat 1955, Duke 1972, Duke et al 1975, Lewis 1953). These flies also transmit Leucocytozoon infections of poultry and water fowl (Bierer 1954, Jones et al 1972, Kissam et al 1973). Instances of cattle being killed by intensive feeding of *Simulium* flies have been reported and hordes of these flies deteriorate the quality of outdoor living (Fredeen 1973). Loss of milk production in dairy cattle has been documented due to heavy outbreaks of these flies (Anderson and Voskuil 1963). *Simulium* flies are the cause of great human misery and their bites can produce both local and systemic allergic reactions in humans (Jamback 1973).

S. vittatum, the main problem species in the Lower Colorado River Basin, feeds readily on bovines, equines and other mammals and causes a great deal of discomfort and restlessness in these animals. Secondary infections are rampant in areas infested with these flies, and large scarred areas are readily visible on these animals. This species also bites humans, and some individuals experience severe allergic manifestations. After feeding of the female, a droplet of blood seals the wound which becomes reddish, tender to touch and itches severely for 2-4 days.

To assess the economic and public health impact of *S. vittatum* in the Lower Colorado River Basin, 300 question-

Figure 2.—Blooded female *S. tescorum* feeding on a human host.

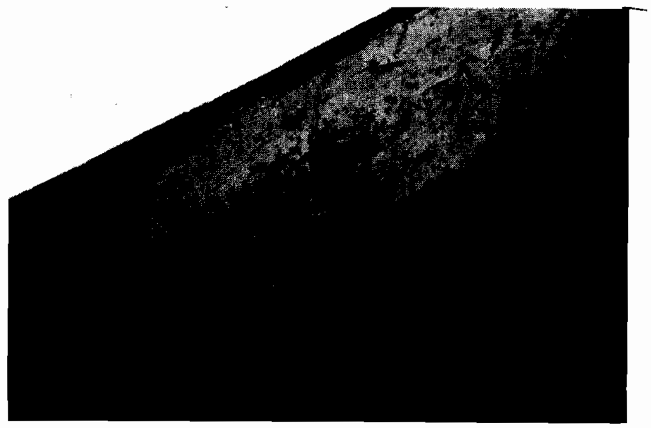

Figure 3.—Resting masses of *S. vittatum* on a boat dock in the Colorado River, just above the water line during the heat of a hot summer day. Similar numbers rest in bank vegetation, shrubs and on boats, equipment, etc; when disturbed, they fly into humans, darting into the face, ears, nose and eyes.

naires were passed out to residents in the Bullhead City, Holiday Shores, Riviera and Davis Dam areas in Arizona and South Point area in Nevada. A high proportion (180) of these questionnaires were returned. Some of the pertinent querries and the (%) of respondents answering each item are as follows:

Economic and Public Health Significance.

1. Reside near Colorado River: permanently - 91, occasionally - 6, visitor - 3.
2. Have resided at the River: 1 yr - 14, 2 yrs - 29, 5 yrs - 23, 5+ yrs - 30.
3. The gnats are bothersome: all the time - 30, winter - 3, spring - 29, summer - 18, fall - 15, rarely - 5.
4. The gnats bite: all the time - 29, sometimes - 45, rarely - 26.
5. Gnats are bothersome in the: morning - 42, midday - 7, late afternoon - 34, at night - 17.
6. React to their bites: severely - 41, moderately - 33, slightly - 23, none - 3.

7. Gnats bite on the: head - 30, trunk - 2, legs - 13, all over - 24.
8. Have sought medical attention due to the bites of gnats or their invasion in the nose, ears, and eyes: yes - 55, no - 45.
9. Animals and pets are pestered by the gnats: yes - 55, no - 45.
10. The gnats hamper outdoor activity: severely - 63, moderately - 27, slightly - 6, never - 4.
11. While fishing, swimming, water skiing, or hiking, the gnats annoy: severely - 64, moderately - 28, slightly - 5, not at all - 3.
12. Gnats are a detriment to outdoor living and pleasure in the Lower Colorado River Basin: yes - 93, no - 7.

It should be noted that the questionnaires were passed out predominantly to permanent residents. Most of the people responding had lived in the Mohave Valley area for 5-10 years (2). Most of the residents were bothered by the gnats in the spring, summer and fall (3). This is further documented by our observations on the pestiferous nature of the biting gnats where highest female host seeking and male swarming activity takes place in the warmer months. This does not mean that gnats are not a problem in the winter months. The biting activity of the gnats, as indicated from question 4, seems to be more overrated than we have observed. We believe that the layman cannot distinguish readily between actual biting and hovering and crawling of the gnats on the human body.

