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THE IMPACT OF EPA ON MOSQUITO CONTROL PROGRAMS

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There are various regulatory sections of the amended Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) which are of concern to you, so it might be well to review the status of these sections preparatory to a question and answer session which will follow Dr. Hazeltine's response. He and a few other members of your Association have been in touch with us at EPA to help us produce regulations which are clear and generally acceptable. May I remind you all that input is solicited so that we can produce the best possible regulations to permit the continued use of vital pesticide chemicals, while still protecting man and the environment from adverse effects. The enactment of the FIFRA amendments two years ago has made it essential that we increase cooperation in implementing new programs to meet the needs of our times.

The first priority of the Environmental Protection Agency is the protection and enhancement of the environment. The emphasis on environmental values is of critical concern to the future of vital, irreparable, natural resources. However, we at EPA appreciate the importance of proper use of pesticides in the control of pests in public health programs, in agriculture, and in other areas. We fully recognize that pesticide chemicals are vital to our continued state of abundance and good health.

The new pesticide law has placed increased responsibility on the Environmental Protection Agency and on each state. It provides additional tools to develop pesticide regulatory programs which will protect and enhance the quality of the environment, while at the same time permitting the continued abundance of food and fiber and quality of health so long enjoyed. Congress recognized the need for proper use of available talent and expertise in the field of pesticides. The new Act returns more decision-making responsibility to people close to the problems.

Now let's discuss the progress we have made toward the goal of implementing the Federal Environmental Pesticide Control Act (FEPCA) amendments by October of 1976. Section 4 concerns Certification of Applicators which is certainly of concern to you. Certification is expected to improve the degree of professionalism and skill in your fine ranks, but preliminary to this discussion of Agency's intent would be in order. The goal of EPA is to strive to achieve an overall increase in environmental quality. It is important for all of us to recognize that EPA was created because of increasing public and governmental concern about the dangers to the health and welfare of our nation caused by environmental deterioration. Man has always been aware of the transience of the natural environment. Troubadours and poets have long lamented the passing of beauty; but while the poets of old lamented the passing of beauty, 20th Century man laments the passing of our natural resources due to his own carelessness, improper planning and waste. William Ruckelshaus, first Administrator of EPA, said when the Agency was formed:

"More and more people within our society want to participate in the development of a new environmental ethic -- a way of life which will allow us to retain and improve the life-enhancing features of technology without repeating and intensifying the mistakes of the past. A central role of the United States Environmental Protection Agency is to support this national effort and to help change those habits and those obsolete viewpoints which led to our current confrontation with gross pollution and threats of irreversible environmental damage."

Knowing these priorities of the Agency, you can better understand the goals of the Office of Pesticide Programs, and our concern with the effect of these substances on the environment. However, we recognize the vital importance of pesticides in our daily lives, and the concerns of the Mosquito Control Association in maintaining the tools necessary to the proper accomplishment of your professional services. We are for pest control too, and fully cognizant of the disasters which could occur were pesticides abandoned. We would be foolish to eliminate our pest control arsenal, but more than foolish if we did not recognize that these products of advanced technology must be regulated to assure the health and welfare of the nation at large. The knowledge gained in the last decade has taught us that pesticide residues can pose serious problems, that they can persist in the environment, that pesticides can travel miles, even hemispheres, from the site of original application, that they can bioaccumulate and magnify in the food chain, and may effect serious adverse health consequences in non-target animals, including man. The public has demanded that government protect its interests by regulating potentially harmful products; EPA through administration of FIFRA strives to fulfill this vital mandate.

We count on you heavily in developing regulatory procedures. We have common goals which we can pursue together: we are both interested in effective pest control, and we are both interested in accomplishing pest control without adverse effects on non-target organisms.

Section 4 of the regulations governing Certification of Applicators was published in the Federal Register on October 9, 1974 and has been fairly well received. The Amended FIFRA specifies that standards for Certification of Applicators "shall provide that to be certified, an individual must be determined to be competent with respect to the use and handling of pesticides, or to the use and handling of the pesticide or class of pesticides covered by such individual's certification." We believe that the regulations are inclusive, reasonable, fulfill Congressional intent, and reflect genuine concern for human health, the environment, and the importance of the proper use of pesticide products. Many expressed an interest that there be a "grandfather clause" in the regulations, since many commercial applicators have had extensive experience and are licensed by a number of states. Our view of this issue is that certification under Section 4 constitutes a first-time entry into regulation of pesticide use on a national basis and that there is no statutory authority for an across-the-board grandfather clause. Licensing of applicators has, in some locales, consisted of nothing more than the purchase of a business license. EPA believes that state plans for applicator certification should be considered and evaluated on a case-by-case basis to determine whether a state's present or past licensing procedures do in fact meet Agency standards. If EPA is satisfied that a state's standards do in fact meet ours, that state may provide that applicators so licensed or certified be initially certified under the new program without reexamination.

Many expressed concern that our proposed regulations encouraged states to establish stricter standards than those imposed by EPA. We have stipulated that standards "must conform and be at least equal to" those of EPA. It is not the Agency's intention either to encourage or discourage states from exceeding the Federal standards since this matter is a state prerogative.

The Certification standards clearly identify the broad areas of knowledge with which an applicator should be familiar prior to certification. I emphasize here that we do not intend to suggest that an extensive amount of rote memory work should be required for certification. It is our contention that the certified applicator should have a storehouse of general knowledge to enable him to understand the nature of problems that may be encountered and/or avoided. The eight areas of knowledge specified in the regulations, i. e., label comprehension, safety, environment, pests, pesticides, equipment, application techniques, and laws and regulations, are imperative to the proper application of hazardous products. As operators under Category 7, industrial, institutional, structural and health related pest control, you will be particularly concerned with having a practical knowledge of a variety of pests, including their life cycles, types of formulation appropriate for their control, and methods of application that avoid contamination of food, damage and contamination of habitat, and exposure of people and pets. In short, to be certified, an applicator must demonstrate competence in dealing with pest problems in situations in which he will most likely be operating. This is only reasonable and will require competence an experienced applicator should have, and that which an inexperienced applicator most assuredly should desire to attain -- EPA regulations aside -- before using restricted products.

Probably you are interested in the status of EPA regulations for the Development and Submission of State Plans for Certification of Pesticide Applicators. These were published on January 13, 1975.

These revisions are designed to ensure that State Plans for Certification meet the requirements of Section 4, and they explicity identify other important elements needed for a state to implement and maintain an effective certification program. While some states do not currently have the appropriate legislative authorities needed to implement proper state plans, those in this category are moving toward correcting deficiencies. State plans must be explicit, and must conform to the standards prescribed in the Certification Regulations. We anticipate that states will submit sample questions or sample examinations which can adequately reflect the adequacy of the state program.

Concerning reexamination provisions, it is important that certified applicators continue to meet the demands of changing technology. Pest control is a dynamic industry and is likely to become even more so. A number of options are available to ensure continuing competence, including requirements for periodic reexaminations, the administering of special examinations as significant new developments emerge, or a system of attendance at conferences, workshops, or scheduled training sessions. The matter has currently been left flexible, and states will only be asked to include suitable provisions in their plans.

Training is not addressed by Section 4, rather by Section 23. But training obviously is crucial to the proper certification of both private and commercial applicators by October 1976. Training programs are most important in assuring that applicators meet competency standards. The Department of Agriculture and EPA have requested approximately $10 million for Fiscal Year 1975 and $12 million for Fiscal Year 1976 to be devoted to training and certifying applicators. Unfortunately, we have no assurances of getting this amount, though we'll continue to be optimistic. We have developed a Core Manual for Training Applicators, which should be of much assistance in training efforts. We are emphasizing to the lead agencies in the states that all resources -- the Cooperative Extension Service, educational institutions, industries, and associations such as yours -- MUST be identified and utilized to the maximum.

Fees for certification is a matter entirely within the states' purview. We anticipate that some states will elect to charge minimal administrative cost fees, as is done currently in many places. Supervision will depend on state policy in many instances. Section 4 Regulations specify that the availability of the certified applicator must be directly related to the hazard of the situation in which pesticides are used. There are many times when "direct supervision" by a certified applicator can consist of detailed guidance concerning the application of a pesticide and a provision that the certified applicator shall be available if needed. However, if the label requires direct application by the certified applicator, or if a state decides that certain operations must be conducted personally by a certified individual, those requirements will apply.

Many persons expressed concern about EPA's policy toward the "use inconsistent with the label" provisions of Section 12 of the Amended Act. This important Section of the Amended Act was intended to provide the Agency with the vital legal authority to prosecute misuse of pesticide products. Pesticide misuse was felt by the Congress to be a significant source of adverse environmental and health effects from these chemicals, and an important consideration in the legislative history leading to the 1972 Amendments. Prior to these Amendments, EPA administered the Act under the frustrating circumstance of having the statutory authority to register pesticide products, but not having authority to ensure that registered directions and precautions crucial to the proper application of hazardous products were followed. It is in the best interests of the public, the applicator, and the environment at large that all pesticide products be registered with EPA for their intended purposes, and that users adhere to the registered label in dealing with pest problems.

However, there are some significant problems confronting certain user groups such as yours if a strict interpretation of the "use inconsistent with the label" provision is enforced. There are important issues such as using less pesticide than the label calls for, or using pesticides against insects which are not mentioned on any registered label; these point up a dilemma which we are anxious to resolve. Jim Agee, the Assistant Administrator for Water and Hazardous Materials, is concerned that we fully explore these problems with the hope that they can be expeditiously identified and resolved. The entire misuse problem is under close scrutiny, and you have our assurance that we understand your concerns and are confident that they can be met with reason and good sense.

Our initial effort in this regard, dated November 27, 1974, had some shortcomings and Bill Hazeltine was quick to point out Public Health considerations that had been neglected. Many comments, including his, are now under review.

Section 3, concerned with Registration and Classification of pesticides, is of concern to your group. Comments received on proposed Section 3 guidelines, recently published, are currently under review. Let me discuss the registration activity with you generally.

The U.S. Environmental Protection Agency currently has registered some 34,000 pesticide products. The products include the chemical types as follows: insecticides, 44 percent; herbicides, 20 percent; disinfectants, 17 percent; fungicides, 15 percent; rodenticides, 3 percent.

The number of uses covered by these products is in excess of 250,000. There are more than 1,200 chemically active ingredients in the 34,000 registered products.

Registration is the U. S. Government's method for control-use of pesticides to ensure that these chemical and biological agents are as safe and effective as possible, employing present technology.

Although pesticides are deliberately put into the environment for a beneficial purpose, it should be kept in mind that many of them may be pollutants.

EPA has a program of monitoring pesticides to determine levels and trends of residue in soil, water, air, plants and other organisms where residues may accumulate. Most pesticides manufactured or imported into the United States, and certain ones shipped to other countries, are registered under the Federal Insecticide, Fungicide and Rodenticide Act.

The registration process for pesticides begins when an applicant submits a request for registration along with test data to show that when used as directed, the pesticide:

1. Will not injure man, animals, crops, or damage the environment.
2. Is effective against the pests listed on the label.
3. Will not result in illegal residues on food or feed. Pesticides used on food and feed crops must have a residue tolerance established prior to their registration.

A tolerance in the United States represents the upper level of pesticide residue which can lawfully remain on food or feed when it enters commerce. Tolerances also apply to imported commodities.

When EPA registers a pesticide product, it assigns a registration number which is required on the label. The label also bears safety precautions, application limitations, and directions for use. This information must be explicit as to pests controlled, when and where the pesticide may be used, how it is to be mixed and applied, and what precautions should be taken in handling. In some instances, the label specifies how many days must pass before a crop may be harvested after the last treatment or before reentry by work crews.

Much of the data used in the labeling process is obtained in connection with an experimental use permit. This enables the registrant to obtain data on the effectiveness of the pesticides from limited field testing after the chemical has been shown to have pesticidal value.

New chemicals and new uses of old chemicals often are required to go through this permit process before being considered for registration. Pesticides imported from other countries must meet all the requirements of the U. S. pesticide law.

Under the 1972 amendment to FIFRA, pesticides will be classified under two major categories: general use and restricted use.

A restricted use means that a pesticide must, in most cases, be applied by or under the direction of a qualified applicator, that is, one who has been tested and certified under his State's certified applicator program. Pesticides will be classified for restricted use if they are highly toxic or persistent in the environment and can cause unreasonable adverse effects on the environment, or to the applicator, in the absence of additional controls.

All other pesticides will be classified for general use.

I know you want to know what pesticide products will be in the restricted category, but there isn't much I can tell you at this time since the classification portion of Section 3 of the regulations has not been completed. However, in late November of last year, preliminary information was supplied state regulatory people to help them estimate the number of private applicators they might have in their individual states, as follows:

"This memorandum is to indicate the use classification of a number of widely-used pesticides. Although the analysis was based upon only a partial consideration of all the factors that enter into the classification decision, it should prove useful to state regulatory officials in determining the magnitude of state certification programs required to train and certify applicators."

Initially chosen for examination were pesticides that were heavily used on major crops, according to the 1971 USDA farm survey. This list was pared down by eliminating pesticides for which data were not currently or easily available.

Proposed Section 3 regulation requires that the following factors be considered before classifying a pesticide formulation for general or restricted agricultural use:

1. If the formulation has been assigned a toxicity category rating of one highest toxicity it is a candidate for restricted use. Otherwise it is a candidate for general use classification.
2. If the formulation, on the basis of its acute toxicity and use rates, presents pre-determined hazards to fish and wildlife it is a candidate for restricted use. Otherwise, it is a candidate for general use.
3. Even though the pesticide is a candidate for restricted use, if the labeling, packaging, use history or type of formulation indicate that exposure can be minimized, then the pesticide will be classified for general use. Conversely, pesticides that are candidates for general use may be classified as restricted if these same factors indicate a sufficient hazard is present.

Only steps 1 and 2 were followed in this analysis. The review for exposure (step 3) was not included and hence the results indicate only initial estimates of the final product classification.

The following results were obtained:

Insecticides

General use:

Bux (except for rice application which would be a restricted use)

Carbaryl

Restricted use:

*toxaphene (an environmental hazard)

Methyl parathion (human and environmental hazard)

*Parathion (human and environmental hazard)

phorate (human and environmental hazard)

*Carbofuran (human and environmental hazard)

Herbicides

General use:

Atrazine

2. 4-d (except for aquatic use, which would be restricted)

Propachlor

Alachlor

Fungicides

General use:

Copper Sulfates

Maneb

It must be emphasized that a pesticide product will be classified only after it is subjected to a labeling review. Therefore these results should be interpreted only as giving a preliminary indication of final classification. With few exceptions, formulations of chemicals that have been shown as general use will have that classification. A number of formulations of chemicals that are now classified as "restricted use" may ultimately be classified "for general use".

*A number of granular formulations of these products may be classified as general use on the basis of individual labeling reviews.

Registered products must be reregistered every five years to ensure that they meet current standards. For example, scientific data developed subsequent to registration may call into question earlier evidence of safety, requiring additional precautions or restrictions on use of a product. The Administrator of EPA is empowered by the law to deny registration, cancel or suspend previous product registrations to protect the public interest.

Matters of prime consideration in EPA are environmental damage and risks from using pesticides, as well as the economic factors. Pesticides may be banned if the risks and costs of their use outweigh the economic and social benefits.

While pesticides are valuable tools, we are interested in improving methods of pest control. One of the more promising of these is the integrated pest management concept, an approach that employs a combination of techniques to control the wide variety of potential pests that may threaten crops. It involves maximum reliance on natural pest population controls, along with a combination of techniques that may contribute to suppression. For example, cultural methods and pest-specific diseases, such as bacilli that kill cabbage loopers and the imported cabbage worm as well as other caterpillars. Such bacilli are presently being tested for use against the Douglas Fir Tussock Moth. Some other techniques of integrated pest management are resistant crop varieties, sterile insects, attractants, augmentation of parasites or predators, or chemical pesticides as needed.

A pest management system is not simply biological control or the use of any single technique. Rather, it is an integrated and comprehensive approach to the use of various control methods that takes into account the role of all kinds of pests in their environment, possible interrelationships among the pests, and other factors.

The search for improved methods goes on as does EPA's continued vigilance over the pesticides now in use.

A section of the Act directly related to Section 3 is Section 24. Section 24(c) is of special concern to all of you. "A State may provide registration for pesticides formulated for distribution and use within that State to meet special local needs if that State is certified by the Administrator as capable of exercising adequate controls to assure that such registration will be in accord with the purposes of this Act and if registration for such use has not previously been denied, disapproved, or cancelled by the Administrator. Such registration shall be deemed registration under Section 3 for all purposes of this Act, but shall authorize distribution and use only within such State and shall not be effective for more than 90 days if disapproved by the Administrator within that period."

In providing for Federal registration of all pesticides, the Congress recognized that there are many pesticide uses, particularly on minor pests and specialty crops, for which there is no Federal registration. In many instances this situation reflects the fact that the pesticide manufacturer or formulator considers the cost of seeking and obtaining Federal registration of such uses to be disproportionate to potential profitability. Nevertheless, farmers and others rely on such minor uses as solutions to many locally important pest problems.

The legislative history of the 1972 amendments indicates that it was the intention of the Congress that Section 24(c) be employed to help deal with the minor use problem.

Specifically, Senate Report No. 92-838, dated June 7, 1972, said:

"The purpose of this subsection is to give a State the opportunity to meet expeditiously and with less cost and administrative burden on the registrant the problem of registering for local use a pesticide needed to treat a pest infestation which is a problem in such State but is not sufficiently widespread to warrant the expense and difficulties of Federal Registration."

We are now in the process of developing proposed regulations and guidelines to facilitate State registration without compromising any important safety considerations. They are designed to permit States to issue registrations covering not just minor uses in the usual sense, e. g., for use on pests or crops not listed on an EPA-approved label, but also special local needs of other kinds, including, for example, needs for special directions for use of pesticides to accommodate unusual local conditions.

EPA recognized that most States will not be able to obtain certification under Section 24(c) prior to the effective date of EPA's regulations for implementation of Section 3. To prevent needless disruption of State registration programs, particularly in relation to minor uses, we anticipate that an interim authorization procedure will be permitted. This procedure would enable eligible States to register pesticides to meet special local needs for a period of not more than 90 days after the effective date of the Section 3 regulations.

Such interim authorization would be available only to States that have been issuing pesticide registrations based on their own independent consideration of efficacy and environmental and human health hazards. Our proposed regulations will, we anticipate, provide instruction advising how a State that has been conducting such a program can request interim authorization. We will continue to solicit State opinions as our proposals are developed.

Section 5 deals with experimental permits. Anyone can apply to EPA for an experimental permit. If food and feed are involved, tolerance must be determined. Provisions also allow States to issue experimental permits under provisions to be set by EPA. These will generally be for special local needs. Section 5 regulations all are being finalized by the Agency.

There are other sections which may be of concern but maybe we can touch on them in our question session.

Summing up our look at pesticides today, let's keep several things in mind. First, we are always seeking to achieve the best possible balance of pesticide use. This is the goal not only of EPA but also of state programs, industry, and users who have the best interests of the nation and the environment at heart. It is also the goal of the Amended FIFRA. At the same time, our knowledge of pesticides . . . and their impact on the environment . . . is constantly changing. With this in mind time is important. It is important in order that we can best serve your interest as pesticide users while protecting the environment that sustains us all.

As a parting word, thank you for inviting me to participate in your Annual Meeting, and let me emphasize that the lines of communication are always open between us. As I said earlier, we share many common goals -- let us strive to increase the natural harmonies which exist between us, and to refrain from stereotyping ourselves as "regulator" and "regulatee". Your ideas and cooperation are appreciated by EPA. I trust that we will continue to keep our thoughts and discussions moving, and that together we will make Amended FIFRA a smooth and viable reality. Many thanks.