The diurnal rhythmic activity (pest) as shown in question 5, is in agreement with quantitative sampling data gathered on female pest activity and male swarming. However, it is certain that the respondents have not differentiated between late afternoon and dusk. The night pestiferousness is probably the one taking place soon after sunset. As a rule, *S. vittatum* in the Mohave Valley area does not show much activity after dark except being attracted to lights in small numbers.

In item 6, the extent of severe reaction to bite is overrated and here, again, distinction between a bite and hovering, landing and crawling is difficult to make. The regions of the body where the gnats bite and/or crawl are accurately pictured by the answers.

The extent of medical help sought (statement 8) by the respondents (11%) does not agree with information from medical files of one clinic in one of the infested areas (see below). It is probable that self-medication practice is included in answering this question, or the patients seek medical help elsewhere than in the Valley. Response to question 9 is realistic in view of the zoophilic nature of *S. vittatum*.

In question 10, the overwhelming majority feel that the gnats hamper outdoor activity. It is interesting to note that the level of response to the problems in question 10 and 11 is essentially the same. Question 12 draws exactly similar response as in 11 to the same problem posed in a different way. This bears out the objectivity of the respondents evaluating the gnat problem which they have been experiencing for the past 5 years or so.

At the end of the questionnaire was a simple question: What do the gnats do to you? Explain. Responses to this question were very interesting. These comments and the (%) distribution of respondents are as follows:

Comments:

1. Gnat bites itch, are painful, and often cause unsightly welts or sores that take a week or longer to heal, welts burning sensation, tender to touch, (30%).
2. The gnats are a continuous source of annoyance, irritation, and hazardous while driving, (27%).
3. The gnats swarm around the face, often getting into ears, mouth, eyes, and hair, (22%).
4. The gnat problem causes people to stay indoors, preventing them from enjoying many outdoor activities, (13%).
5. The gnat problem has hurt many outdoor recreational and related businesses and has given the area a "bad reputation", (4%).
6. The gnats are not a problem, (4%).

These above comments are some representative ones in response to the above question. Many other comments were also made which, due to lack of space, cannot be discussed here.

MEDICAL DATA.–Information on gnat bites, allergy, and impaction was provided by Drs. Lindsey and Ritter, physicians practicing in the Davis Dam area of the Colorado River. Data from randomly selected gnat-bite cases were evaluated, using 600 files. Some interesting trends were observed:

1. The number of patients visiting the clinic for gnat bites and allergy constituted about 3% of the total caseload of 10,000 or so patients. This is a considerable proportion of the total population affected by gnat bites and associated syndromes of allergy and secondary infections.
2. Most of the patients requiring medical attention because of gnat bites and impaction visited the clinic during emergency hours.
3. The age of patients seeking medical aid for gnat-related illnesses was 2-70 years. There was no significant difference by sex.
4. Treatment mostly required removal of impacted gnats, treatment with antibiotics to prevent or cure secondary infections and administration of corticoids for allergic and other manifestations.
5. Most of the patients were local residents, although a few from southern California, Las Vegas, and Phoenix area were also treated at the clinic. It is logical that most tourists and visitors will seek medical help in their own home towns after leaving the river area, as the severe symptoms associated with gnat bites do not develop for 4-5 days after the bite.
6. The bites treated at the clinic occurred from February to November. No cases were reported in December and January. This agrees with the biting and host seeking activity trends of the females that we have observed over the past 2 years.
7. Some of the symptoms and causal agents diagnosed by the physicians are as follows:

Foreign object in ears, eyes, sinuses, look like insects.
Eye swollen, discharging, bite on lid, etc.
Bite on knee, swollen, red, can't bend.
Bite on face, eye swollen.
Hand swollen, wrist tender, apparent gnat bite, itching.
Outer ear swollen, allergic reaction to gnat bite.

Insect bites on arms and face, swollen, inflamed.

At work, bitten by insect on ear, swollen, allergic reaction.

Fishing, bitten on arm, large area swollen, has allergy to insect bites.

Bitten on lip, swollen, can't move it.

The case history shows that the symptoms of swelling, itching, burning, pain and secondary infections sometimes lasted for 3 weeks. In general, the symptoms after appropriate medications, disappeared within a week or so.

NUISANCE.– In addition to biting man and animals, the huge numbers of *S. vittatum* on the river and along both banks create a serious nuisance problem. The flies form loose or compact swarms along the banks using trees, bridges and man himself as markers. During the heat of the day, they rest in trees, shrubs, lawns, vegetation or on boats, docks (Figure 3), patios, buildings and other water and land base structures. When disturbed, these flies hover, dart and hit the human body, frequently making impact into the eyes, ears and nostrils.

The flies also enter houses, restaurants, business establishments and create somewhat serious nuisance problem indoors. Compared to the outdoor problem, this aspect is of minor importance at this time. The flies have been noted to mass migrate as far as 20-30 miles, pestering distant communities at certain times of the year.

Aside from the medical and economic importance of blackflies along the Colorado River, they are greatly annoying to man and domestic animals. Swarms and massive resting and flying numbers of these flies, at times, create conditions deteremental to outdoor leisure activity and enjoyment of the natural scenic beauty of the lower Colorado River Basin.

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A PROGRESS REPORT ON INSECTICIDE RESISTANCE IN HOUSEFLIES IN CALIFORNIA

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In a previous study of resistance completed in 1971 (Georghiou et al. 1972) we concluded that field populations of house flies *(Musca domestica)* in California exhibit a mosaic pattern of resistance consistent with the extent of insecticide usage in each geographic area. Resistance occurred at a higher level and involved almost every available insecticide in such areas as Moorpark where fly control in poultry ranches is extremely intense, whereas it was minimal at Brawley where climatic conditions and a rural environment impose less frequent recourse to chemical treatments.

We have continued our investigations and we now report on the evolution of resistance through 1975 in three representative situations: a poultry ranch (S&K) at Moorpark, Ventura County; a dairy (Excelsior) at Chino, San Bernardino County; and a cattle feedlot at Meloland, Imperial County. As in the earlier studies, tests were carried out by topical application of insecticides to 3-day old female flies of generations F_1 to F_3 after colonization. The results are expressed as resistance ratios at the LD_{50} (or LD_{95}) with reference to a standard susceptible strain (MAIDM).

In Table 1 are indicated the levels of resistance obtained toward each of the four principal insecticides on which fly control in California has depended in recent years, i.e., dimethoate, naled, dichlorvos and stirofos. The evolution of resistance from 1970 to 1975 at the Moorpark poultry ranch is illustrated in Figure 1. It will be noted that resistance at this ranch toward each of the four compounds exceeds the 10-fold level which is normally considered as the threashold for effective fly control. At the Chino dairy, with the exception of dimethoate which has been used extensively in many dairies, resistance is lower than at Moorpark, but sufficiently high to present control problems. Resistance is still low and less extensive at Meloland, although it now fully involves such "older" compounds as malathion, diazinon and ronnel. However, there is no evidence of resistance to dichlorvos or to stirofos.

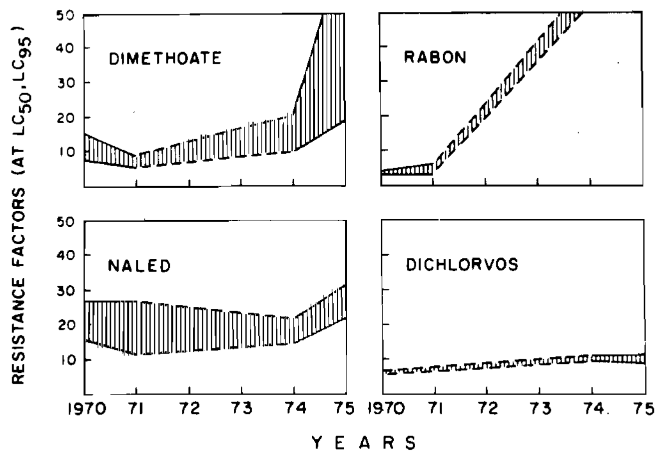

Figure 1.–Resistance factors toward dimethoate, naled, stirofos (=Rabon®) and dichlorvos (at LC_{50}, lower line, and LC_{95}, upper line) at S&K Poultry Ranch, Moorpark, California, during 1971-75.