RESPONSE TO EPA

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There are certain areas of concern relative to EPA and its administration of the pesticide laws. The EPA has almost exclusive judgment on pesticide use, labels, registration. There are civil and criminal penalties for any use inconsistent with the strict wording of the label. The regulations allow a reduction of up to 40% on the fine, for a person who pleads guilty. Civil penalties can be set up to $1,000 for a private applicator for each violation.

Use inconsistent with the label is one of the real keys to the federal law. The EPA as yet fails to acknowledge or allow a reasonable deviation from the label. However, congressional intent seems to require some individual judgment, and imposes a "person of ordinary intelligence" guideline on what constitutes a use inconsistent with the label.

EPA has moved to block or preempt state registrations of some pesticides, such as parathion. The label registration by one supplier, for example, was turned down for mosquito control at 0.1 lb. per acre. The registrant was told that EPA would accept a registration of methyl parathion at .04 lb. per acre, yet we know that this lower rate would not provide effective control; the experts in EPA were willing to permit a label at this rate, without regard to current control uses and needs. In fairness, subsequent contact by EPA suggested that the 0.1 lb. per acre rate would be accepted, if the registrant would reapply. Obviously some of the people who are making decisions do not really know what is happening in the field. With all the requirements of efficacy information, the authorities were relying on an old document called the "compendium", which stated that .04 lb. per acre of granular material could be used to control mosquitoes, but unfortunately mosquitoes in the field cannot be killed at this rate.

The concern in this restriction on use ties to the provision of the law which prevents State registration for any use which has been cancelled or blocked. Even registrations which are withdrawn, seem to be included in the restriction.

EPA has denied the use of DDT for tree hole mosquito control, although the law giving the Agency authority for restricted beneficial use authorizations is now in effect. Butte County Mosquito Abatement District especially has been harmed by this action by EPA.

Some of the Federal Agency edicts ignore factors such as risk-benefit balance. Sometimes it seems that judgments are based more on political than on scientific information. The matter of essentiality is of special concern. This says that a registration will not be granted if there are other registered chemicals or methods which will work; the new use is "not essential". This is supposed to prevent the use of alleged hazardous materials, but this provision was not authorized in the law; it was even flagged out as what should not be done. The basic issue is that as many pesticides as possible should be kept available for use. If registrations are disallowed, if manufacturers are harrassed to the point that they will not bother trying to retain registered uses, then the control agencies have nothing -- or to put it another way, we may have to go back to the "good old days".

Applicator certification is not the same issue for mosquito control workers as it is for agricultural workers. The knowledge of the life history of an insect is not important to protect the health of a worker and such excess requirements may be challenged as unnecessary for the safe application pf a pesticide. Requirement of pest life history knowledge under other laws seems reasonable, but it is not required under the Federal Law and its worker safety provisions.

The major move to Federalize, to preempt the authority of States, is obvious and unfortunate. In California, the cooperative agreement between the Department of Health and the local mosquito control agencies has been an outstanding document, prepared and implemented by the Department of Health for and with the local agencies. It had the concurrence of the Department of Food and Agriculture. It protected the health of people. It accepted a different risk-benefit balance because the risks were different from those of agriculture, and the benefits were different too. Unfortunately, after a number of years of highly successful use of this program, it appears probable that the Federal program will now move in and preempt this cooperative program. By restricting, and requiring use precisely according to the labels, mosquito control agencies would no longer be able to adapt broadly used chemicals to their specific, more specialized needs.

It appears that EPA wants a strong, exclusive control of the registration of pesticides, and this does not seem right. Any registration which has been cancelled, suspended or denied by the Federal people is unavailable for State registration. If application is not made to EPA to hold the State registration, it will be cancelled according to a recent announcement, and therefore it will be blocked for State registration. This technicality could stop many mosquito control programs, and its potential should be recognized and challenged.

It seems necessary for all of us, separately and collectively, to take this matter to the right people and to demand results - by saying "We have problems and we expect you to work with us to get these problems solved". I suggest we need to use political pressure to sell the health protection aspects of pesticides, and the place to do this is in the media, as well as the legislature, both State and Federal.

Positive suggestions which should be given consideration by officials in EPA are these. It is not fair to complain without offering constructive alternatives:

1. Recognize that pesticides have health benefits as well as health risks.
2. Allow competent local individuals to use pesticides beneficially. This is especially important relative to uses inconsistent with the label.

3. Move away from the concept that "EPA knows best". Obtain input from others and then use it.
4. Recognize that there are reasonable limits for risks. Nothing is without risk, and the present "no tolerable risk" stance is unreasonable.
5. Obtain advice from people who know what the problems are and who know how to make programs work. It takes no expert to destroy a program - the expert is one who can make it work.
6. Move away from so-called Scientific Advisors who have a track record of proven unreliability. These people often claim to be environmentalists.
7. Be reasonable about alternative means of control - do not discard the old even if the replacement is proven better.
8. Recognize the need for broad spectrum chemicals which can be economically made and can be used selectively. Narrow spectrum chemicals that will control only one or a limited number of species are usually economic losers.
9. Effective chemicals must be available, as well as registered, to back up biological programs. Integrated control, which mosquito control agencies have been practicing, is based on a harmonious use of natural and chemical controls. A pest outbreak means that natural controls have failed to control.
10. Recognize that risk benefit balances must be allowed at each level of decision making. If the man in the field is a true professional, he will be able to determine the best way to solve the problem, and when necessary, the best pesticide to use. Labels do not kill pests.
11. Apply the present law as it was intended, and get away from Agency fascination with old legal opinions and programs which Congress considered, and did not approve.
12. Start making decisions on the scientific merits, in place of being overly concerned with the political mileage of the decision.
13. Restrictions to protect people's health should not be used to prevent protection of people's health.

CALIFORNIA OCCUPATIONAL SAFETY (CAL OSHA) AND ITS IMPACT ON MOSQUITO CONTROL PROGRAMS

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The federal Occupational Safety and Health Act (OSHA) was signed into law four years ago. This Act preempted all 50 states out of whatever business they were conducting in the field of occupational safety and health. At that time about one in six states including California had a fairly good program of its own.

The federal law is a very tough law. California followed with a very tough law, too, called the Occupational Safety and Health Act of 1973. The California law matches or in some instances exceeds the federal law. These laws have changed the philosophy of governmental surveillance in areas of occupational safety and health. The complexity of the total program is such that the potential impact has not yet been analyzed or evaluated. Since its Act was passed, California has moved ahead, trying to develop an acceptable state plan.

This California Act is a "compliance administration" type of law - a law with teeth in it. The absence of such a law created a criticism of our Division of Industrial Safety for many years. The law was there, but it was based on a friendly, persistent, persuasive approach. There was a big stick to use if desired. Now the Division does not worry about having the big stick. Its job is to look, evaluate, to determine very strictly whether or not what it sees, hears, smells or tastes in the way of exposure to hazard by an employee meets a system of the most complicated legal imperative possible. These are safety rules, regulations and orders. They are produced by using some basis as a background, then getting committees together to see if they can make sense out of them and put them into language which can be understood, then supplied to 150 compliance and safety engineers located in 22 districts and 4 area offices, in 6 regions, supervised by 9 administrative executives. No one in California is located very far from one of the offices, from which an investigation will be forthcoming in the event of an accident which is covered by these regulations. Mosquito abatement districts, even though they are public entities for which the normal kind of inspection cannot produce a citation, nevertheless are subject to criminal action which a plaintiff may wish to bring against them.

The Division has a thousand employees, all public servants, all working in an extremely complicated field involved with exposures of many kinds: machines, tools, equipment, processes, hazardous substances, all of which are covered by a variety of rules and laws. The Division is working under a new law which tells it what it must do. It is working with a revised set of safety rules which must be as effective as those of the federal government. The job as an enforcement agency will be done according to the legal demands upon the Divisions.

Public agencies are fortunate compared with the private sector. There is a new complaint law in California which provides that any time a complaint is received from any person or employee, the Division must respond to that complaint within 72 hours. When it responds, regardless of the specifics of the complaint, the compliance man must make a wall-to-wall inspection. It is unlikely that a mosquito abatement district will be visited by a compliance man from the Division of Industrial Safety unless it comes as a result of a complaint, or unless it comes as a result of a serious accident. Thus the public agency, while fortunate compared with the private agency, nevertheless will experience the impact in a way never before contemplated.

Managers in each district should contact the nearest office of the Division and request 4 or 5 simple publications, then do a little homework:

- There is a digest of the California Occupational Safety and Health Act.
- There is a safety publication that goes into detail on guarding machines, on tool work, etc.
- There is a publication which explains the Division's entire operations.

In the Division are 18 consultants, available to respond to requests from public and private agencies. These consultants have expertise and for the most part they are right.

Mosquito abatement districts should become intimately involved in safety programs, and should consider their vulnerability in the event of a complaint or bad accident. They will be augmented by the stimulus and guidance of their compensation insurance carrier, the California State Compensation Insurance Fund.

RESPONSE TO CAL OSHA

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Everyone agrees that a safe working place is in order. There is an issue, however, relative to the risk-benefit balance. Recent statements indicate that fiscally a human life is worth about $400,000.

Most of the regulations of the Division of Industrial Safety and of Cal OSHA are good and should be followed. But nothing we do is without some risk. Some of the regulations should be questioned. One challenge for example, is that you cannot do in your working area what you and others can do at home. For example, in the working area you cannot have lye or pesticides where there is food. You can not keep food and pesticides in the same room, yet we keep sanitizers, bleach and lye under the kitchen sink at home.

There are questions relative to legislative and State department actions. Are mosquito abatement districts classed as agricultural or otherwise? Who specifically in the State Health Department represents that Department in advising the Department of Food and Agriculture? The Statute specifies only that one Department shall advise the other.

Until a judge referees in the conflict, the matter of clothing, washing facilities and so forth will not be resolved. I refer here to pending litigation.

The Butte County MAD in just 6 months has expended its total annual budget for laundry and professional services. Application has been made to the State for reimbursement, under the revenue and taxation code, which states that mandated local costs should be paid for by the agency which mandates them. But the Department of Health and the Department of Food and Agriculture have said "We cannot pay the bill". Our District has not given up - it would like to recover these costs because they are excessive to a very successful program which was initiated in 1966, a program which has worked well under less stringent and expensive regulations. Certainly the guideline suggestion that workers using more toxic phosphate insecticides be bled once a week for cholinesterase test seems excessive, especially when actual exposure is minimal or nonexistent. Even the requirement of bleeding for test of any worker who gets sick within 24 hours of handling toxic organophosphates seems excessive, but there could also be a benefit; this could well reduce infringement on sick leave benefit, if the employee knew he was going to have to provide a blood sample as part of the price.

HEALTH AND SAFETY REGULATIONS AND THEIR IMPACT ON MOSQUITO CONTROL PROGRAMS

William A. Betts

California Department of Food and Agriculture

Agricultural Chemicals and Feed, 1220 N Street, Sacramento, California 95814

The State Department of Food and Agriculture is writing a plan to certify private commercial applicators in California. It is reasonably certain that EPA will accept this plan inasmuch as the Department already has programs that are equal to or exceed EPA standards. The Department is working closely with the Vector Control Section of the California Department of Health to provide the best input into the certification program for mosquito control workers.

EPA has listed many restricted materials, for which mosquito control operators should be certified if they wish to use them. However, California also has a list of many restricted materials, and in some ways this may be more restrictive than EPA's list. Thus EPA permits 2,4-D to be listed as a general-use material, except for aquatic uses, but in California this herbicide causes many problems due to drift and high phytotoxicity, therefore California will continue to make all 2,4-D uses restricted.

Pesticide training sessions have been held in all areas of California, with the cooperation of the University of California staff. Over 300 persons, mainly county farm advisors and agricultural commissioners, have been provided detailed course work so they could give training courses to all interested in pesticide worker safety. Several slide sets have been prepared by the Department, the University of California, and EPA to show an employer what his responsibilities are and an employee what kind of training he should get.

The legislature introduced many bills on pesticides in recent years, culminating in AB 246 which establishes the responsibilities of a number of agencies relative to pesticide worker safety. This bill required the Department of Food and Agriculture to adopt pesticide worker safety regulations. The bill also required that these regulations must be based on recommendations of the California Department of Health. A cooperative study by the Department of Food and Agriculture and the Department of Health identified in 1973 1,474 pesticide-related illnesses. The two most hazardous occupations were the mixer-loader and the ground applicator, while the most hazardous activity was removing the pesticide from its original container. Most of the regulations which have been developed are aimed at these two groups of workers and this activity. The two most hazardous pesticides have been parathion and phosdrin.

Pesticide regulations were first adopted as an emergency measure in January, 1974. A few months later, after additional hearings, the regulations were made permanent. They have been in effect for over a year. These regulations apply to all pesticide users except those in structural pest control, the home use of pesticides, and several very specialized uses.

There are four categories of pesticides. Category 1 materials carry the signal words "Poison" and "Danger", plus a skull and crossbones symbol. These are highly toxic. Category 2 materials carry the signal word "Warning"; these are moderately toxic. The regulations are directed primarily towards these two categories, since they have caused almost all the problems. Category 3 materials carry the signal word "Caution"; these are of low toxicity. Category 4 materials, which are relatively nontoxic, carry no signal word. "Keep out of the reach of children" is on all labels.

A closed mixing system is required in certain situations. This provides a system in which the pesticide is removed from its original container and transferred into a closed mixing tank without exposure of any person to the pesticide. Many mosquito abatement districts already have this for their airplane loading.

A closed loading system provides a means of transferring a pesticide from a mixing tank into an applicator vehicle by a closed system of hoses, pipes and couplings.

A minimum age has been established for persons using category 1 and 2 pesticides. Those under 18 are not permitted to mix or load these materials unless closed mixing and loading systems are used. There are no age restrictions on the application of pesticides.

Employers are required to verify that their employees are adequately trained. The training record of each employee should be on file in each agency - the Department can supply a suggested form. This form is also signed by each employee. If an inspection is made of the facilities, one thing the Inspector will want to see is the employee training record. The training should cover safety procedures relative to the pesticides in use; safety clothing; common symptoms of pesticide poisoning; dangers of eating, drinking and smoking while handling pesticides; emergency medical care; medical supervision; applicable laws and regulations.

The employer must make prior arrangements for emergency medical care - this may be the emergency room at the local hospital, but any approved medical facility may be satisfactory. There is a requirement that there be a posted sign at the work site indicating where to go for emergency medical care - personnel should know where to go without asking their supervisor. A sign could be taped to the dash or the door of the vehicle which the employee uses.

The only occasion when an employer will have to maintain medical supervision is when an employee works with a category 1 or 2 organophosphate or carbamate pesticide more than 30 hours in a 30 day period. This involves obtaining the services of a physician to provide the supervision. The California Department of Health has developed guidelines to help physicians carry out the medical supervision program. These guidelines should be reviewed by the employers and the employees so all will know their responsibilities. One recommendation is that there be a pre-employment examination to help determine if a person is really suitable to work with pesticides. There are some ailments which would create a special risk to an employee who works with organophosphate or carbamate pesticides. The guidelines state that cholinesterase testing should be done at the same laboratory by the same method. Tests should include both plasma and red cell cholinesterase determinations. A pre-exposure base line should be determined at least 30 days before exposure to organophosphates. The base line is an average of two tests at least 72 hours apart but not more than two weeks apart. If the results of these two tests differ by more than 15%, a third test should be run and the average calculated from the closest two.

How are the tests used? If the red blood cell cholinesterase drops 40% from the base line, the employee should be removed from exposure to the pesticide. The same action should be taken if the plasma cholinesterase drops 50% from the base line. If either plasma or red cell cholinesterase drops 30%, the agency should take a close look at the work practices of the employee. If an employee has been taken off pesticide use because of lowered cholinesterase, he can be returned to work when he gets back to his base line range, which is interpreted to be within 20% of his pre-exposure base line. The physician should determine the frequency of blood testing according to the amount of exposure, the type of equipment, the safety practices, and the past history of the firm or of the particular employee.

Regulations control procedures for working alone. These apply only to persons who work with category 1 pesticides. These employees in the daytime must have some type of supervision every two hours, and in the nighttime every hour. "Supervision" means phone contact, radio contact, or any other acceptable contact with the agency office or supervisor.

A pilot who is using category 1 or 2 organophosphate or carbamate pesticides cannot load his own aircraft or mix his own pesticides unless he has closed mixing and loading systems. If he uses an open system, a helper must do the mixing and loading.

The requirement for a change room has caused much concern. This does not necessarily have to be a room - just a change area. The regulations require this when the pesticide usage calls for medical supervision - that is, when an employee uses category 1 or 2 pesticides for more than 30 hours in a 30 day period. A fancy place is not required - it can be a sink where a person can wash at the end of the day and put on clean personal clothes in which to go home. There must be water, soap and towels available.

Protective clothing is a major part of the regulations. This can be expensive, however there are available for purchase disposable coveralls which do not cost very much. They can be kept in a plastic sack in the vehicle where they will stay clean until and if needed. Labels on the containers note the precautions which should be taken and what protective clothing or equipment should be worn for certain uses. One may need a waterproof hat, a face shield, an apron, rubber boots and rubber gloves. Coveralls should be worn on the outside of boots. Some applicators, with some pesticides, probably will not have to wear anything special except clean coveralls each day and rubber gloves.

The Department has developed a Pesticide Safety Information Series, numbered 1 through 10. These are available on request. The first covers interpretation of the label. The others cover more specific topics, such as parathion and phosdrin handling, paraquat, methyl bromide, cholinesterase testing, and so forth.

Regulations also cover the maintenance of equipment. There have been many cases of persons working on planes or ground equipment who became ill because they did not realize that the pesticides were hazardous. Since January 1, 1975, flexible hoses under pressure must be shielded or replaced with something rigid to assure that nothing will break or slip. This is a problem especially with aircraft, because if something should break the pilot cannot jump out.

In July, 1975, there will be a requirement that a valve be located at the end of a hose which carries a pesticide from a mix tank to an applicator vehicle. This is to prevent material remaining in the hose from running out on the ground or on the pant leg of the loader. After January, 1976 there also must be leakproof caps or hatches on applicator vehicles to prevent splashes if the vehicle hits a bump.

In January, 1977, there will be in effect a requirement to provide some external means to determine the internal capacity of a pesticide tank, such as a sight guage. Also on that date there will be a requirement that all loading of liquid category 1 pesticides and certain category 2 pesticides be via a closed system.

Pesticide illnesses in 1974 were substantially reduced over previous years, especially in the loading and mixing areas. Illnesses in 1974 were 1,150 compared with 1,474 in 1973. There were seven incidents in mosquito abatement district workers. There were cases of a hose coming loose and the applicator getting sprayed, cases in which oil sprays blew back into the applicator's face because of wind, a case in which a pilot had too much exposure to organophate insecticide, etc.

Agricultural Commissioners are the persons who are most likely to inspect mosquito abatement districts and to examine the records and equipment. The Department of Food and Agriculture will help to coordinate the work of these Agricultural Commissioners.

UNIVERSITY OF CALIFORNIA RESEARCH PROGRAM ON MOSQUITOES

PANEL

Dr. James L. Hardy, Associate Professor of Medical Virology . . . . School of Public Health, Berkeley

Dr. Mir S. Mulla, Professor of Entomology and Entomologist . . . . Department of Entomology, Riverside

Dr. Charles H. Schaefer, Lecturer and Entomologist in the Experiment Station . . Department of Entomological Sciences, ... Berkeley, and Director of the Mosquito Control Research Laboratory, Fresno

Dr. Robert K. Washino, Associate Entomologist and Lecturer . . . . Department of Entomology, Davis

Dr. Telford H. Work, Professor of Infectious and Tropical Diseases . . . . School of Public Health, Los Angeles

MODERATOR

Dr. Carl J. Mitchell, Coordinator of Mosquito Research . . . . Cooperative Extension, Davis

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THE UNIVERSITY OF CALIFORNIA'S RESEARCH PROGRAM ON MOSQUITOES — AN OVERVIEW

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"Mosquito control today is in a state of crisis." This is the blunt assessment of an expert panel recently convened by the National Academy of Sciences in Washington, D. C. to discuss this problem.[1] The panel pointed out that mosquito-borne diseases such as malaria, filariasis, yellow fever, and dengue are still rampant in many parts of the world. The crisis in mosquito control is related to an overdependence on synthetic chemical pesticides during the past 30 years and ensuing resistance to many insecticides in several areas. The panel cited the example of encephalitis-transmitting mosquitoes in California (principally *Culex tarsalis*) having become "resistant to virtually all larvicides" in some areas. The irrigated pasture mosquito, *Aedes nigromaculis*, a major pest in the Central Valley of California also is resistant to pesticides in many areas. Elsewhere in these Proceedings organophosphate resistance in the *Culex pipiens* complex in California is documented.