We have been especially impressed by the rather rapid rise in resistance toward stirofos at Moorpark and Chino. Resistance toward this compound was practically nonexistent in any of 8 geographical areas studied in 1970-71 (Georghiou et al. 1972). However, because of the demonstrated ability of *Fannia* spp. flies to transmit the Newcastle

Table 1.– Resistance to insecticides in house flies, 1975.

Insecticide	Susceptible NAIDM LD_{50} (µg/♀)	Resistance ratios* at LD_{50} Meloland (UC)	Chino (Sterk)	Moorpark (S & K)
Dimethoate	.012	5.7 (16.6)	23.3 (46.8)	18.3 (68.4)
Naled	.019	3.5 (5.3)	6.8 (11.0)	22.1 (31.0)
Dichlorvos	.036	1.5 (1.9)	3.1 (3.4)	8.1 (11.2)
Stirofos	.068	0.5 (0.7)	4.6 (66.6)	100
NRDC 161	.00018	0.78 (0.94)	1.6 (2.2)	2.8 (4.0)
NRDC 161 + p.b.	.00015	0.59 (0.79)	1.5 (2.6)	2.1 (3.1)
Orthene	.032	4.1 (14.5)	71.9 (375)	169.0 (790)
Orthene + p.b.	.032	2.4 (8.3)	15.3 (24.4)	28.1 (50)

*Resistance ratio (RR) = $\frac{LD_{50} \text{ R Strain}}{LD_{50} \text{ S Strain}}$ (In parenthesis, RR at LD_{95}).

disease virus (Rogoff et al. 1975), stirofos in combination with dichlorvos was applied extensively in 1972-73 to ranches which were to be depopulated by the Newcastle Disease Eradication Teams (Bram et al. 1974). This measure, and the subsequent use of stirofos by the ranchers upon resumption of poultry rearing operations, is most likely responsible for the rapid development of resistance to this compound.

In the course of these investigations we have also examined the activity of several new chemicals against flies. Two of these, typifying the importance of novelty of chemical structure for effectiveness against resistant flies, are indicated in Table 1. Orthene®, an organophosphate (O,S-dimethyl acetylphosphoramidothioate), although as toxic as dichlorvos to the susceptible strains (LD_{50} 0.032 and 0.036 μg/♀, respectively), is 71.9 and 169-fold less active against the Moorpark and Chino strains, respectively, due to cross-resistance. In contrast, NRDC 161, a member of the novel family of synthetic pyrethroids (which have not yet been employed in practical fly control in California) exhibits nearly equal toxicity against susceptible as well as resistant flies. Its remarkable activity against house flies, even at dosages 200-fold lower than dichlorvos (LC_{50} 0.00018 μg/♀ vs. 0.036 μg/♀) suggests that this and related chemicals hold considerable promise for future control of organophosphorus-resistant house flies.

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CHIRONOMID MIDGE INVESTIGATIONS IN A CONCRETE LINED DRAINAGE SYSTEM IN SOUTHERN CALIFORNIA[1]

Arshad Ali[2], Mir S. Mulla[2] and Frank W. Pelsue[3]

Studies on the nuisance aquatic chironomid midges in the Coyote Creek drainage system initiated in August, 1974 are continuing. This waste-water drainage system, situated in Orange and Los Angeles Counties, California, is composed of 2 main channels (the North Fork and the East Fork) and several small tributaries. It is open and concrete lined over most of its course (Figure 1), receiving mainly domestic and industrial waste water rich in organic and inorganic materials. These materials settle out in varying quantities on the concrete lining of the channels, forming ideal substrates or habitats for breeding of chironomids, supporting midge larvae at nuisance levels. Five types of substrates, forming different midge biotopes, were noted in this drainage system. These were categorized as follows: bare concrete surfaces, filamentous algae, detritus, fine sand, and sand and gravel (Ali and Mulla 1975).

The present studies were initiated with the main objective of accumulating basic information about the biology, ecology, distribution (spatial and seasonal), succession and abundance of the midge fauna in the system. Such basic understanding of the problem is essential to the development of management strategies and practical procedures for control of the nuisance midges.

Since August, 1974 to February, 1976, biweekly quantitative benthic samples were taken from 9 stations established along the 12-mile stretch of the drainage system, using a modified Surber sampler fitted with a 500μ pore net.

Water temperature and current velocity were measured at each site while sampling. Daily max. and min. air temp. data and measurements of rainfall in the area were also recorded.

Larvae belonging to the genera, *Cricotopus* spp., *Chironomus* spp., *Dicrotendipes* sp., *Paralauterborniella* sp., *Tanytarsus* sp., *Tanypus* spp., and *Procladius* sp. were taken during this study. Determination of these immatures beyond the generic level was not possible, due to the lack of suitable keys. However, adult midges belonging to the species *Cricotopus bicinctus* (Meigen), *Cricotopus sylvestris* (Fabr.), *Chironomus decorus* Joh. complex, *Dicrotendipes californicus* (Joh.), *Paralauterborniella subsincta* (Townes), *Tanypus grodhausi* Sublette, *Tanypus carinatus* Sublette, and *Procladius denticulatus* Sublette were taken in the area.

Larvae of *Cricotopus* spp. were the most abundant midge group in the Coyote Creek system, and were present year-round. *Cricotopus* spp. formed more than 82% of the total midge fauna taken from August, 1974 - February, 1976; their monthly composition ranging between 65-99% of the total midges taken each month. Two distinct larval peaks were noticed between August - October, 1974. Similarly, in 1975, 3 peaks were recorded between June - September. These larval peaks coincided with the higher water and air temperatures. In 1974, the highest number of *Cricotopus* spp. larvae were taken in August when the average larval density amounted to 2000/ft^2, and in 1975, the highest density was recorded in June when on the average 1300-1400 larvae/ft^2 were recovered. During winter (November - February) each year, *Cricotopus* spp. declined in the channels with the decreasing water and air temperatures. However, marked reductions in benthic midge populations during winter usually followed heavy rainfall in the area.

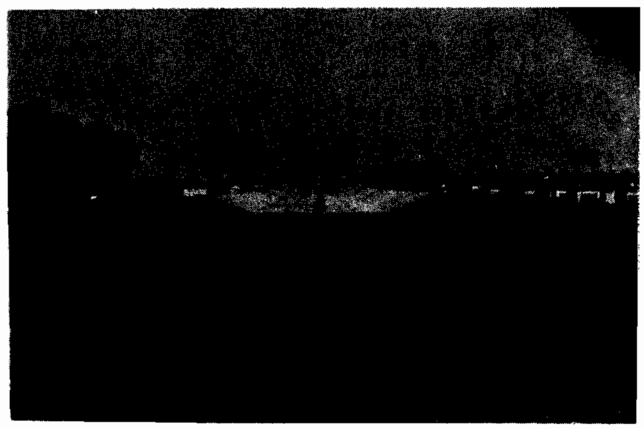

Figure 1.–North Fork of Coyote Creek in Los Angeles County, California, located in residential-industrial area.

Figure 2.–Part of the East Fork of Coyote Creek adjacent to residential-industrial establishments, Los Angeles County, California, where the substrates from the channel were removed.

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Chironomus spp. larvae occurred in the drainage system during August - November. In 1974, their monthly composition ranged between 1-28% of the total midges during August - November, and in 1975, when present, they did not exceed 8% of the monthly total midge collections. Each year, *Chironomus* spp. appeared in early August and their numbers gradually increased all through August and September, peaking in late September and declining thereafter in October and November. After November, *Chironomus* spp. occurred sporadically in negligible numbers. In the peak period in September 1974, the average larval density of *Chironomus* spp. ranged betwen 400-500/ft^2, but during the same period in 1975, their numbers were low, not exceeding 80 larvae/ft^2.

Although larvae of *Dicrotendipes* sp., like *Cricotopus* spp., were present throughout the study period, they were rare in the channels from December - April. *Dicrotendipes* sp. formed about 9% of the total midges taken during the study and were more common from May - November, 1975. During this period, their monthly composition ranged between 2 - 34% of the total chironomids taken each month. In 1975, 2 larval peaks, one in July - August and the other in October - November were attained. The former peak was more pronounced than the latter, and it preceeded the peak in the other chironomines, *Chironomus* spp., by a few weeks. The average larval density of *Dicrotendipes* sp. at the time of earlier peak exceeded 600/ft^2, while during the 2nd increase in October -November their density averaged to 50 larvae/ft^2.

The tanypodines, *Procladius* sp. and *Tanypus* spp. combined were less common, forming less than 1% of the total midges taken from August, 1974 to February, 1976. Although they were more numerous from May - October, their average larval density remained below 25/ft^2. Larvae of the genus *Paralauterborniella* sp. were also less common. They were noticed only from August - November and formed 0-5% of the total midges taken in each of these months. Their average density never exceeded 30/ft^2. *Tanytarsus* sp. were rare, only a few specimens occurred during the summer months.