During the period since the end of the second World War, the people of California have been fortunate in experiencing a high level of mosquito control and relative freedom from epidemics of mosquito-borne disease. The development of insecticide resistance and increasingly stringent restrictions on the use of some pesticides, however, makes continuing control more difficult. Consequently, increasing demands are being placed on the University to develop new control technologies and improve existing ones. The University is responding to this demand by intensifying research efforts in a variety of areas which may lead to the discovery and development of additional methods of control. Highlights of these research endeavors and important accomplishments by University of California scientists are summarized in the following papers.

Many of the activities and results described by the panel have been supported by a special appropriation from the State Legislature. Currently this totals $300,000 per year. These funds for mosquito research are shared by the UC Schools of Public Health at Berkeley and Los Angeles, UC Agricultural Experiment Station, and UC Cooperative Extension. Additional support comes from local Mosquito Abatement Districts, industry, and state and federal sources.

A University-wide Advisory Committee on Mosquito Research coordinates the expanding research and educational programs on mosquito control. Its members are James B. Kendrick, Jr., Vice President, Agricultural Sciences and Director of the Agricultural Experiment Station (Chairman);

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[1] "Mosquito Control: Some Perspectives for Developing Countries." A report of an Ad Hoc Panel of the Advisory Committee on Technological Innovation, Board of Science and Technology for International Development, Office of the Foreign Secretary. National Academy of Sciences Publication, Washington, D. C., March 1973, 63 pp.

Carl J. Mitchell, Extension Specialist – Mosquitoes (Secretary); A. Ralph Barr, Professor of Medical Entomology, School of Public Health, UCLA; Richard W. Emmons, Public Health Medical Officer, California State Department of Health; William E. Hazeltine, Manager and Entomologist, Butte County Mosquito Abatement District and Chairman, California Mosquito Control Association Research Committee; Edmond C. Loomis, Extension Parasitologist; Powers S. Messenger, Professor and Chairman, Department of Entomological Sciences, UC Berkeley, and Chairman, Entomology Research Coordinating Committee; Lloyd E. Myers, Associate Deputy Administrator, Western Region, Agricultural Research Service USDA; Richard F. Peters, Chief, Vector Control Section, California State Department of Health; William C. Reeves, Professor of Epidemiology, School of Public Health, UC Berkeley; and William M. Rogoff, Research Leader, Western Region, Agricultural Research Service, USDA.

The University-wide Advisory Committee receives input directly from the California Mosquito Control Association Research Committee and the Entomology Research Coordinating Committee from within the Agricultural Experiment Station. Chairmen of the latter committees serve as *ex officio* members on the University-wide Advisory Committee.

Local mosquito control agencies in California continue to support and collaborate with UC investigators in addition to conducting their own research on applied control. Their assistance and cooperation have contributed to the results reported in the following papers and are sincerely appreciated. Collaboration from the Vector Control Section, California State Department of Health, has been substantial and is gratefully acknowledged. Investigational and educational programs on mosquito control in California also are being conducted by the U. S. Department of Agriculture and the State Department of Food and Agriculture. We are grateful to each of these agencies for their support and assistance.

ARBOVIRUS RESEARCH PROGRAM AT THE UNIVERSITY OF CALIFORNIA, BERKELEY[1]

James L. Hardy, William C. Reeves, and Sister Monica Asman

University of California

Department of Environmental and Biomedical Health Sciences and the Naval Biomedical Research Laboratory

School of Public Health; and Department of Entomological Sciences, College of Natural Resources

Warren Hall, Berkeley, California 94720

The Arbovirus Research Program in the School of Public Health at the University of California, Berkeley, continues to have as its primary objective the acquisition of a sufficient depth of knowledge of the factors that are essential for maintenance of arbovirus transmission cycles to allow the development of effective disease control programs. A research endeavor with such a broad objective can only be successful if its limited resources at any one time are applied to more specific and immediately attainable goals. There must be a constant reevaluation of specific research aims and alteration of priorities if research results or changes in problems that confront mosquito control agencies dictate such changes.

During the 1960's a major emphasis on our research program was to elucidate mechanisms that allowed the long-term maintenance of overwintering of arboviruses in a temperate climate. The gradual disappearance of encephalitis viruses, especially western equine encephalomyelitis (WEE) over an extensive part of the Central Valley of California during the 10 year period made further field observations difficult and relatively nonproductive. This development, at least in part, dictated a change in our research direction. Concurrent to the above event, *Culex tarsalis* developed widespread resistance to organophosphorus (OP) insecticides in many areas (Georghiou et al. 1969, Womeldorf et al. 1972). Thus, we asked the question "Is it a coincidence that WEE virus disappeared from the Central Valley at a time when *C. tarsalis* was becoming more difficult to control?" Studies during the last 4 years revealed that colonized and field-collected *C. tarsalis* varied significantly in their ability to become infected with and to transmit WEE virus. Since changes in vector competence could account for the observed changes in the prevalence of WEE virus, we revised our research program to study vector competence and its potential application to the genetic control of *C. tarsalis* and encephalitis.

Currently, our specific aims in order of priority are: 1) to identify genetic and nongenetic variables that affect the efficiency of arbovirus transmission cycles, 2) to evaluate the feasibility of genetic and other biologic approaches for control of arbovirus vectors in the field, 3) to determine how arboviruses overwinter in a temperate climate, 4) to characterize new arboviruses isolated in California including their ability to cause disease and 5) to develop statistical models to describe the dynamic interactions between arboviruses, vectors, and hosts during epidemics. Each of these specific aims will be discussed briefly.

[1]This research was supported in part by Research Grant AI 03028 from the National Institute of Allergy and Infectious Diseases; Contract No. N00014-75-C-0157 from the Chief, Office of Naval Research; and Contract No. DAMD17-74-C-4128 from the Commander, U.S. Army Medical Research and Development Command.

GENETIC AND NONGENETIC VARIABLES THAT AFFECT THE EFFICIENCY OF ARBOVIRUS TRANSMISSION CYCLES.–The basic components of an arbovirus transmission cycle are depicted in Figure 1. Relatively little attention has been given in arbovirus programs to the potential relationship between genetic or physiological changes in the vertebrate hosts and the changes in the ecology of arboviruses. For example, avian and mammalian hosts, as well as vectors, have been exposed to ever-increasing amounts of insecticides and herbicides during the past 30 years which might have altered their ability to serve as competent hosts of encephalitis viruses. Alternatively, decreases in arbovirus prevalence could be related to genetic changes in the viruses, such as temperature sensitive or host-range mutants. We are conducting several experiments in these areas but time will not permit us to present them.

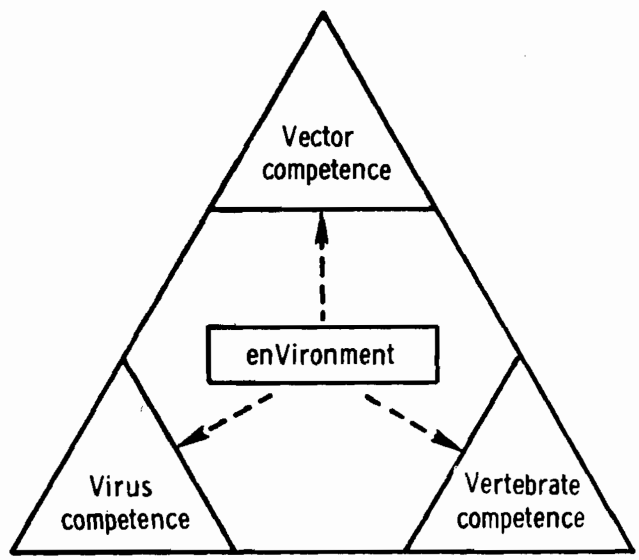

Figure 1.–Basic ecologic components of an arbovirus transmission cycle.

Our primary emphasis is to elucidate factors that affect the ability of mosquitoes to transmit arboviruses; i.e., vector competence, and to determine which factors are under genetic control and may have application for the control of encephalitis through the genetic manipulation of vector populations. Our current concepts of factors that could affect vector competence are shown in Figure 2. Some of these factors control the exposure of the vector to infected vertebrates and could be under genetic control. For example, it has been observed that the host preference of *C. tarsalis* shifts during the summer from feeding almost exclusively on birds to feeding on both birds and mammals, primarily ungulates and jackrabbits, and this shift is not cor-

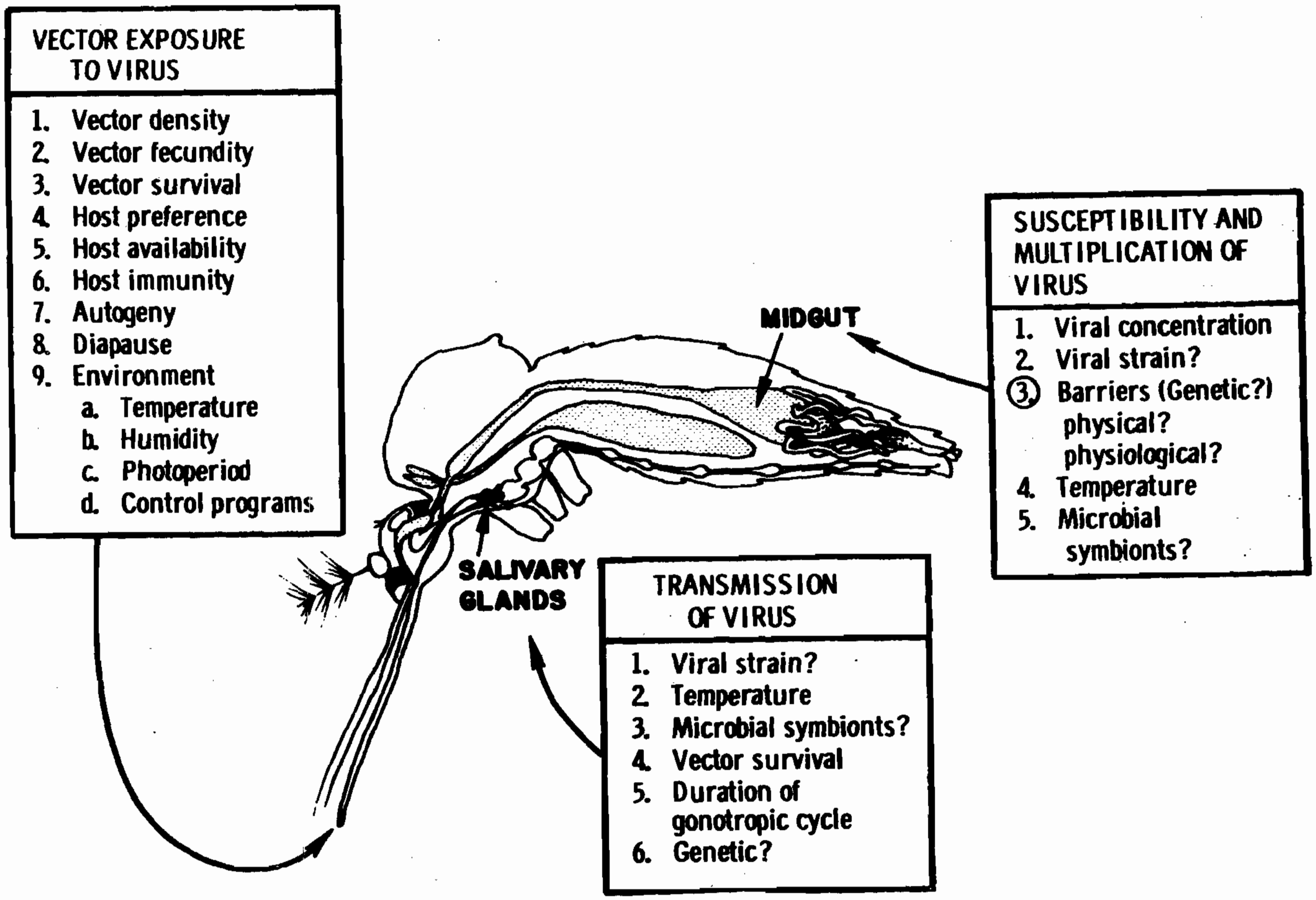

Figure 2.–Factors affecting vector competence of mosquitoes to arboviruses.

related with host availability (Tempelis et al. 1965). If the host preference of *C. tarsalis* is under genetic control similar to that of *Anopheles gambiae* in Africa, (Gillies 1964) then ungulate feeders would be poor vectors since these mammals are dead-end hosts of encephalitis viruses as they are for human malaria.

Autogeny is probably an inherited trait and high autogeny rates in a population of *C. tarsalis* would dampen an arbovirus transmission cycle since females would not have to take a blood meal before oviposition. Diapause potentially is also under genetic control and the gene for nondiapause would be lethal for *C. tarsalis* populations in the Central Valley and other areas with cold winters since the vector must undergo diapause to survive the winter period. We are also studying the vector competence of female *C. tarsalis* derived from temperature-sensitive and temperature-tolerant larvae or OP treated and OP nontreated larvae to determine their potential value in genetic studies.

Field studies on the vector competence of *C. tarsalis* with WEE virus suggests that susceptibility is genetically inherited. To date we have been able to select within 8 generations several variants that are completely or highly resistant to infection with WEE virus after feeding on viremic chicks. We will initiate studies soon to determine the mode of inheritance, if any. Resistance of *C. tarsalis* and several other *Culex* species (to WEE virus) appears to be associated with a "gut barrier" since all *Culex* mosquitoes thus far tested have equal susceptibility when virus is introduced by intrathoracic inoculation. We are currently studying the physical and/or biochemical basis of the gut barrier utilizing the *Culex tarsalis* - *Culex peus* - WEE virus model.

Variability in capacity to transmit WEE virus also has been observed with colonized and field-collected *C. tarsalis*. For example, we have a colony of *C. tarsalis* in which only about 50% of the infected females can transmit WEE virus by bite after 14 days extrinsic incubation and in each case where it has been examined nontransmitting females contained about 100-to-1,000 fold less virus than transmitting females. Thus, it is possible that the abilities to become infected with and to transmit virus are under separate genetic control. Currently, we are attempting to select a virus susceptible but nontransmitting variant of *C. tarsalis* to determine where the virus is multiplying within the mosquito and if the ability to transmit virus is genetically controlled.

Indigenous viruses of *C. tarsalis* (Richardson et al. 1974) and rickettsial-like symbiotes in *C. pipiens* (Yen and Barr 1971) could influence their vector competence with arboviruses at the susceptibility, multiplication and/or transmission levels. If these indigenous microorganisms do affect vector competence, then they might be used as a biologic control mechanism for encephalitis provided that they can be introduced into and maintained in vector populations. We are actively engaged in research in these areas.

FEASIBILITY OF GENETIC AND BIOLOGIC APPROACHES TO THE CONTROLS OF ARBOVIRUS VECTORS.–Our inability to effectively control *C. tarsalis* with

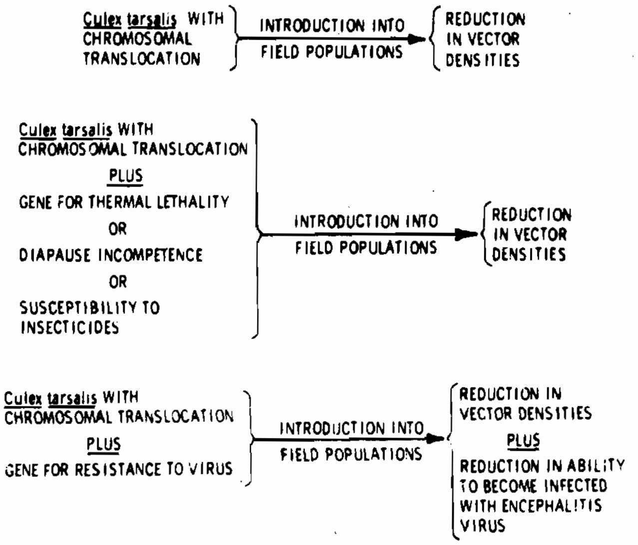

Figure 3.–Potential application of genetics to encephalitis control programs in California.

licensed chemical insecticides in some areas, as well as the objections that are raised to the contamination of our environment with these chemicals, has necessitated a search for alternate mechanisms for control of this important vector of disease. An alternative approach to mosquito control that has been widely advocated in recent years is the genetic control of vector populations (Whitten and Foster 1975). One genetic method that we are evaluating is chromosomal translocation and insertion of this strain with its desirable genes into field populations could decrease vector competence for arboviruses, increase susceptibility to organophosphorus insecticides, increase autogeny, etc. When this "tailor-made" mosquito is introduced into and perpetuated in field populations of *C. tarsalis*, the chromosomal translocation theoretically would result in a 60 percent or greater reduction in the vector population through the mechanism of genetic incompatibility and the surviving mosquitoes would be incompetent vectors of the arbovirus in question.

Obviously, the success of this approach to mosquito control requires numerous preliminary studies that we have or will initiate. The genetics of *C. tarsalis* is under intensive study and we have developed a strain of *C. tarsalis* with a chromosomal translocation which Sister Monica Asman reported at last year's meeting of this association (Asman 1974). She will soon initiate studies to determine to what degree the induced translocated chromosomes can be successfully incorporated into the genomes of other populations of *C. tarsalis.* Concurrently, we are looking for sites with geographically isolated populations of *C. tarsalis* where the bionomics and competence of the vector will be thoroughly studied before introduction of the translocated strain. For the introduction of the translocated mosquito strain into the field population, we propose to produce large numbers of egg rafts in the laboratory and rear the mosquitoes in outdoor ponds. Studies initiated during the past summer began to develop the methodology for this procedure. As many as 1,000,000 eggs were produced in 1 week in a 4 cu ft cage and over 10,000 adults were hatched from a 24 sq ft plastic lined pond in 1 generation. We would hope to have our first field trail of this method of mosquito control within the next 5 years.

Butte Co. MAD	Dr. W. E. Hazeltine
Calif. Dept. of Health	Dr. R. W. Emmons
Kern MAD	Mr. R. H. DeWitt
	Dr. R. L. Norland
Sutter-Yuba MAD	Mr. E. E. Kauffman
U. C. Berkeley	
Dept. of Entomological Sciences.	Dr. D. P. Furman
	Dr. E. S. Sylvester
Naval Biomedical Research Lab.	Dr. N. A. Vedros
U. C. Davis	
Dept. of Entomology	Dr. C. J. Mitchell
	Dr. R. K. Washino
U. C. Los Angeles	
School of Public Health.	Dr. A. R. Barr

Figure 4.–Cooperative agencies and persons in the arbovirus research program (partial listing).

MECHANISMS FOR OVERWINTERING OF ARBOVIRUSES.–We still have a vested interest in elucidation of mechanisms which allow arboviruses to overwinter in a temperate climate. During the past 10 years we have been able to identify the primary vector and vertebrate host of many of the arboviruses that occur in California. However, we recently found that 2 separate but related transmission cycles exist for WEE virus in the Sacramento Valley, one involves jackrabbits, *Aedes melanimon* and *C. tarsalis*; the other involves *C. tarsalis* and wild birds (Hardy and Bruen 1974). At this point, we do not know how grey squirrels became involved in the cycle or which cycle is initiated first. We propose to continue these studies to evaluate the relative importance of alternative primary vectors and vertebrate hosts of WEE virus.

We believe that arboviruses arose originally either as viruses of invertebrates that acquired the ability to infect vertebrate hosts or as viruses of vertebrates that have acquired the ability to infect invertebrates. It is also conceivable that these viruses produce a chronic infection in their natural hosts. Thus, we are using a combined field and laboratory approach with selected arboviruses to determine if they persist as chronic infections in either their primary mosquito vector or their primary vertebrate host. Examples of models being studied include the Turlock virus - House Finch - *Culex tarsalis* model and the *Aedes* sp. (e.g., *Aedes melanimon* and *Aedes sierrensis*) - Jamestown Canyon virus model. We have searched for but have no evidence that any mosquito-borne arbovirus is transmitted transovarially in California though such transmission has been reported for viruses in the California group in other areas (Watts et al. 1974).

We are also interested in determining the vector and host ranges of low and high temperature viral mutants as a possible means of long-term persistence of arboviruses in nature. For example, we would expect viruses that are transmitted transovarially in mosquitoes to be low temperature mutants. We are also evaluating the possibility that St. Louis encephalitis (SLE) virus is a high temperature mutant of Rio Bravo virus from bats.

CHARACTERIZATION OF NEW CANDIDATE ARBOVIRUSES AND RELATIONSHIP TO DISEASES.–As an arbovirologist moves into new field study areas, tests different species of vectors and hosts, or changes isolation systems in the laboratory, he frequently isolates new viruses, some of which turn out to be arboviruses. We currently have at least 3 new candidate arboviruses that have been isolated from *C. tarsalis* collected in the Sacramento Valley. Studies are in progress to determine their experimental vector and vertebrate host ranges, their natural history through retrospective serologic surveys utilizing large banks of serum from wild birds, wild mammals, domestic mammals, sentinel chickens, etc., and their potential importance as agents of undiagnosed disease in humans and horses.

DEVELOPMENT OF STATISTICAL MODELS.—All data derived from our arbovirus field studies, mosquito light trap records from almost all mosquito abatement districts in the state, and confirmed cases of encephalitis in man and horse have now been put into our computer data bank. This information, that now covers up to 20 years of records in some areas, is available to graduate students and others who are interested in developing statistical models of arboviruses. As an example, these have modeled the dynamics of *C. tarsalis* populations and annual basic cycles for WEE virus (Moon 1973). Another student is completing a study of the correlation between the MAD light trap records for *C. tarsalis* and the occurrence of clinical cases of WEE and SLE. Preliminary summaries indicate a close correlation and extend our confidence in the findings we presented several years ago.

This use of records from our research, the State Department of Health and your trap records indicate clearly the collaborative nature of our research and how we share interests that can rapidly carry research findings into practice. Figure 4 indicates the extent of agencies in this collaborative effort.

A SUMMARY OF CURRENT RESEARCH ON MOSQUITOES BY PERSONNEL OF THE U. C. AGRICULTURAL EXPERIMENT STATION AT BERKELEY

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DIVISION OF ENTOMOLOGY AND PARASITOLOGY.—Dr. R. H. Dadd is conducting basic research on mosquito physiology. His research objectives are to develop detailed information on mosquito larvae and adults with respect to: (a) feeding and its regulation, (b) digestion, and (c) nutritional requirements. The major areas of his research are as follows:

A. Determinations have been made on the rates of larval ingestion of solids during filter feeding. This depends on:

(a) The mechanical properties of food particles (their size, shape, and concentration in water).

(b) The presence of soluble gustatory stimulants (powerful stimulation of filtering is produced by crude organic materials; experiments using defined chemicals show that nucleic acids will cause this effect).

B. Liquid ingestion by larvae. Dr. Dadd has found that bulk liquid ingestion, or drinking, occurs if water is rendered slightly viscous by low concentrations of suitable colloids.

C. Larval digestion. A current study is underway on the digestive functions of mosquito larvae. The types of enzymes present and their physiological requirements, e.g. pH, are being determined.

D. Nutrition. With the knowledge of larval feeding mechanisms described above, it became possible to rear *Culex pipiens* sp. in defined media and hence to determine specific nutrient requirements. Detailed information on the requirements for sterols, nucleotides, and amino acids has been done on *C. pipiens*, but partial success has also been accomplished in rearing *Culex tarsalis* in defined media.

E. Diet Fortification. The ability of larvae to develop on various materials used in crude diets is being compared. As specific requirements become known, as mentioned above, the possibility of adding supplements to make poor materials adequate is being investigated. For example, yeast powder is a poor diet alone, since its sterol (ergosterol) cannot be utilized by *C. pipiens*, studies in which yeast powder is fortified with cholesterol (a sterol specifically required by *C. pipiens*) are underway.

DIVISION OF BIOLOGICAL CONTROL.—Dr. Richard Garcia is conducting mosquito research in three areas:

A. Research on *Aedes sierrensis*. This important nuisance species has recently been implicated as a vector of heartworm of dogs. Emphasis has been placed on obtaining accurate data on the biology of this species in anticipation of developing a life table to help implement an integrated control program. In Marin County it was found that the fungus, *Beauveria tenella*, causes mortality against immature stages of *A. sierrensis*, but this fungus was not effective in field trials against *C. tarsalis* and *Anopheles freeborni*. Research to determine the necessary conditions for the effective utilization of the fungus is continuing. The fungus has the potential of being mass-produced at a low cost.

A trapping system using CO_2 and a host (rabbit) was developed for capturing adult *A. sierrensis* and has proved useful for monitoring adult populations. Mark and recapture studies with *A. sierrensis* were conducted to establish patterns of movement. Most of these studies were conducted at Blodgett Experimental Forest. The trap and release studies were aimed at determining how long released *A. sierrensis* remained near residential areas and how far they will move. Further studies are aimed at establishing movement patterns from emergency sites and longevity of adults. It is anticipated that these studies will provide a better basis for adult control programs.

B. Research on Notonectids. Systems for harvesting and temporary storage of notonectid eggs were developed; the biologies of five notonectid species in northern California have been worked out in general with respect to breeding activity and development. Research is being conducted in the area of flight behavior, microhabitat selection, and the relationships to other aquatic predators.

C. Research on a Water Strider. Studies indicate that the water strider, *Gerris remigis*, might be used in conjunction with notonectids for mosquito control. It occurs abundantly in certain temporary waters and can significantly reduce mosquitoes emerging from temporary ponds. More research emphasis will be placed on *G. remigis* and its role in mosquito suppression.

Dr. Clifford Johnson is conducting research on the mosquitofish, *Gambusia affinis*. Several years ago Dr. James Hoy of the USDA Laboratory in Fresno began conducting cooperative evaluations on the potential of *G. affinis* for controlling rice field mosquitoes. The chief problem he encountered was in obtaining enough mosquitofish for the field experiments. After the termination of Dr. Hoy's work, the U.C. Agricultural Experiment Station placed high priority on hiring someone to develop means of mass-rearing *G. affinis*. Dr. Clifford Johnson was hired to conduct this research. An abandoned water treatment plant (Chenery facility) near Concord, California, was leased to provide a physical site for this research.

Dr. Johnson's research on *G. affinis* can be considered in three phases:

PHASE I -- Mass-rearing.—Mass-rearing of slightly less than 100,000 *G. affinis* was accomplished last summer. Ideal reproductive conditions were established in the outside basins at Chenery. Reproductive experiments were conducted to determine optimum conditions. Research to increase production will be carried out next year.

PHASE II -- Overwinter holding of fish.—Experiments are being conducted to determine factors involved in mass mortality and how they can be alleviated. Minimal conditions for survival are being determined. Such research is being conducted in both laboratory tanks and in outside tanks and earthen ponds at Chenery, Modesto, and Colusa.

PHASE III -- Effectiveness of *G. affinis* as a control agent in rice fields.—Experiments are being conducted (the first last summer) on effectiveness of different numbers of *G. affinis* as a control agent in rice fields (80 and 25 acre fields) within the Modesto area. These are isolated rice fields and should provide excellent data.

Cost analyses are being kept for all experimental procedures of rearing and holding. So far the rearing program in the summer appears very economical, and the holding program looks promising.

U.C. MOSQUITO CONTROL RESEARCH LABORATORY.—This group is a field unit of the Division of Entomology and Parasitology at Berkeley. The laboratory is composed of eight persons and is housed in space leased from the California Department of Health in Fresno. Dr. C. H. Schaefer and Dr. T. Miura are the principal investigators. The primary objectives of the Fresno Laboratory are to: (1) comprehensively evaluate mosquito control agents, (2) to work with cooperators to obtain the necessary data required by EPA for registration and, (3) to develop biological data on target and nontarget species that will allow for integrated control programs. For new, promising control agents, simultaneous data are collected as to effects on target and nontarget organisms, and determinations are made on the stability of the control agent in the habitats where it would be used for mosquito control. During the past several years, the laboratory has conducted extensive studies on Altosid®: this investiment now appears justified, as the EPA has recently registered Altosid for mosquito control use. The laboratory is now in the process of conducting extensive evaluations on TH6040, another chemical agent that is now under consideration for commercial development.

Other developments at the Fresno Laboratory during 1974 are as follows:

1. In cooperation with the Kern MAD, a complex of 32 experimental ponds was constructed in Kern County.
   An underground observatory has been constructed and is being installed in one of the new ponds to allow direct observations on behavioral activities of target and nontarget species under field conditions.
3. A high performance liquid chromatograph has been purchased and, in conjunction with other analytical equipment, will allow for quantitative analyses of new chemical control agents.
4. Studies on the overwintering physiology of adult mosquitoes continued with studies on the precursors which *A. freeborni* adults utilize for the production of overwintering energy stores.
5. Studies on the amounts of types of lipids in *Aedes nigromaculis* larvae, pupae, and eggs are currently in progress.
6. A five-year study on the potential use of soil amendments for improving water penetration on impervious, alkaline pastures is now in the final phase.

MOSQUITO RESEARCH PROGRAM WITHIN THE AGRICULTURAL EXPERIMENT STATION AT THE UNIVERSITY OF CALIFORNIA, DAVIS

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In recent years, many changes have taken place on the Davis campus of the University of California. For example, the expansion of the Health Sciences Complex has included the establishment of a new School of Medicine and a general expansion of the School of Veterinary Medicine. Permanent facilities for the School of Law have long since been completed on the banks of Putah Creek. The construction of Briggs Hall has also been completed and presently houses among others, the Department of Entomology. These along with other physical changes appear miniscule, however, in comparison to changes in student enrollment which has increased from 6,444 (1963) to 12,594 (1969) to 16,248 (1974).

Despite the many physical changes so clearly evident on the Davis campus, the research being undertaken here still retains a strong "Aggie College" orientation in that the Agricultural Experiment Station's activities constitute a very substantial part of the Davis campus research program. This program revolves around the College of Agricultural and Environmental Sciences which presently encompasses 23 major Departments. For mosquito control research, emphasis centers around the Departments of Entomology, Agricultural Engineering and most recently, Agricultural Economics -- with liberal drawing of resources from other major departments (i.e., Environmental Toxicology, Water Science and Engineering, Agronomy, etc.). Although the results of the mosquito research program have national and in some instances, international impact, the objectives of most studies are focused on mosquito problems most prevalent in northern California. Both urban and rural problems emanating from the use of water for recreation, agriculture and industry purposes have been subjects of intensive research. In many instances, the activities discussed below have been in collaboration with the University of California School of Public Health, State Department of Health, State Department of Agriculture, U. S. Department of Agriculture, U. S. Public Health Service and with numerous individual mosquito abatement districts. For the purpose of summarizing the Davis activity touching on mosquito control, projects will be summarized in alphabetical order of the principal investigators.

EVALUATING MACHINES, TECHNIQUES AND METEOROLOGICAL FACTORS IN RELATION TO MOSQUITO CONTROL.—This is a project primarily aimed at examining all of the factors concerned with application of chemical larvicides and adulticides for the control of mosquitoes. Work by project leaders N. B. Akesson and W. E. Yates in Agricultural Engineering has been continuous since 1959 under grant funds from mosquito abatement districts and the U. S. Department of Agriculture as well as from funds made available through the Agricultural Experiment Station. Current activities revolve around the following 7 points: (1) Design and construction of a low noise level cold aerosol machine mounted on an International Scout vehicle; (2) Redesigning and improvement of an aircraft high pressure (200-400 psi) spray system; (3) Atmospheric studies for development of a turbulence sensor system to aid local mosquito abatement districts in determining vertical temperature lapse rates, or sensing of an overhead warm inversion layer; (4) Initiation of mathematic analyses (modeling) of the downwind distribution of aerosols released by ground and aircraft applications; (5) Continuation of drop size studies with additional data on various 2-fluid systems and rotating spinners as used for producing cold fogs or aerosols; (6) Review of techniques employed by local mosquito abatement districts on closed systems and safe handling of chemical insecticides; (7) Determination of adverse effects of mosquito control chemicals by monitoring insecticidal applications and correlating the results to clinical response of individuals exposed to any of the materials.

MOSQUITO SYSTEMATIC STUDIES.—This is a long-term study on California mosquitoes by R. M. Bohart in collaboration with R. K. Washino. The work should culminate in a book which will cover the taxonomy, distribution, biology and public health importance of the 48 species of mosquitoes presently recorded from California.

MOSQUITO PHYSIOLOGY STUDIES.—Physiology studies by C. L. Judson have centered around 3 specific areas of mosquito research. The effect of topical application of various analogues of Juvenile Hormone on reproduction of female aedines is presently under study. In particular, the basic mechanism on the breakdown of oogenesis in the JH-treated-adult females is being investigated. This study augments the more applied aspects of the work on JH analogues being conducted in field studies by other investigators at Davis, Fresno and Riverside.

A second area of study in mosquito physiology relates to chemical composition of the blood meal and subsequent egg production. The numbers of eggs produced per unit of ingested blood varies with the type of blood ingested by the adult female mosquito. By feeding different combinations of various blood fractions, an effort is being made to identify the fraction(s) responsible for the difference. Chemical analyses are being made of the amino acid and lipid compositions of these fractions.

Another area of study involves the testing of various chemicals for possible ovicide action against aedine eggs. This represents a re-activation of a program begun several years ago and is an effort to determine some of the characteristics required by a mosquito ovicide. Compounds chosen for certain characteristics are tested for ovicidal action as fumigants.

Table 1.—Summary of mosquito research projects within the Agricultural Experiment Station activities at the University of California, Davis.

Investigators	Department	Project
Akesson, N. B. and W. E. Yates	Agricultural Engineering	Field studies to evaluate machines, techniques and meteorological factors in relation to mosquito control.
Bohart, R. M.	Entomology	Systematics of mosquitoes in California.
Judson, C. L.	Entomology	Physiological mechanisms controlling ovipositional behavior of mosquitoes. Chemical manipulation of aedine mosquito egg hatching.
McClelland, G. A. H.	Entomology	Quantitative field estimation of adult population parameters of *Aedes nigromaculis*. Competition among mosquitoes in the human environment.* Energy and cost analysis of the cattle-mosquito irrigated pasture system.
Moore, C. V. and M. S. Sarhan	Agricultural Economics	An economic analysis of mosquito abatement strategy and investment policy for narrow spectrum pesticides in California.
Washino, R. K., Case, T. J. and M. L. Birch	Entomology	Applied field research on mosquito control agents in northern California. The ecology and experimental host range of *Lagenidium giganteum* in California. Identification of host blood meals in arthropods.*

*Supported by extra-mural funds.

ECOLOGY AND GENETICS OF SOME AEDINE MOSQUITOES.—One of G. A. H. McClelland's major projects has been a study to understand the ecological and genetic relationship between populations of *Aedes aegypti* inhabiting houses and those breeding independently of man, and the interactions between *A. aegypti* and other mosquito species with which it shares its breeding habitat. The results may greatly assist in elucidating various aspects of the epidemiology of yellow fever and dengue.

A mark-release-recapture study of *Aedes nigromaculis* in California irrigated pastures has provided much data on survival, population size and dispersal that are presently being analyzed. Short distance dispersal (less than 500 m) was found to be generally downwind.

Feasibility studies for monitoring livestock losses due to pasture mosquitoes revealed more the potential difficulties than providing any preliminary data. Separation of the effect of horn fly from that of mosquitoes was a primary problem.

AN ECONOMIC ANALYSIS OF MOSQUITO ABATEMENT STRATEGY AND INVESTMENT POLICY FOR NARROW SPECTRUM PESTICIDES IN CALIFORNIA.—The objectives of Economists C. J. Moore and M. S. Sarhan are: (1) to develop a methodology and criteria for evaluation of minor use of insecticides and (2) to determine the divergence between a socially desirable level of investment and the observed level of private investment in narrow spectrum mosquitocides research and development.

A mathematical-economic model has been developed defining the demand and supply variables to be estimated. The investigators are now in the process of refining this model and gathering data from a limited number of local mosquito abatement districts and the California State Department of Health.

ECOLOGY AND CONTROL OF MOSQUITOES.—In developing integrated strategies for management of mosquito populations, the activities of investigator R. K. Washino centered around the following: (1) Entomologic and sociologic studies on the extent of discomfort inflicted by pest mosquitoes; (2) Comparison of 3 standard methods for measuring adult mosquito populations including parity profile of the female; (3) Optimum condition for mass-rearing *C. tarsalis* were studied utilizing ponds of various designs; (4) A study of the ecology and experimental host range of the aquatic fungus, *Lagenidium giganteum*, a promising biological control agent for possible use in California rice fields.

With co-investigator T. J. Case, intensive field studies were continued in California rice fields. Larval population on *Culex tarsalis* utilizing mark-release-recapture techniques revealed patterns of survivorship and insight as to causes of natural mortality. The same techniques were applied in additional studies which dealt with the interaction of mosquito larvae to natural and introduced predators (both invertebrate and vertebrate) and to insecticide applications. A multi-variate factor analysis was also run to characterize aquatic organisms in rice fields most influential in both total faunal composition and mosquito larval abundance. Three formulations of insect growth regulators (IGR) were tested against *C. tarsalis* and *Anopheles freeborni.* The adverse effects of IGR on non-target organisms were also investigated.

Additional studies on IGR included (1) their efficacy in a slow-release formulation in urban catch basins and (2) to prematurely terminate diapause in overwintering adult mosquitoes.

An accurate knowledge of the host range of mosquitoes is essential in studies of mosquito-borne diseases. Identification of mosquito blood meals requires a test which is sensitive enough to detect partially digested blood and specific enough to identify the various hosts. With co-investigator M. L. Birch, a method is being developed which involves crystallization of certain blood components in the blood meal samples from the mosquito midgut and comparing the crystal structure with that of known material.

CURRENT RESEARCH ADVANCES ON THE INTEGRATED MANAGEMENT OF MOSQUITOES

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The research program on mosquitoes at the University of California, Riverside, is primarily concerned with the applied aspects of mosquitoes and their control. The aims of this program are to study, formulate and develop integrated control strategies against various species of mosquitoes prevailing in diverse habitats.

Current programs are placing emphasis on the development of biological control agents and chemical control strategies including the use of conventional and novel chemical substances regulating mosquito populations. Studies are also under way to elucidate the physiological mechanisms involved in the development of resistance to the various groups of chemical control agents.

The results of these research programs dealing with mosquitoes and related problems will be presented by the research staff of UCR at this meeting. A total of 19 scientific papers are listed on the program and will be presented by the researchers in person. The highlights of these findings which I am going to touch upon were furnished by the various researchers. These researchers will be available to answer any questions during the course of this conference.

PATHOGENS.–In the field of insect pathology Dr. Brian Federici, a recent addition to our faculty in the Division of Biological Control, is devoting a substantial portion of his research time to investigations on the potential role mosquito pathogens may play in integrated control programs. Currently, he is studying the potential of both viruses and fungi of the genus *Coelomomyces*. Laboratory studies with cytoplasmic polyhedrosis viruses, nuclear polyhedrosis viruses, and iridoviruses have indicated that none of the known viruses of these types have much control potential.