In September - October, 1974, spatial distribution of chironomids in relation to the 5 prevailing types of substrates in the Coyote Creek drainage was studied. Quantitative benthic samples from each of the 5 substrates in different sections of the channel system were collected on 4 different occasions.

At the time of this study, midge larvae belonging to 5 genera were present but *Cricotopus* spp. and *Chironomus* spp. were the 2 most abundant groups, forming more than 97% of the total midges taken on any occasion.

On all 4 sampling dates, highest numbers of *Chironomus* spp. were recovered from fine sand (42-80% of the total *Chironomus* spp. at all sites) or filamentous algae (10-45% of the total *Chironomus* spp. at all sites) and least were taken from bare surfaces (less the 1% of the total *Chironomus* spp. at all sites). Bottom materials consisting of detritus or coarse sand and gravel were relatively poor in *Chironomus* spp. fauna. In each of these 2 microhabitats, *Chironomus* spp. did not exceed 12% of the total *Chironomus* spp. taken at all sites on any occasion.

The distribution pattern of *Cricotopus* spp. was different from that of *Chironomus* spp. On all 4 sampling dates, *Cricotopus* spp. were most abundant in detritus (58-78% of the total *Cricotopus* at all sites) showing a strong correlation with this type of substrate. Filamentous algae supported a fair number of *Cricotopus* spp. (7-21% of the total *Cricotopus* spp. at all sites) and fine sand contained 7-12% of the total *Cricotopus* spp. taken during the study. Bare surfaces and coarse sand and gravel were poor in *Cricotopus* spp. midges. In each of these 2 substrates, *Cricotopus* spp. did not exceed 8% of the total *Cricotopus* spp. taken from all sites on any occasion.

Subsequent to the establishment of substrate type and midge faunal relationship, the effectiveness of mechanical removal of substrates for the control of chironomids was studied in June - July, 1975. Approximately 500 cubic yards of substrate materials from half a mile long section of the East Fork were mechanically removed (Figure 2) using an Eimco loader equipped with ballon tires and a hydraulic loading bucket. Fine sand and detritus were the 2 predominant types of substrates in the area cleared. Pre- and post-removal benthic samples from 3 sites in the half mile long section were taken. Check area was located upstream in the uncleared area.

Midge larval densities in check and cleared areas were over 1300/ft^2 prior to the removal of substrates. *Cricotopus* spp. and *Dicrotendipes* sp. predominated and formed more than 89% of the total midge fauna. Pupae constituted more than 8% of the total immature midges. Samples taken 3 days after substrate removal showed a reduction of 88% in the total midges. After 10 days, a reduction of 63% was recorded and after 17 days, the total reduction decreased to 17% only. Satisfactory midge control lasted for 2 weeks or so and benthic midge populations recovered completely after 4 weeks. The possible factors responsible for midge recolonization in this situation are discussed elsewhere (Ali et al. 1976).

In addition to the above studies, diurnal periodicity in the common midge groups was also studied. Drift samples at one site below the confluence of the North and the East Fork were taken on 2 separate occasions in 1975. Each time sampling was carried out over the entire 24 hour period, taking a 10-minute sample every alternate hour during daytime and collecting one sample every hour during the night.

Changes in water temperature, current velocity and width and depth of water during the 24 hour sampling period were recorded. Time of sunset and sunrise was also noted.

Data acquired showed an increase of the common midge (i.e. *Cricotopus* spp., *Chironomus* spp. and *Dicrotendipes* sp.) larvae and pupae in drift immediately after darkness, and their high numbers in drift were maintained at least until midnight.

From the studies undertaken thus far, described above, it can be concluded that *Cricotopus* spp., *Chironomus* spp. and *Dicrotendipes* sp. are the 3 groups of midges abundant in the Coyote Creek drainage during the midge annoyance period in summer and autumn months. *Chironomus* spp. in this biosphere, were associated with fine sand or filamentous algae and *Cricotopus* spp. were strongly correlated with detritus. Mechanical removal of these substrates eliminated most chironomid larvae and pupae associated with these substrates, offering good potential for integrated operational control of the nuisance midges in this drainage system and elsewhere.

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