The work on *Coelomomyces*, although at a very basic stage now, suggests that these fungi deserve serious consideration as control agents. Epizootics resulting in high mortalities (95-100%) in larval populations are well documented in the literature. However, it has only been within the last year that workers at the University of Washington demonstrated that *Coelomomyces* fungi require copepods as obligate alternate hosts. Since coming to Riverside, Dr. Federici has found that the copepod *Cyclops vernalis* is an alternate host for *C. punctatus*. During the next few years he will concentrate on this system and another system involving *C. psorophorae* and *Culiseta inornata*. His objectives include determining complete life cycles for these fungi, the development of methods for mass production of mosquito-infective units, and a realistic evaluation of the role these fungi may play as biological control agents in mosquito control programs. This research is relevant to California mosquito problems in that a strain of *Coelomomyces* has been found in Utah which causes mortalities of up to 100% in populations of *Culex tarsalis* larvae and is also cross-transmissible to species of *Aedes* and *Culiseta*. It is planned to initiate surveys for new viruses and other pathogens of mosquitoes within the near future.

Reesimermis nielseni is a mermithid nematode which can parasitize and kill the larval stages of 57 species of mosquitoes under laboratory conditions. Research on this promising mosquito parasite is conducted by Dr. E. G. Platzer and his graduate student Becky Brown of the Department of Nematology. This research is part of a 5 to 7 year program on the biological control of mosquitoes with mermithid nematodes. The goal of this program is to develop and maintain a facility for the mass cultivation of *R. nielseni* and to study the ecological requirements of this mermithid nematode to develop rational guidelines for its use in natural control of mosquito populations.

They have established a culture system for autogenous *Culex pipiens* and can produce 100,000 mosquitoes weekly. Most of these are used for infection with *R. nielseni* and the culture system can produce 50,000 post-parasitic nematodes per week. After maturation, the post-parasitic female nematodes lay eggs on a sand substrate and these eggs can be stored for up to six months on moist sand. The mass production techniques have to be improved for much greater yield of the parasites. Major efforts have been directed toward the basic biology of *R. nielseni*. Findings of interest to this group are: (1) The infective nematode larvae tolerate the copper and Endothal based algacides at concentrations used for algae control. (2) The typical concentrations of cations and anions found in the fresh-waters of North America do not affect the motility of the infective larvae. (3) Light or darkness do not have any significant effect on the infectivity of the infective stages. (4) Temperature effects on the survival of the infective stages have been studied: the optimum temperature for survival is 11-12 C but the optimum temperature for infection is at 21 C.

PREDATORS.–Dr. Fred Legner and his graduate student, Mr. Yu, and others of the Division of Biological Control are engaged in research on the effectiveness of various fish and invertebrate predators of mosquitoes. They have made significant contributions to the ecology of the desert pupfish *Cyprinodon macularius* which is an effective biological control agent for mosquitoes. The ecological implications of this fish as well as *Gambusia affinis* are being studied now. It seems that the desert pupfish is a more desirable mosquito predator and, therefore, trials to stock and establish this fish in some areas are under way now.

The studies on Hydra have progressed considerably. Mass production techniques are sufficiently developed to yield large populations of these for dispersal and application in certain nonpolluted waters of permanent to semi-permanent nature. Hydra do well in habitats supporting emergent vegetation such as rice fields and others.

Two planaria species (*Dugesia dorotocephala* and *D. tigrina*) hold great potential for the control of mosquitoes and chironomid midges. Current problem in utilizing these biotic control agents at field level is the lack of suitable mass culturing technique. The planaria when disturbed by the presence of prey organisms such as mosquito larvae, secrete a slimy substance which acts as a toxic or paralytic agent on the prey. This biological chemical elaborated by planaria is so quick-acting that most of the prey larvae succumb in a few minutes. Dr. Legner and Dr. Y-S. Hwang, an organic chemist, are investigating the chemical nature of this natural product. If chemically known, it is quite conceivable that the toxic and paralytic agent might be employed in mosquito control programs. Studies on the species specificity, stability and potency of this product are under way now.

RESISTANCE.–The primary thrust of Dr. George Georghiou's research involves the elucidation of the phenomenon of mosquito resistance to chemicals. The immediate objectives are: (1) toxicological and biochemical characterization of resistance, (2) updating knowledge on the evolving spectrum of resistance, (3) identifying chemicals which are effective toxicants or synergists on resistant populations. Investigations on the mode of action of the chemicals involved are an integral part of this research. The long-term objective of Dr. Georghiou is the formulation of control approaches which would reduce or eliminate the possiblity of development of resistance.

The most significant finding of Dr. Georghiou's research program in 1974 has been the identification of organophosphorus multi-resistance in *Culex 5-fasciatus.* Strains from Hanford, California, were colonized in October 1974 and found to be strongly resistant to Abate, chlorpyrifos, parathion, fenthion, and malathion. As in the case of OP-multi-R *C. tarsalis,* these strains are susceptible to carbamate insecticides. Research with synergists and radio-labelled chemicals revealed that in both species resistance is due to enhanced metabolism of the organophosphates. This contrasts sharply the situation in *Anopheles albimanus* which has developed among mosquitoes a unique mechanism of resistance, namely "reduced sensitivity" of the target enzyme AChE toward OP and carbamate insecticides.

Further research on *C. tarsalis* has confirmed the recalcitrance of the species to development of resistance toward carbamates: selection of a highly OP-resistant strain (with 45x resistance to parathion) for seven generations had no effect on the level of susceptibility toward carbamates.

Several insecticides received from WHO or the chemical industry were tested for their toxicity to insecticide-resistant strains. Available strains include *C. 5-fasciatus* susceptible, propoxur-R, fenthion-R, and OP-multi-R; *C. tarsalis* susceptible, OP-multi-R, and Altosid-R; and *Anopheles albimanus* susceptible and OP/Carb.-multi-R. A biodegradable DDT analog (OMS 1476) showed good activity on all but the propoxur-R strain of *C. 5-fasciatus.* The organophosphates Celamerck S-2957 and Cyanox were highly toxic to larvae but were affected by cross-resistance in all the OP-multi-R strains. Ciba-Geigy 18809, a derivitized carbamate, was effective on OP-R strains but less toxic to carbamate-R strains. Least influenced by OP, carbamate, or DDT resistance were Cismethrin and Biopermethrin, two potent synthetic pyrethroids.

Studies on the mode of action of TH-6040 have produced information relevant to the field application of this IGR chemical. The compound showed nearly identical activity against five strains of *C. tarsalis* and four strains of *C. 5-fasciatus*, irrespective of existing insecticide resistance in the strains. It was demonstrated that activity is congruent with ecdysis, that sensitivity of the four larval instars is approximately equal, and that a considerable proportion of eclosed adults are incapable of completely separating from the puparium.

OVERCROWDING FACTORS.–Recently we isolated and identified the natural chemicals produced by overcrowded larvae of mosquitoes. We know now that older larvae of *Culex pipiens quinquefasciatus* Say, under overcrowded conditions elaborate a number of chemicals known as overcrowding factors which play a role in regulating mosquito larval populations. These factors manifest toxic and growth-retarding effects in younger larvae. Dr. Y-S. Hwang and others have elucidated the chemical nature of these biologically active compounds.

The hydrocarbon fraction contains heptadecane, octadecane, 1-methyloctadecane, and 8-methylnonadecane. Synthetic samples of these compounds show some biological activity against mosquito larvae. Structural isomers of methyloctadecane and methylnonadecane were also synthesized and evaluated. Among these, 4-, 7-, and 9-methylnonadecanes showed the greatest activity and LC_{50} in the range of 1-2 ppm and LC_{90} about 10 ppm. This rate of application might seem high, but combining these with the other components of overcrowding factors one gets good synergistic action.

The carboxylic acid fraction contains 2-methyl and 2-ethyl substituted aliphatic carboxylic acids. To confirm the biological activity of branched chain carboxylic acids, a number of carboxylic acids with various structural types were evaluated. Bioassay showed that branched-chain carboxylic acids in general possess good activity inducing mortality in younger larvae while straight-chain carboxylic acids show no or little activity. The structural-activity studies of 2-ethyl, 2-butyl, and 2-hexyl substituted carboxylic acids reveal that the level of activity of these compounds seems to be dependent upon the total number of carbon atoms in the carboxylic acids. Methyl esters of these branched-chain carboxylic acids are being studied. Methyl esters of the branched-chain carboxylic acids are more active than the corresponding acids. It is therefore concluded that esterification of the acids increases the biological activity.

The overcrowding factors are produced by mosquito larvae for regulating their own populations. These chemicals according to their structure seem to possess little toxicity to man or domestic animals. They are highly specific and hopefully will not disrupt ecological equilibirum in an aquatic ecosystem.

It is quite possible that the overcrowding factors or their analogs could be devleoped as specific larvicidal agents for mosquito control.

Insect Growth Regulators

LABORATORY.–Emulsifiable concentrate and encapsulated formulations of the IGR R-20458 were evaluated in the laboratory against 4th instar *Culex p. quinquefasciatus* Say at 0.1 and 0.5 ppm. The encapsulated thickwall formulation at 0.1 ppm produced complete inhibition of emergence for 9 days of the experiment. The EC and encapsulated thinwall produced complete inhibition of emergence for the first day only. Inhibition of emergence was less than 56% for the 3-, 6-, and 9-day intervals. At .5 ppm only the

EC and the thickwall formulations produced complete inhibition for the 9-day period, the thinwall formulation yielding less than 90% for days 3, 6, and 9.

RO-8-3165, RO-10-3108, RO-10-3683, RO-10-6425, and RO-20-3600 (all EC4 formulations) were evaluated at 0.1 ppm against the larvae. The first 2 compounds yielded mediocre inhibition of emergence only for the first day after treatment. Inhibition was very low for day 4 and 8 of aging. RO-10-3683 produced 100% inhibition on day 0, and 88% and 74% inhibition on day 4 and 8, respectively. The last 2 compounds yielded high level of inhibition on day 0, but inhibition declined on day 4 and 8.

EC and encapsulated formulations of RO-20-3600 were evaluated at 0.05 and 0.1 ppm. The EC formulation yielded consistent inhibition on day 0, 4, and 8. The encapsulated formulation was inferior.

Methoprene or Altosid was evaluated as EC, encapsulated SR10 and encapsulated SR10F (with charcoal) at 0.01 and 0.05 ppm. The 2 encapsulated formulations were similar in their performance but superior to the EC formulation.

GRANULAR FORMULATIONS.–4 granular formulations (0.5%) of the IGR TH-6040 were evaluated in the laboratory. During the first 2 days after addition of granules to water, release was low. On the 3rd day the level of release into the water was high enough to produce complete inhibition with a final theoretical concentration of 0.05 ppm. All carriers (Florex, Celatom and Emathlite) yielded similar releases. However, Celatom with large particle mesh released the material somewhat slowly.

Granular formulations of the IGR R-20458 yielded good inhibition of emergence at 0.5 ppm 1 day after addition of granules. Level of inhibition was low to mediocre 2, 4, and 8 days after addition of the granules.

A--STAGNANT WATER MOSQUITOES.–Two formulations (EC and semi-gelled) of RO-10-3108 and EC formulation of CGA-13353 (Ciba-Geigy Co) were evaluated in field ponds at Oasis, California, against larvae of *Culex tarsalis* at the rate of 0.25 and 0.5 lb/acre. Formulations of the former compound performed similarly, yielding 85-95% inhibition of emergence in the population isolated 2 days post-treatment. Low level of inhibition was obtained 6 days post-treatment. The 2nd compound was ineffective at the rates used.

A comparison of the EC and WP formulation of HE-24108 at the rate of 0.5 and 1.0 lb/acre in ponds against *C. tarsalis* showed the two to be similar. Inhibition of emergence at the lower rate was mediocre, while at the higher rate, excellent inhibition of emergence (82 +%) was obtained up to 11 days posttreatment. Level of effectiveness declined considerably when tested 17 days after treatment. The WP formulation, however, is the most economical, since high emulsifiable concentrates cannot be formulated.

Since the 2 encapsulated formulations of Altosid SR10 were found to be equally effective in the laboratory, they were then tested under field conditions during cool weather against *C. tarsalis* and *Culiseta inornata* at the rate of 0.1 lb/acre. The standard SR10 formulation was superior to the SR10F (with charcoal) against *Culex tarsalis.* The standard SR10 yielded complete inhibition on day 2, 5, and 8 posttreatment, while the SR10-F gave only 79% inhibition on day 8. *C. inornata* was more susceptible, its inhibition of emergence being complete with both formulations at all 3 intervals. Mean maximum and mean minimum water temperature during the study period was 74 and 58°F, respectively.

During warm weather (mean max. and mean min. water temp. 89 and 70°F, respectively) the above 2 Altosid formulations at the same rate (0.1 lb/acre) produced high level of inhibition of emergence of *C. tarsalis* 2 days posttreatment. As found previously, Altosid is shorterlived at higher temperatures than at low temperatures. Another study during high water temperatures against *C. peus* produced similar results as with *C. tarsalis.* The formulations inhibited emergence 2 days posttreatment but not 4 days after treatment.

The 3 formulations of the IGR R-20458 (EC, encapsulated thinwall, and thickwall) and the encapsulated formulation 2S were evaluated in ponds at 0.5 lb/acre against larvae of *C. tarsalis* and *C. inornata.* Low to mediocre level of inhibition of emergence was obtained with all formulations. None of the formulations was superior to any other and none gave satisfactory control even initally, i.e. 2 days posttreatment.

GRANULAR FORMULATIONS OF TH-6040.– Since the granular formulations of TH-6040 proved effective in the laboratory, these were then studied in ponds against *C. tarsalis* larvae. The material was applied at 0.1 lb/acre. All four granular formulations (0.5% on Florex 15/30) proved more or less equally effective, Celatom 15/30 formulation being the most effective. All formulations yielded complete inhibition of emergence for 10-11 days after treatment.

B--FLOODWATER MOSQUITOES.–The IGR's TH-6040, HE-24108, methoprene or Altosid and R-20458 were evaluated against the floodwater mosquito *Psorophora confinnis* in irrigated pastures. TH-6040 WP gave complete control of this species at 0.005 lb/acre. HE-24108 WP at 0.25 and 0.5 lb/acre produced high level of control. Altosid SR10 yielded complete control at 0.025 and 0.05 lb/acre. The encapsulated formulation (2S) of R-20458 yielded almost complete control at the rate 0.25 lb/acre. These IGR's, especially TH-6040 and Altosid, offer good potential for effective and safe control of floodwater mosquitoes.

EFFECT ON NONTARGET ORGANISMS.–EC and WP formulations of the IGR TH-6040 were evaluated in the field for their short-term effects on several prevailing groups of arthropods. At the rates of 0.025 and 0.1 lb/acre neither formulation produced short-term effects on diving beetle adults and larvae, mayfly naiads (which are the most susceptible group to OP insecticides) and dragonfly naiads (mostly *Anax* sp.). However, a number of diving beetle larvae (Dytiscidae) were found dead or in stress at the surface of water in the WP treatments and not in the EC formulation. In another experiment where granular and WP formulation of this compound were studied, no noticeable effect was observed on mayfly and dragonfly naiads. Ostracoda populations were not affected either. However, copepods (*Cyclops* and *Diaptomus*) were slightly depressed, but their population recovered 11 days posttreatment. Cladocera (mostly *Daphnia* sp.) were severely affected up to 11 days posttreatment, but their population recovered 15 days after treatment.

In terms of toxic hazards of this IGR, this material is much less hazardous than most commonly used OP mosquito larvicides. The OP larvicides in general induce heavy mortalities in mayfly naiads, dragonfly naiads, Cladocera and Copepoda.

Mosquito Larvicides

FIELD EFFICACY.—A synthetic pyrethroid NRDC-143, which was found to be highly effective against larvae and pupae in the laboratory. was studied in the field against *Culex peus*, *C. tarsalis*, and OP resistant *Aedes nigromaculis*. Unlike other synthetic pyrethroids, this material yielded excellent control of *Culex* species at the rate of 0.025-0.05 lb/acre. This compound was more effective against highly OP-resistant strains of *A. nigromaculis* in Tulare and Kern Counties, yielding complete larval control at 0.01-0.025 lb/acre. This compound is unique in showing good activity against both larvae and pupae.

Two new organophosphate materials, N-2596, and S-2957, were also highly effective, yielding complete control of *Culex* species at 0.025 lb/acre. The former compound produced good control of OP-resistant *A. nigromaculis* at the rates of 0.05-0.1 lb/acre. Its effectiveness was influenced by the level of OP-resistance in the population. It is quite possible that these two OP compounds will not be effective for too long against OP-resistant strains.

Two other new OP larvicides, CGA-12223 and CGA-15324, were evaluated against *C. tarsalis* and *C. inornata* in the field. The former compound proved effective at the rate of 0.5 lb/acre, failing to yield good larval control at 0.25 lb/acre. This is too high a rate (0.5 lb/acre) for an OP compound to be used in mosquito control. The latter compound proved very effective against these species at the rates of 0.05 and 0.1 lb/acre, yielding larval control for 10 days or so. This compound seems to offer good potential as a larvicide. CGA-18809, a derivatized carbamate, was ineffective as a larvicide at rates as high as 0.5 lb/acre.

EFFECTS ON NONTARGET ORGANISMS.—The synthetic pyrethroid, NRDC-143, caused complete mortality in mayfly naiad in field ponds at the rates of 0.05 and 0.1 lb/acre. The former rate is at the upper limits of larvicidal dosage, and at this rate the mayfly population recovered to full capacity 16 days after treatment. Copepods were affected by the higher rate but not the lower and Ostracoda were not affected.

The organophosphate S-2957 also caused severe mortality of mayfly naiads at 0.05 and 0.1 lb/acre rates, the population not recovering for up to 16 days after treatment. Copepods and ostracods were slightly affected, recovering 16 days after treatment.

CGA-12223 at 0.05 and 0.1 lb/acre (larvicidal rates) completely decimated mayfly naiads, no recovery noticed 14 days after treatment. Dragonfly naiads, however, were not affected at these rates, nor were diving beetle larvae (predaceous) and adults. CGA-15324 at 0.05 and 0.1 lb/acre also decimated mayfly naiads, but not dragonfly naiads or diving beetle larvae and adults. As is well known, the mayflies, which are grazers of the freshwater habitats, are severely affected by OP compounds. The derivatized carbamate CGA-18809 at 0.1 lb/acre (much lower than larvicidal rate) affected mayflies slightly, but not the other two groups. This material, as discussed earlier, does not have any mosquito larvicidal potential.

ALIPHATIC AMINES.—These simple and readily available compounds are finding more uses in mosquito control programs. For the past several years, the aliphatic amines have been employed as cationic surfactants and as additives to hydrocarbon larvicides. Due to the current shortage of petroleum products, the cost of larvicidal oils is getting beyond the reach of some districts. There is a great need now to develop concentrate formulations of aliphatic amines so that these can be used with water as the carrier and sprayed over mosquito breeding sources. With these objectives in mind, several aliphatic amines were evaluated as concentrate formulations applied with and without water.

Ground applications of Alamine 11, Armeen L-15 and Duomeen L-15 at 0.5 lb/acre active yielded good control of larvae and pupae of *C. tarsalis* and *C. peus*. At the same rate all three controlled larvae of *A. nigromaculis*. Alamine 11 also controlled pupae, while Armeen L-15 and Duomeen L-15 yielded 90 and 83% control of pupae, respectively. At a higher rate (1.0 lb/acre) both materials yielded complete control of pupae. It is the unique feature of these compounds to control both larvae and pupae.

Aerial application of concentrates of aliphatic amines in oil as ULV treatments failed to control larvae of *A. nigromaculis* in irrigated pastures. Further studies are needed to develop effective concentrate formulations of aliphatic amines.

MOSQUITO RESEARCH AT THE UNIVERSITY OF CALIFORNIA, LOS ANGELES

TROPICAL AND SUBTROPICAL MOSQUITOES OF IMPORTANCE TO CALIFORNIA

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In terms of longevity, Professor John Belkin's taxonomic and bionomic study of tropical mosquitoes is the most substantial accomplishment in mosquito research at UCLA. In the former Zoology Department, Dr. Belkin undertook extensive studies of the taxonomy, bionomics and distribution of the mosquitoes of the South Pacific. This culminated in the publication in 1962 by the University of California Press of the two-volume work on "Mosquitoes of the South Pacific (Belkin 1962).

This initial model of personal field collections in the tropics, rearing and fixing morphological slides of all instars of both sexes, and examination of material in collections scattered all over the world, provided the format for a much larger undertaking initiated in 1962. This is on the taxonomy, bionomics, and distribution of Mosquitoes of Middle America. The northern and southern limits of Middle America are being considered flexible in accordance with the distribution of tropical and subtropical mosquito species, which at times extend into the temperate zones of North and South America.

Because Dr. Belkin recognized the vastness of the undertaking and its obvious evolutionary nature, at the outset he determined that the contributions would be published periodically under a nominal series, Contributions of the American Entomological Institute. This has led to a cumulative reference bibliography of 43 papers to date (Appendix I). The contents are of interest and value for a diverse community of professional, academic and research entomologists. The work is supported under contract of the Department of the Army; the biological aspects by a grant from the National Institute of Allergy and Infectious Diseases. What is emerging is a unique contribution to the reference sources of importance to medical entomology and especially vector control. For instance, when California was threatened with the northwestern extension of epidemic and epizootic VEE (Work 1972), the contribution of Adames (1971) was the only source of reliable information on *Deinocerites* mosquitoes which had been implicated as an important vector in the 1971 outbreak in Texas.

It is expected that ultimately the information, with illustrations, will be collated into reference volumes which will replace the outdated works of Howard, Dyar and Knab (1912-17) (Dyar 1928).

CYTOPLASMIC INCOMPATIBILITY.–Initially supported by the California Department of Public Health, and subsequently transferred to the University of California, Dr. A. Ralph Barr has been colonizing strains of different genera and species of mosquitoes in attempts to cross-breed for evidence of a genetic basis for cytoplasmic incompatibility. This is a term associating inherited mating type to cytoplasm of the egg formed in the female mosquito ovary. If the combination of sperm produces sterile eggs or others that do not hatch, a condition of cytoplasmic incompatibility is said to exist. This phenomenon initially promised to be an approach to biological limitations of natural production of mosquitoes. As the years passed, there has been a continuous emission of information on various markers associated with varying levels of incompatibility, but no demonstrable chromosomal character associated with incompatibility.

In 1970, a student, Janice Yen, looking at reproductive tissues of incompatible strains of *Culex pipiens*, observed what appeared to be viruses. Subsequent experiments utilizing antibiotics established the susceptibility of these organisms and therefore eliminated them from characterization as viruses. Search of the literature determined that many years ago Marshall Hertig had observed similar particles that he described as *Wolbachia pipientis* (Hertig 1936). The key result of Ms. Yen was that the application of antibiotics to breeding cultures eliminated or diminished the apparent cytoplasmic incompatibility (Yen and Barr 1973).

Further use of electron microscopy has visualized a larger, therefore different *Wolbachia* organism by another student, John Wright, in the testicular cells of the Tafahi strain of *Aedes scutellaris.* Whether this, too, is associated with reproductive incompatibility awaits appropriate antibiotic experiments.

While one can facetiously conclude that the best way to increase mosquito production would be to fog with broad-spectrum antibiotics, it emerges from this work that control of parasitism by manipulating these as yet biologically uncharacterized agents would be one approach to inhibit actual breeding of mosquitoes (Yen and Barr 1974).

ENCEPHALITIS TRANSMISSION IN ARID ZONES.–Epidemic encephalitis in California has been sufficiently frequent, severe and costly to have supported development of mosquito abatement programs that cover large areas of the state, particularly in the San Joaquin and Sacramento Valleys. While this is an abiding and continuously threatening public health problem in California, much has been learned through the classic research and accomplishment of Dr. W. C. Reeves and colleagues of the University of California, Berkeley, and those associated with Mr. Richard F. Peters and Richard Emmons in the State Health Department in Sacramento and Berkeley. It is also acknowledged that additional knowledge on arbovirus transmission of human pathogens that produce central nervous system disease as well as other disease syndromes, has been accomplished through epidemic investigation and long-term basic studies in other states, in association with the U.S.P.H.S. Center for Disease Control, and abroad by national laboratories, Rockefeller Foundation-supported efforts, the World Health Organization and various military research teams and facilities.

From this collation of research results and epidemic experiences on a global basis, concepts and perceptions have emerged which frame hypotheses that are relevant to the dynamics of encephalitis virus transmission in California. One major hypothesis is that when human endeavour drastically changes arid regions by introduction of irrigated water for production of crops, environments are created which support mosquito breeding and supply food and habitat for vertebrates in which viruses are circulated. This not only results in exposure of the increased human population required to work the new agricultural production, but on occasion attracts large numbers of susceptible people for recreational and retirement purposes. It is therefore important to: (1) determine whether vector species exist and do transmit pathogenic arboviruses; (2) determine their dynamics so that a threatening threshold can be established beyond which vector control must be initiated; (3) elucidate the mosquito-vertebrate maintenance and transmission cycle, so that any link amenable to interruption can be considered for a preventive program; (4) determine key sites and mechanisms which are the most sensitive indicators of transmission; (5) define reliable foci of transmission which can be used for evaluation of continually needed measures of vector control; and (6) establish a continuous surveillance which will detect new areas of transmission, invasion or emergence of new pathogenic arboviruses or extension of previously defined enzootic and endemic areas.

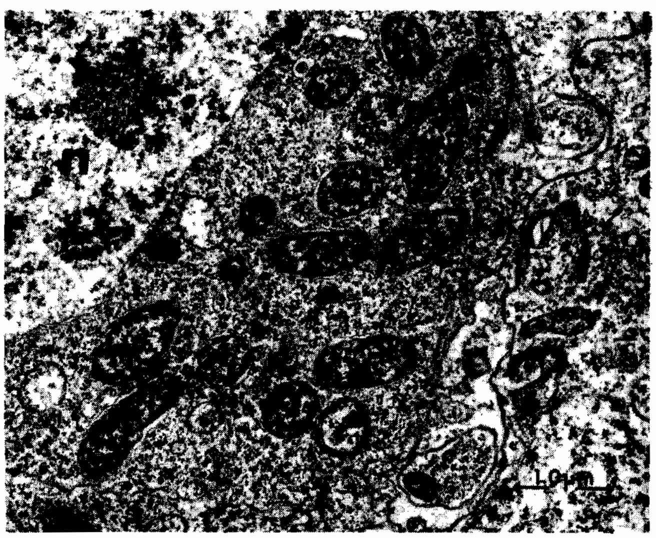
The arid zones of Southern California pose such problems which are relevant to the health and welfare of more than twelve million people who either live in these areas or frequent them for recreational or other purposes. In 1967, we undertook to determine whether *Culex tarsalis*, the recognized arbovirus encephalitis vector in California, was active throughout the year in the Imperial Valley, and whether it transmitted arbovirus encephalitis, in contrast to the summer seasonal activity well recognized and defined in Kern County and other areas of the great Central Valley, where mosquito activity essentially ceases with the onset of cold weather. These investigations were undertaken by graduate students, trainees supported by grants in the training program of the U.S.P.H.S. National Institutes of Health (TOI-AI-00132).

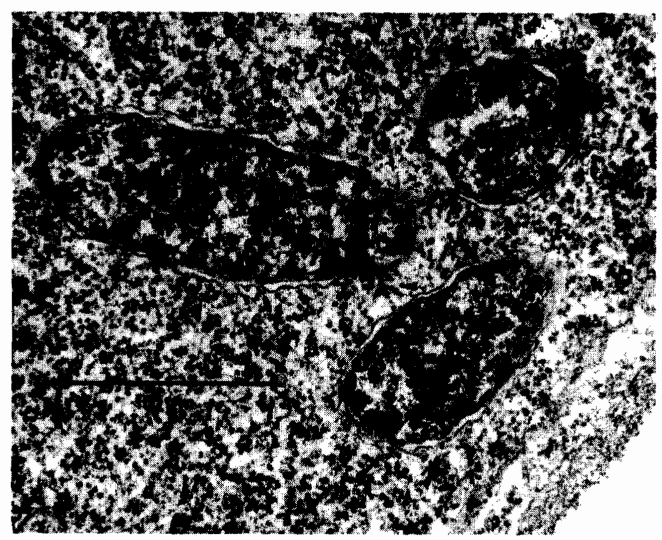
Year around, *Culex tarsalis* activity was defined, as was the winter transmission of California encephalitis virus by *Culiseta inornata* mosquitoes. A bimodal curve of *Culex tarsalis* activity was observed, peaking in late spring and early fall. The enigmatic Turlock virus has appeared frequently in *Culex tarsalis* pools but as yet not according to any recognizable pattern. One other virus isolated but not yet identified is known to be a member of the *Bunyamwera antigenic* group. At no time did we fail to catch *C. tarsalis* mosquitoes which indicated that they were active every night of the year. About 90% of the total catch consisted of *C. tarsalis*. Over a seven year period SLE Virus was isolated every year: in 1972 as early as May and in 1970 as late as November. But as yet no evidence of SLE virus transmission has appeared in the winter and early spring months. This could be attributable to low yield per trap night in the cooler months, but may suggest either annual reintroduction or maintainance by means other than mosquito transmission.

On the basis of this finding, the State Health Department's Vector Control Section initiated more widespread surveillance for arbovirus transmission in the arid zones of the California border. Review of four years' accumulated data in 1971, indicated that our study area was at the periphery of a more centrally located emission of WE and SLE viruses. Further reconnaissance located the Finney-Ramer Wild Life Refuge, an aqueous habitat more centrally located astride the Alamo River northeast of Brawley. Simultaneous studies of the transect running the length of the river from Finney Lake to Salton Sea indicated that there were no foci of mosquito transmission of viruses more constant than at Finney Lake.

General dissemination throughout Imperial Valley was demonstrated in the combined data of Harvy Magy's Vector control surveillance and our border search in 1972 (Bown and Work 1973).

The reliability with which SLE virus appears at Finney Lake each year called for intensive quantitative studies that were commenced in January 1973. (Clark, Work and Moss 1974). The State Surveillance turned up SLE infected *C. tarsalis* mosquitoes, April 4, 1973, along the New River on the west side of Imperial Valley. (Magy, pers. commun.).





Figures 1 and 2.—*Wolbachia*-like micro-organism in spermatogonial cell from 24 hr. pupal testes of the *Aedes scutellaris* complex, tafahistrain. n: nucleus of spermatogonial cell, I: interstitial cell, **arrow**: *Wolbachia*-like micro-organism.

Our quantitative efforts were concentrated in an east-west transect of 15 CDC light traps hung 100 feet apart between Sheldon Pond and the Alamo River. Less than a mile to the north, a grid of 9 CDC light traps 50 feet apart in rows of three was layed out between the shore of Finney Lake and the valley level bluff top. As many as seven nights trapping were accomplished each month. These studies showed that the more exposed transect mosquito-catches peaked in June and precipitously dropped to a significantly lower level than the grid which was maintained until the return of colder weather in early fall. While the grid catches reflected a similar peak in June, a decline was associated with the highest mean maximum temperatures exceeding 100 degrees. The trap night yield in July-August in the grid persisted at a significantly higher level than the transect, perhaps indicating higher protection in the denser vegetation of the grid, or continuous breeding in the marsh shores adjacent to the grid (Clark et al. 1974).

The contrast in virus isolations was even more striking in that the per pool isolation rate for SLE virus dropped in the transect collection, but showed a persistence in the grid which reflected two possibilities: (1) that the more favorable habitat of the grid allowed infected mosquitoes to survive longer, or (2) that the wild mosquito-vertebrate maintained cycle persisted in the aqueous habitat adjacent to the grid.

This obviously required examination of microclimatic associations which was undertaken in 1974 by placing hygrothermometers adjacent to the most reliable trap sites on the transect and in the grid. Over the years, we had observed that excessively strong winds had a marked depressive effect on trap night yields of mosquitoes. Winds will significantly decrease the catch on one or two nights. But on the first relatively calm night the per trap night catches would significantly exceed the average. Because of the transient gusts, localized physical obstructions and air channels - it is considered impossible to relate air movement to transect and grid trap sites in the same way as recorded temperature and humidity. However, recognizing that air movement may be a critical factor, we have directed our attention only to the relationship of temperature and humidity at this time. Quite obviously, identification of almost three months nightly collecting in 1974 has been beyond human capability to complete by now. Initially, the reference site collections for every fifth night were identified, pooled and inoculated (Clark and Work, in preparation).

From the data analyzed so far, it appears that maximum per trap night collections were preceded by the highest mean maximum temperature which may reflect accelerated maturation of larvae and pupae. No correlation with high humidity has emerged as yet other than a higher mean minimum in the grid where much higher trap night yield was maintained. This may confirm the hypothesis that the mosquito vectors tend to frequent more protective conditions for survival which maintain a significantly larger population of infected vectors; or that the more humid environment of the marsh adjacent to the grid maintains a mosquito-vertebrate cycle of transmission throughout the summer.

When the results of *C. tarsalis* pools positive for SLE virus are analyzed, it is again clear that SLE transmission was prolonged in the more humid grid than in the more exposed transect which was subject to lower minimum humidity, greater fluctuation in temperature and humidity, and more exposure to sun, wind and other harsh elements associated with a desert climate.

We can therefore conclude that an arid region which provides adequate humidity with high temperature contributes to *Culex* vector productivity and longevity. The productivity supports a vector population of sufficient numbers to survive the extrinsic incubation period of SLE virus into a significant population of infective vectors. The longevity is a key element in maintaining a mosquito-vertebrate cycle of infection. This strengthens the concept that excessive accumulation of water-by irrigation, rainfall or flooding - combined with excessive temperatures promotes a condition of high incidence of infected *Culex* vectors and a prolonged season of transmission. Such detailed dynamics of encephalitis virus transmission as is emerging from these studies should set the stage for testing various new means for mosquito control in California.

Initial attempts to determine whether the breeding blackbird population was associated with the appearance and increase of virus transmission showed little evidence of increased involvement in collections made in March, April, May and early June 1970. This directed attention to the other vertebrates residing in the Finney habitat: including cottontail rabbits, skunks, racoons, muskrats and *Perognathus* and *Peromyscus* rodents. Sera collected from cottontails in 1971, and in the spring of 1972, had a significant percentage of SLE positives. Intensive collection of cottontails, and in a lesser extent other vertebrates was commenced by Dr. Gary Clark in 1973. Using a low dose highly sensitive mouse neutralization test of 10 to 60 LD_{50}, of those tested only a few sera of any species contained demonstrable antibodies to SLE virus. These animals were collected mainly in the area between Finney transect and grid. In the face of continued high isolation rates of SLE virus from pools of *C. tarsalis*, these results led us to conclude that none of the indigenous mammalian fauna of the Finney-Ramer Refuge is a primary source of virus to the prevalent infected *C. tarsalis* mosquito vector population. The high prevalence of SLE antibodies observed in grebes, bitterns and coots in 1970 returns our attention to water birds of the Finney Lake habitat as the suspect source of SLE virus to *C. tarsalis*.

In 1969, when excessive snowfall resulted in extensive flooding of the valleys, UCLA undertook search for encephalitis arbovirus transmission in Owens Valley. Although no evidence of WEE transmission turned up, processing of *Aedes melanimon* mosquitoes yielded twelve strains of California encephalitis virus. A subsequent dissertation study by Dr. Hazel Wallace, now at UCSF, demonstrated that the G1040 Owens Valley strain was antigenically closely related to the classic reference BFS-283 strain.

Followup studies showed antibodies in kangaroo rats. Postdoctoral students Bowen and Bown demonstrated 3-5 day viremias in *Dipodomys merriami* and experimentally produced mosquito infection and transmission of virus from kangaroo rat to mosquito to kangaroo rat.

Attempts to isolate virus from larvae and pupae collected in 1973 did not yield an agent. However, in 1970, 1971, and 1973, G1040 California type isolates have been obtained from *Aedes melanimon* mosquitoes collected in our defined focus in Owens Valley. In August 1974, 9,306 mosquitoes of which 8,784 were *Aedes melanimon*, were processed. Forty-three pools yielded an isolate. Eighteen strains have so far been identified as G1040 type California encephalitis virus. We therefore have defined a permanently established focus of *Aedes melanimon* transmitted California encephalitis virus. Long term research studies are being developed because of the recognized recreational association of this disease. The Owens Valley is a major recreational area for Southern Californians.

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INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN CALIFORNIA: OCCURRENCE OF ORGANOPHOSPHORUS RESISTANCE IN *CULEX PIPIENS* SUBSPP.

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Chemical control of mosquitoes in California has been mainly dependent upon organophosphorus insecticides for two decades, following the widespread failure of DDT and related compounds against both *Culex* and *Aedes* during the late 1940's and early 1950's (Smith 1949, Bohart and Murray 1950, Gjullin and Peters 1952). Repetitive exposure has resulted in resistance of *Culex tarsalis* and *Aedes nigromaculis* extending to all six of the commonly used organophosphorus compounds; malathion, parathion, methyl parathion, fenthion, Abate, and chlorpyrifos.

Mosquito resistance to an organophosphorus compound in California was first confirmed in 1956, when larvae and adults of *C. tarsalis* from Fresno County were found to be resistant to malathion (Gjullin and Issac 1957). By 1969, *C. tarsalis* larvae from the southern portion of the San Joaquin Valley had become resistant to all the above compounds (Gillies and Womeldorf 1969, Georghiou et al. 1969). Resistance was documented in a second species in 1958 when *A. nigromaculis* larvae were found resistant to parathion (Lewallen and Brawley 1958). By 1968, both larvae and adults had demonstrated resistance, both in the field and in the laboratory, to all the commonly used organophosphorus compounds (Ramke et al. 1969, Gillies and Womeldorf 1969).

During 1974, resistance to several organophosphorus compounds was detected in the two California subspecies of *Culex pipiens*, *C. p. quinquefasciatus* and *C. p. pipiens*. Resistance in this species to an organophosphorus compound was first detected in 1956, when larvae from Kern County were shown to be resistant to malathion (Gjullin, unpublished data). As a part of a program initiated in 1963 to monitor the susceptibility of California mosquitoes, the Vector Control Section of the State of California Department of Health in cooperation with local mosquito control agencies has tested populations of *C. pipiens* subspp. from both controlled and uncontrolled areas throughout the State. By 1970, this surveillance program indicated widespread malathion resistance; fenthion resistance appeared definite although limited mostly to Coastal and Southern California. Occasional high levels of tolerance to parathion and methyl parathion were detected, but these findings appear to indicate the upper range of tolerance for the species rather than induced resistance, since the high levels were not generally accompanied by a change in the slope of the log dosage-probit line. That is the LC_{90}/LC_{50} ratio was less than two fold, demonstrating a lack of heterogeneity considered to be an indicator of incipient resistance (Gillies et al. 1968, Pelsue et al. 1972). The results of previous surveillance against this species have been summarized by Womeldorf et al. (1966, 1968, 1971).

During 1974, mosquito control agencies in Northern and Central California reported control problems with several organophosphorus compounds. It was not possible to test larvae from all the suspect areas, but laboratory tests against larvae collected from Shasta, Solano County, Alameda County, San Mateo County, Turlock, and Kings Mosquito Abatement Districts confirmed resistance to malathion, parathion, fenthion, Abate, and chlorpyrifos. These levels were generally accompanied by a change in the slope of the log dosage-probit line, as evidenced by a greater than two-fold LC_{90}/LC_{50} ratio. Figures 1-3 illustrate the loss of susceptibility to parathion, fenthion, and chlorpyrifos as shown by 1974 tests compared to those of previous years. The LC_{90}/LC_{50} ratio is indicated by "K".

Strains of *C. pipiens* subspp. from Kings and San Mateo County Mosquito Abatement Districts were colonized at the University of California, Riverside where additional tests were performed and reported upon by Georghiou et al. (1975). Case histories of the development and impact of this resistance on mosquito control have been reported for Alameda County and San Mateo County (Dill and Roberts 1975) and Kings Mosquito Abatement Districts (Stewart 1975). During 1975, the Vector Control Section plans to test larvae from additional areas within the State to determine the extent of the resistance problem and to investigate alternative control methods.

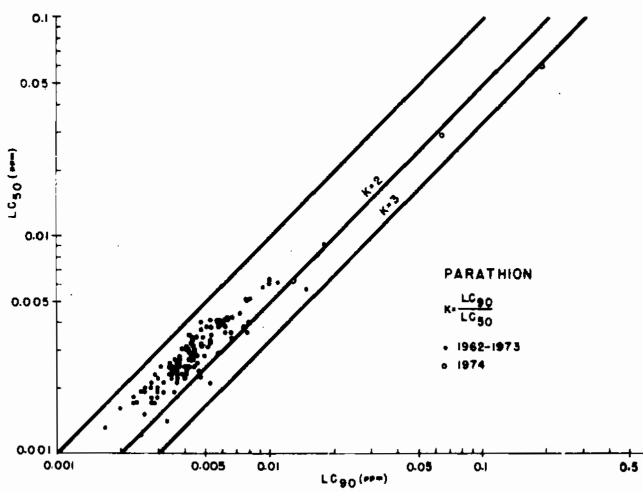

Figure 1.–Susceptibility of larval *Culex pipiens* subspp. to parathion in 1974 as compared to previous years.

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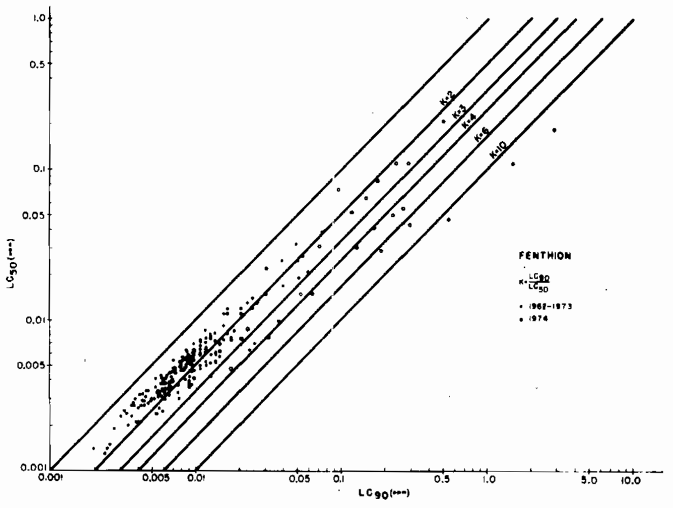

Figure 2.–Susceptibility of larval *Culex pipiens* subspp. to fenthion in 1974 as compared to previous years.

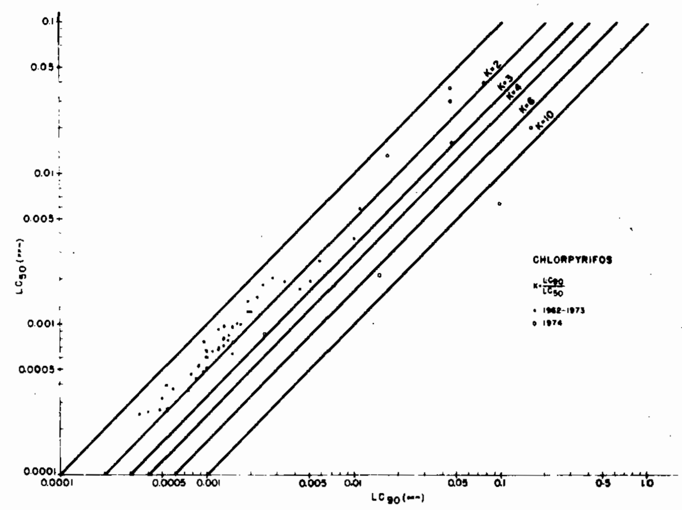

Figure 3.–Susceptibility of larval *Culex pipiens* subspp. to chlorpyrifos in 1974 as compared to previous years.

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ORGANOPHOSPHATE RESISTANCE OF *CULEX PIPIENS* IN TWO URBAN MOSQUITO ABATEMENT DISTRICTS' – A CASE HISTORY

Charles H. Dill[1] and Fred C. Roberts[2]

The house mosquito, *Culex pipiens*, is one of the most important of nineteen species of mosquitoes found in each of these highly urbanized Districts. Their importance stems partially from the potential abundance of these mosquitoes capable of being produced in catch basins, storm drains, septic tanks and sumps, as well as other breeding sources. Another contributing factor is the low tolerance exhibited by the public to this species of mosquito which exhibits a propensity for the indoors, a trait making even low numbers of these mosquitoes obvious.

CHEMICAL HISTORY

The types of sources producing the house mosquito in the Districts have dictated a chemical approach to control. The most important sources are catch basin and storm drain systems found throughout the residential areas. These types of sources are not readily amenable to source reduction methods because of cost factors. The solution to the problem has been achieved through the use of chemicals.

ALAMEDA COUNTY MAD.–In 1973, 92% of the total control effort directed at *Culex pipiens* was chemical in nature. The records suggest that heavy reliance upon chemicals to control this species began early in the District's history.

In 1934, four years after formation of the District, a chemical program was initiated to control the production of the house mosquito in underground drain systems. The program consisted of applying oil to drains and catch basins in the vicinity of complaints but due to the lack of adequate equipment too few were treated and the results were judged to be poor. In 1935 routine oiling of catch basins was carried out in the District with a three-wheeled motorcycle. Approximately 10,000 basins needed treatment, the most productive requiring 8-12 applications per year. The oiling program provided good control of the mosquito population but was suspended with the advent of World War II. The greatest number of catch basins ever treated with oil in one season (46,271) occurred in 1937.

In 1945 DDT was tested in catch basins and found to be highly effective and by 1947 the catch basin/storm drain treatment program was established using DDT rather than oil. The residual effect of DDT was immediately apparent in the operational data. Only 4,072 basins were treated in 1947 compared to an average of more than 30,000 when oil was used. Instead of the 8 or more retreatments that were necessary in the catch basins using oil, only one in 40 catch basins required an additional treatment with DDT. The number of DDT treated basins peaked at 39,546 during 1961. The tremendous increase in DDT treatments since 1947 cannot be attributed only to the addition of new catch basins. Many basins were being treated three or more times per year in order to provide effective control.

In 1964, field tests were made to compare DDT with fenthion. The effectiveness was assessed by the time interval between required treatments. The results of the tests showed fenthion to be more effective.

The District began using fenthion in the basins in 1964. The number of treatments remained approximately the same as with DDT. The total number of catch basins treated per year with fenthion has remained essentially the same to date. This year some of the most productive basins were treated seven times. During the peak breeding season some were treated as frequently as every two weeks. After 1970, Dursban® was used selectively in specified catch basins.

SAN MATEO COUNTY MAD.–There are few historical records available concerning catch basins. It has only been since 1954 that the number of basins treated yearly was published in the annual report, and only since 1968 that records were kept that delineated types and amounts of chemicals used in catch basin work. Fenthion came into general use in 1970, being preceded by diazinon, with an average of three to four applications per season. Diazinon was not used after 1971. In 1973, some areas which were considered to be hot spots, that is, areas where breeding frequently will generate service requests, were treated 5 or 6 times. In 1974, a different approach to catch basin work was initiated. Basins were treated regularly only when they were considered hot spots or they were treated as a result of service requests. This approach resulted in a saving of 1.4 man months (248 hours) to the District and a considerable reduction in the number of basins treated. No appreciable increase in service requests was noted that could be attributed to this change in operational procedure.

DETECTION OF RESISTANCE

Monitoring of susceptibility levels of local mosquito populations was initiated, in cooperation with the Vector Control Section of the State Department of Health, in 1972.

ALAMEDA COUNTY MAD.–The results of some tests on *Culex pipiens* suggested resistance during the first test year (1972). Testing in 1973 was less frequent, but samples containing *C. pipiens* continued to suggest possible resistance. By 1974 full blown fenthion resistance in *C. pipiens* was documented when numerous tests showed high LC_{50} values. When the locations of the samples were plotted on a District map, a distribution pattern emerged indicating a large area of resistance from Oakland south to Fremont. This area of high resistance was bordered on the north and east by more susceptible populations of the same species.

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Upon first glance at District operational data the high levels of resistance might appear to be a laboratory phenomenon, since no increase in service requests and no appreciable increase in control effort of *C. pipiens* is evident. Approximately the same number of catch basins are being treated per year and, most important, only one documented field failure has occurred. Close scrutiny of the recent catch basin treatment program, however, reveals information which supports the laboratory findings. The certified technicians, over the last few years, have become more selective in their treatments of catch basins. Selected catch basins are now treated much more frequently than in the past.

Even more dramatic evidence was provided by interpretation of the results of field tests conducted to establish retreatment schedules for catch basins. On the basis of these tests, operational personnel established retreatment cycles exceeding a month for catch basins in 1964. After 10 years of fenthion usage, treatment cycles on some catch basins were at intervals of less than two weeks.

A review of the District's chemical history strongly suggests the same kind of scenario occurred with DDT. In 1947, the newly acquired DDT was used once a year in most basins. By 1961, records indicate three or more treatments were required. It appears that an impending control breakdown was averted by shifting to fenthion in 1964.

SAN MATEO COUNTY MAD.—Resistance tests began in 1973. Most of the tests run that year in the laboratory were intended to perfect techniques as well as provide susceptibility levels of local mosquito populations. The high levels found during the two year testing period have been confirmed by the Vector Control Section of the State Department of Health, and also this year by Dr. Georghiou of the University of California at Riverside. The field data (catch basin schedules), as in Alameda, gave no suggestion of the developing resistance. The number of treatments per season remained relatively stable as did the number of basins. Resistance levels were fairly uniform throughout the District.

SOLUTIONS

Before solutions could be properly formulated, a thorough analysis of the available data was necessary to provide a clear statement of the problem. Hopefully, one pitfall was averted very early in the process when both Districts avoided defining resistance as the ultimate problem. Resistance, after all, is a well documented, predictable, biological phenomenon. Its appearance simply indicated the existence of inadequacies in the control programs of both Districts. Therefore, the problem in need of solution is to be found in the character of the programs.

Intensive program review sessions were conducted by both Districts during the winter months to establish a basis for the formulation of future control strategies. These independent sessions uncovered a number of common characteristics that appeared central to the problem:

1. Chemicals were by far the most heavily relied upon technique used to control the house mosquito.
2. As long as inexpensive replacement chemicals were available, the chemical in use was changed before resistance induced field failures became apparent.
3. The existing methods used to collect and interpret operational data, and the field inspection procedures, were insufficient to detect control failures.
4. Both Districts lacked clear objectives defining the level of control to be provided. Control operations had evolved over a long period of time to the point that the treatment of many catch basin and storm drain systems occurred whether or not it could be reasonably inferred that they would cause complaints.

The following program was planned for the control of the house mosquito in both Districts for 1975:

1. Efforts will be made to gain the cooperation of public works departments of the various cities in attempt to coordinate their storm drain clean-out programs to supplement our control programs.
2. Treatment of catch basins and storm drains will be accomplished with a larvicidal oil. Abate or Dursban® may be used when *Culex pipiens* is found in sources that cannot be treated with oils, or when adequate control cannot be obtained with a larvicidal oil.
3. Where possible, selected catch basins that had been treated routinely in the past have been designated to be treated on the basis of service requests only.
4. Only those catch basins that, when breeding, and are known to cause service requests will be treated on a routine basis. (The approach outlined in steps 3 and 4 was used successfully by San Mateo County in 1974.)
5. Efforts are currently underway to determine the feasibility of controlling adult mosquitoes at their resting sites.
6. The susceptibility of the house mosquito to various insecticides will continue to be monitored.
7. More emphasis will be given to post-treatment inspections.

SUMMARY AND CONCLUSIONS

The history of chemicals applied to the house mosquito (*Culex pipiens*) in the Alameda and San Mateo County Mosquito Abatement Districts leaves little doubt that we are dealing with insecticide-induced resistance. Both Districts have made significant changes in their control strategies to adapt to the new limitations created by resistance. The new control programs reduce reliance upon chemicals through more selective treatment procedures and more emphasis on alternative approaches. Early indications from the 1974 control program of San Mateo County Mosquito Abatement District suggests this approach may provide significant savings of man-hours.

Other benefits should be derived by a reduction in handling hazards and lowering environmental contamination risks usually associated with the excessive use of chemicals.

These new programs are certainly not without risk. The programs assume that citizens will tolerate a somewhat higher level of the house mosquito than has prevailed in the past, at least during that period when the approved chemicals were highly effective. One should not conclude here that we are lowering standards of mosquito control. We are simply recognizing that in the past an unnecessarily high level of control was established through the use of effective and inexpensive chemicals.

The future of these programs may very well be determined by the effectiveness of public education within the Districts.

CULEX PIPIENS QUINQUEFASCIATUS

RESISTANCE TO CHLORPYRIFOS AND OTHER O. P. COMPOUNDS

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Insecticide resistance is no stranger to Kings Mosquito Abatement District. Only recently it was noted that *Culex pipiens quinquefasciatus* Say had also become resistant to organophosphates. Because of the large number of sources for this mosquito in Kings MAD it is of major concern to keep populations below nuisance levels. One of the major sources for these mosquitoes are dairy drains. The drains vary in size from a few hundred square feet to thousands of square feet and in depth from approximately 5 to 40 feet. Dairy drains contain a large amount of dispersed organic matter. Dursban® has been found to have long residual activity in polluted habitats (Schaefer and Dupras 1970). This long residual activity makes Dursban an excellent insecticide for mosquito larvae in dairy drains. In most areas Dursban treatments gave satisfactory control, but beginning in August 1974 a number of failures were noted in dairy drains. These failures became widespread as the season progressed. Light trap counts, for females of this species, continually increased from August until mid October 1974. Comparing this record with 1973 counts, Figure 1, it is apparent that control measures failed in 1974.

MATERIALS AND METHODS.—The larvae tested were from treated and untreated sources. The information presented here was based on sampled areas. Many more sources are suspected of having resistant populations. Methods employed in the surveillance program have been described by Gillies and Womeldorf (1968). I was unable to maintain a constant 70-75° for 24 hours as is suggested. Temperatures ranged from approximately 70°F ± 10°F. Twenty-five larvae were picked at random from cups and identified to species. All sources showed 25 out of 25 *Culex pipiens quinquefasciatus* except where noted.

RESULTS.—Table 1 lists the data for each source tested by chemical, LC_{50}, LC_{90}, LC_{90}/LC_{50} ratio, and LC_{90} resistant strain/average LC_{90} susceptible strain. Except for Fresno susceptible strain all populations tested are arranged according to increasing LC_{90} level. Source number "1" was treated once in 1974 at the rate of .2 lb fenthion/acre by fixed wing aircraft. An adjacent alfalfa field was sprayed five times in 1974 at the same rate. Spray records for 1972 and 1973 show similar applications of fenthion compared to 1974. Apparently fenthion applications had little effect, if

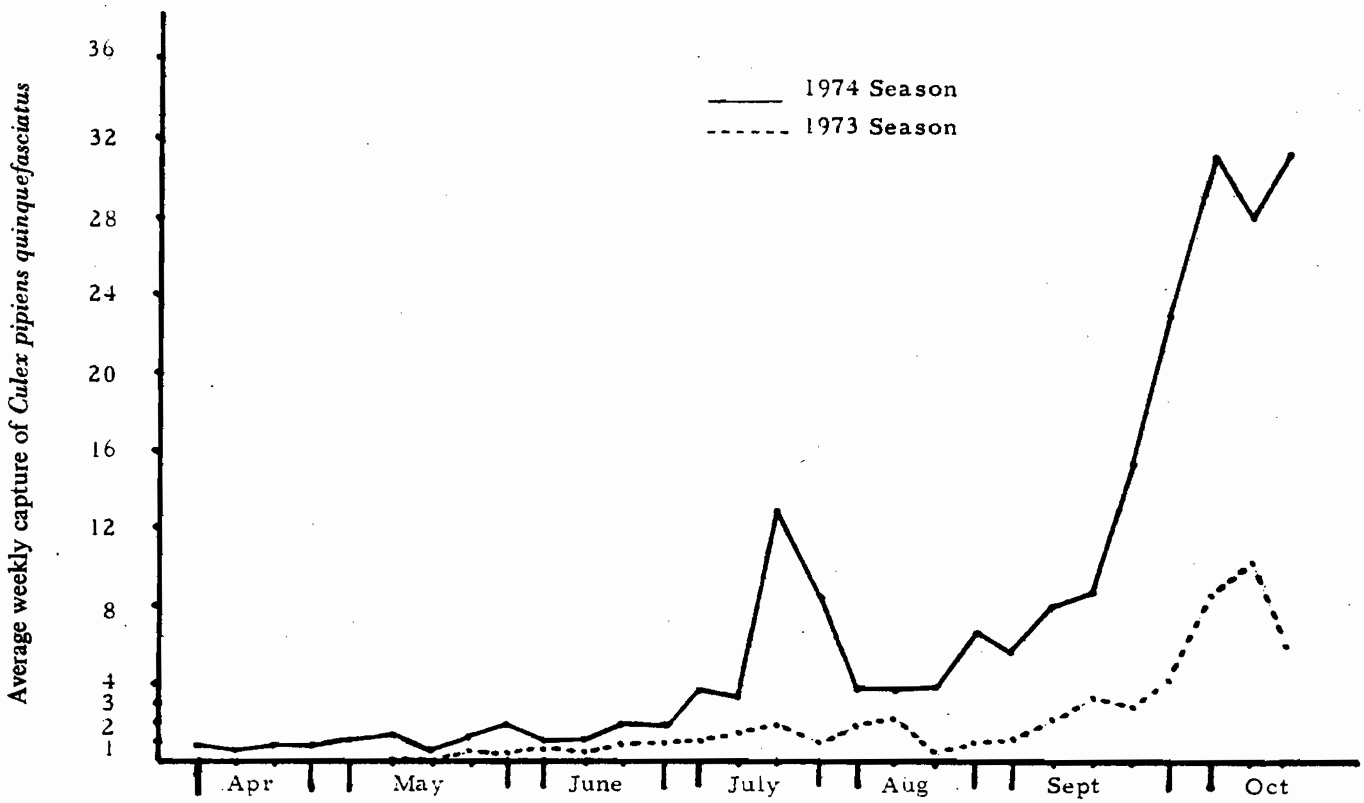

Figure 1.—Average weekly capture of *Culex pipiens quinquefasciatus* females from 15 New Jersey light traps for 1973 and 1974.

Table 1.—Comparison of organophosphorus levels for field-collected larvae of *Culex pipiens quinquefasciatus* with susceptible lab strains.

Source		Chlorpyrifos LC_{50} in ppm	LC_{90} in ppm	LC_{90}/LC_{50}
Fresno Strain	10-30-67	.0008	.001	1.25
Fresno Strain	5- 8-68	.00065	.00092	1.41
Fresno Strain	4-14-69	.0009	.0012	1.33
Fresno Strain	99-16-69	.00052	.00066	1.25
Fresno Strain	1- 7-75	.00098	.0013	1.33
		average LC_{50}	average LC_{90}	average LC_{90}/LC_{50}
		.00075	.0010	1.31

Source	Chlorpyrifos LC_{50} in ppm	LC_{90} in ppm	LC_{90}/LC_{50}	Resistance Ratio (R/S)[a]
1. Toledo Dairy Drain	.0005	.0014	2.8	1.4
2. Pink House Septic System	.00076	.0015	1.97	1.5
3. Spatford Septic System	.00062	.003	4.84	3.0
4. Middleton and Malone Catch Basin	.0014	.0043	3.07	4.3
5. G. Avila Dairy Drain	.0018	.009	5.00	9.0
6. J. Caetano Dairy Drain	.0045	.01	2.22	10.0
7. V. Bettencourt Dairy Drain	.0022	.012	5.45	12.0
8. Giacomazzi Dairy Drain	.0066	.013	1.97	13.0
9. John Avila Dairy Drain	.010	.014	1.40	14.0
10. J. Oliveira Dairy Drain	.0020	.015	7.5	15.0
11. F. Bader Dairy Drain	.013	.017	1.31	17.0
12. Knudson Dairy Drain	.007	.035	5.00	35.0
13. Peoples Ditch-Hanford	.012	.036	3.00	36.0
14. Paulo Dairy Drain	.01	.047	4.70	47.0
15. Camara Dairy Drain	.034	.082	2.42	82.0
16. Lawrence Dairy Drain	.0063	.097	15.3	97.0
17. A. Silva Dairy Drain	.0016	.14	87.5	140.0
18. Bezerra Dairy Drain	.020	.160	9.0	160.0

[a] LC_{90} R-strain/Average LC_{90} S-strain.

any, on the mosquito larvae to develop cross resistance to Dursban. Source number "2" and "3" are septic tank systems that have never been treated with organophosphates. The Spatford septic tank, source number "3", is adjacent to cotton fields. These fields were sprayed twice with Thimet® 10G, organophosphate, at the rate of 3 lb/acre in April and September, before the resistance test was made in November. The three fold resistance level to Dursban is slight but it could be due in part to the Thimet applications. Source number "4" is a typical catch basin that had been sprayed with 1% Dursban 20-30 granules every two weeks for the entire summer. No larval development was noted prior to testing in November. Spraying stopped in early October. Source number "5" is a dairy drain located near peach and nectarine trees. The dairy drain itself wasn't sprayed this year and only twice last year with .2 lb fenthion/acre. Numerous applications of parathion, Diazinon, Dibrom® and Lannate® were applied within a two mile radius of this dairy drain in 1974. The resistance ratio of nine could be due to these applications of organophosphates but in my testing I noted 8% *Culex tarsalis* Coquillett. It is my opinion that this latter condition brought about the level of resistance noted, since *Culex tarsalis* populations have been resistant to Dursban in Kings MAD for several years. I will have to test this source more extensively in order to make a better judgement of resistance.

Sources "6" through "18" were treated an average of five times with 8 to 60 oz. Dursban EC (4 lb AI/gal) per source. Dursban concentrate was poured into one part of source and allowed to spread throughout the volume of water. Two of these sources, "12" and "15", were checked for agricultural chemical applications. Source number "12" had 93 applications of organophosphates and carbamates within a 2 mile radius, prior to resistant test. Source number "15" had 137 applications of the same classes of insecticides within the same radius. Due to the variety of insecticides used in and around sources "6" through "18" it is difficult to determine if cross-resistance to Dursban is occurring. Figure 2 shows the geographical distribution of sources tested.

Using the interpretation of resistance for *Aedes nigromaculis* Ludlow (Gillies, Womeldorf and White 1968) the LC_{90}/LC_{50} ratios can be explained for *Culex pipiens quinquefasciatus*. Homogeneous susceptible populations generally have an LC_{90} approaching but not exceeding 2 times the LC_{50}. As the proportion of more resistant individuals in the population increases, the LC_{90}/LC_{50} ratio increases to greater than 2. When resistance is well established the LC_{90}/LC_{50} ratio again approaches 2 due to the elimination of the more susceptible individuals from the population. This shows that source number "2", Table 1, is a homogenous susceptible population. Sources "8", "9", and "11" are homogenous resistant populations. Thus the majority of populations tested are heterogenous resistant populations and have not as yet reached the homogenous resistant level.

Fenthion is rarely used for control of *Culex pipiens quinquefasciatus* larvae in Kings MAD. Because of this I listed only three tested sources. Source number "1" was treated four times in 1974 with fenthion .2 lb/acre, prior to testing. Sources "2" and "3" were never treated with fenthion in 1974. Source number "2" was treated five times with Dursban EC (4 lb AI/gal). Four applications of 30 oz.

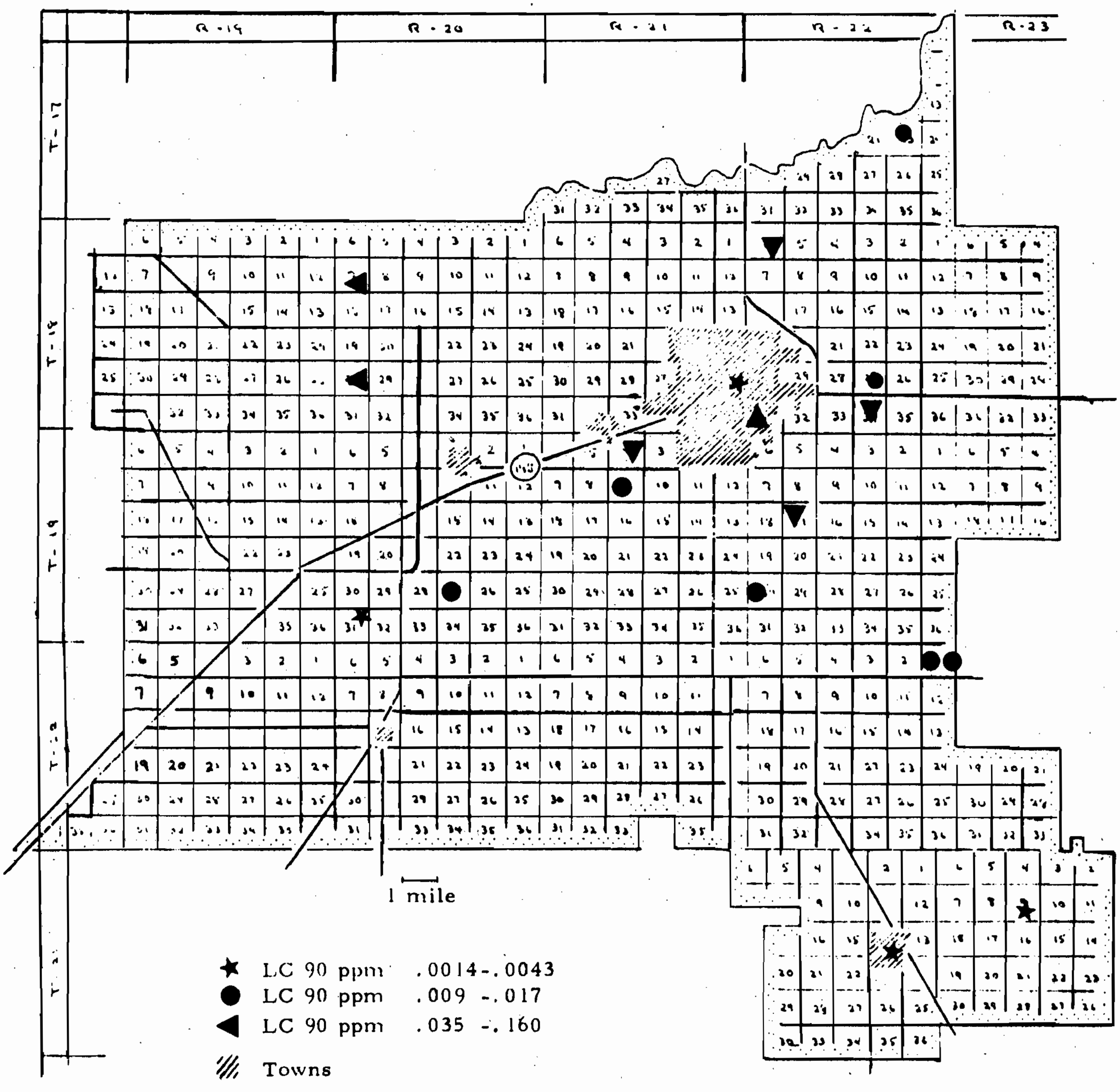

Figure 2.–Distribution of sources tested by LC_{90} ppm.

and one application of 60 oz. were applied prior to testing. Source number "3" was treated three times at an average application of 13 oz. A moderate resistance level for parathion, methyl parathion or fenthion has been determined to be .01 ppm (Gillies et al 1974). The LC_{50} levels, Table 2, show .47 to 7.4 times this level.

DISCUSSION.–Oils were sprayed in dairy drains, when resistance was noted. Unless some other insecticide is found to replace Dursban, oils will have to be substituted for Dursban applications in the future. Unfortunately oils have little residual activity. This means dairy drains will have to be treated almost weekly instead of every three or four weeks as was the case with Dursban concentrate.

Catch basins produce large numbers of *Culex pipiens quinquefasciatus.* Dursban 1% sand granules, 20 - 30 mesh, are sprayed into these potential sources every two weeks between March and October. I checked hundreds of these, in 1974, for failures to Dursban. After finding only one or two failures I wondered why resistance had not developed like it had in the dairy drains. Through the water department in Hanford, I learned that the average catch basin holds 50 gallons of water. By calculating the application rate in pounds of actual concentrate, .000625 lbs/catch basin, I determined that the theoretical initial concentration was 1.49 ppm. This concentration is far above the resistance levels in Kings MAD for this mosquito and insecticide.

ACKNOWLEDGMENTS

I would like to thank the personnel of Kings MAD for informing me of failures and collecting larvae. I especially appreciate the help of Patricia A. Gillies, California Department of Health, Vector Control Section, Fresno.

Table 2.–Comparison of organophoshorus levels for field-collected larvae of *Culex pipiens quinquefasciatus.*

Source	Fenthion		
	LC_{50}	LC_{90}	LC_{90}/LC_{50}
1. T. Avila Slough	.0047	.018	3.8
2. Knudson Dairy Drain	.074	.097	1.31
3. A. Silva Dairy Drain	.074	.15	2.03

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EVIDENCE OF ORGANOPHOSPHORUS MULTIRESISTANCE IN *CULEX PIPIENS QUINQUEFASCIATUS* AND *CULEX PIPIENS PIPIENS* IN CALIFORNIA

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Of major interest during 1974 has been the emergence of organophosphorus multiresistence in *Culex pipiens quinquefasciatus (= fatigans)* in the San Joaquin Valley. Because of the grave significance of this phenomenon we have devoted a large share of our time and resources to its investigation. Below we describe briefly the results obtained.

a. Spectrum of resistance.–Bioassay data on two strains (Camara and Knudsen) collected from dairy drains near Hanford, California, in September, 1974, indicate the presence of resistance involving the main organophosphates (OP) used up to now in mosquito control. Especially high resistance, computed at LC_{50} levels, was found at Camara for chlorpyrifos (52.2x), fenthion (48.9x), Abate (116.7x), methyl parathion (24x) malathion (16.4x) and parathion (12.9x) (Figures 1-3). Resistance extends to related materials by virtue of cross resistance as indicated by a 12.4x increase in LC_{50} for fenitrothion, a compound structurally related to methyl parathion, and an 83.3x increase for chlorpyrifos-methyl. Neither of these has been employed for mosquito or agricultural pest control in the area. Thus the population has developed a true case of OP multiresistance involving compounds of diverse chemical configuration.

As an adjunct to this study we also investigated a report of possible OP resistance in *Culex pipiens pipiens* in San Mateo and Redwood City (Figures 1-3). We have found that resistance in these populations is at present limited to fenthion at modest levels of 8.3x-13.2x. However, low levels of tolerance are also evident toward the other common OP's tested, and thus future trends in susceptibility of these populations must be closely monitored.

b. Precipitating factors.–There could be no doubt that the development of multiresistance is the consequence of prolonged and intensive selection by the multitude of chemicals used in mosquito control. Data on insecticide usage against mosquitoes in California, summarized in Tables 1-3, indicate that high levels of resistance are found toward insecticides used in the largest quantities, i.e. parathion, malathion, fenthion and methyl parathion. Indirect selection by agricultural insecticides is also implicated in resistance: data assembled from computerized records available at the University of California, Davis, indicate that twice as large a quantity of OP's was applied within a 3.4-mile radius from Camara as from Knudsen, consistent with the detection of higher resistance at Camara. At the specific source of each collection, we have found that the breeding sites had been treated during 1973 and 1974 with 4 to 8 applications of chlorpyrifos per year, utilizing a dose of approximately 1 lb. active ingredient per acre, i.e. nearly 10 times the normal rate. From the information summarized above, we conclude that the observed multiresistance contains the ingredients of long-standing selection by a variety of insecticides applied directly and indirectly against the population, climaxed by the specific pressure exercised at the core of the population by the repeated application of chlorpyrifos.

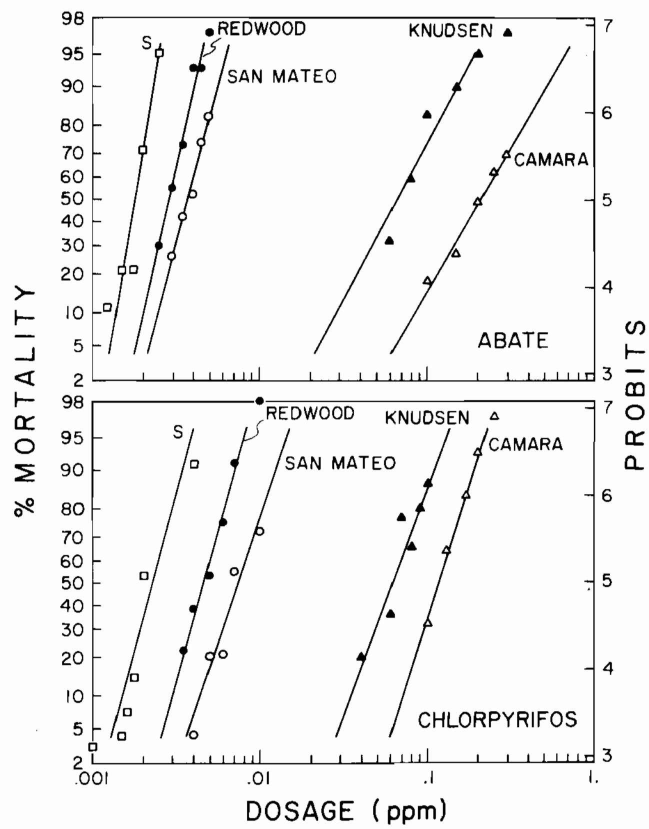

Figure 1.–Relative susceptibility to Abate and chlorpyrifos in *C. p. quinquefasciatus* (S. Knudsen and Camara strains) and *C. p. pipiens* (San Mateo, Redwood City strains) in California, 1974.

c. The role of synergists.–In order to gain an insight into the mechanisms of resistance a number of OP's were tested with the synergists piperonyl butoxide (p.b.), S,S,S,-tributyl phosphorotrithioate (DEF) and triphenyl phosphate (TPP). P.b. exhibited practically no synergism (or antagonism) indicating that mixed function oxidase (MFO) enzymes are not involved in resistance. In contrast, DEF exhibited a remarkable degree of synergism of OP's in the R strain resulting in almost complete elimination of resistance. In all cases, DEF restored the toxicity of the insecticide to near the level found in the S strain. This effect was most pronounced in the case of chlorpyrifos. These results, in addition to suggesting that hydrolytic esterases (inhibited by DEF) are responsible for resistance, provide clues to possible countermeasures against the R strain.

d. Alternative chemicals against resistant *C. quinquefasciatus*.–Because of the pressing need to identify substi-

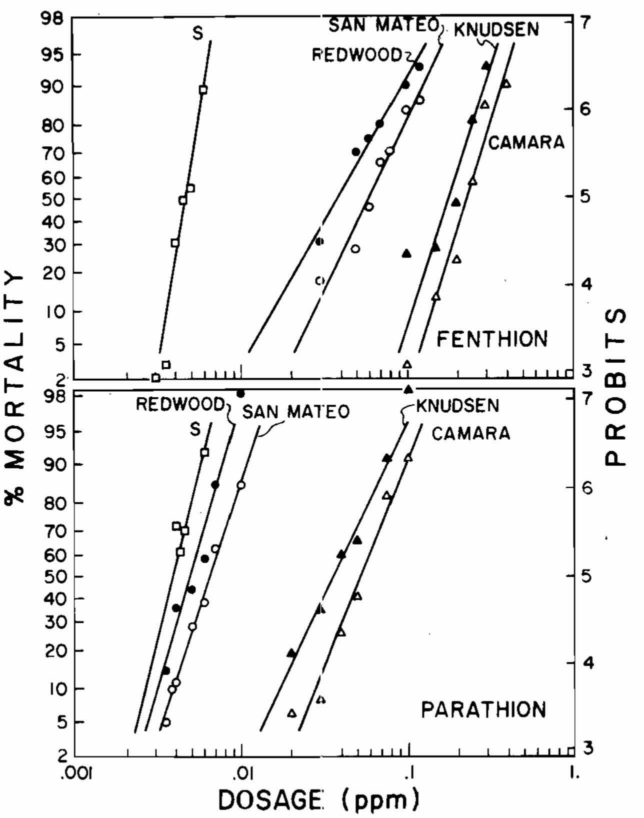

Figure 2.—Relative susceptibility to fenthion and parathion in *C. p. quinquefasciatus* (S, Knudsen and Camara strains) and *C. p. pipiens* (San Mateo, Redwood City strains) in California, 1974.

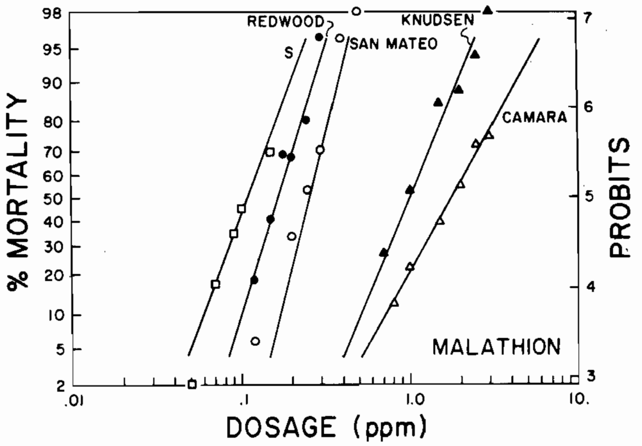

Figure 3.—Relative susceptibility to malathion in *C. p. quinquefasciatus* (S, Knudsen and Camara strains) and *C. p. pipiens* (San Mateo, Redwood City strains) in California, 1974.

tute chemicals for possible use against the resistant population, several experimental compounds available under the WHO Testing Program, representing OP's, carbamates, organochlorines, pyrethroids and IGR's, were tested.

The results indicate that the broad spectrum of OP resistance discussed above extends also at relatively high levels to new experimental OP's as Chlorphoxim, Cyanox and Celamerck S-2957. Compound Ciba-Geigy 18809 is suspected to act as a carbamate after cleavage of the phosphate moiety, thus demonstrating a relatively low resistance ratio of 3.6x. Similarly, OMS 1653 and 1657, two N-sulfenylated derivatives of the carbamates carbofuran and propoxur, re-

Table 1.—Relative usage of various insecticides against *Culex* spp. mosquitoes in the Central Valley of California during 1960-69.[a]

	Percent Within Year Indicated									
Insecticide	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969
Organochlorine										
DDT & DDD	42.9	30.1	24.4	14.3	3.7	3.7	0.3	0.3	1.0	0.4
Perthane		9.8	11.5	7.2	6.9	12.9	13.6	23.0	9.2	16.0
Thiocyanate										
Lethane									9.2	3.5
Organophosphate										
Malathion	40.1	47.6	37.3	46.1	58.3	47.1	37.6	9.2	5.0	5.7
Parathion	10.5	15.8	21.9	28.1	26.1	26.8	20.7	21.2	18.7	15.2
Methyl parathion	6.4	4.5	4.2	2.8	3.4	5.2	15.2	24.4	19.5	31.6
Fenthion	0.1	0.2	0.6	1.4	1.4	4.0	12.0	21.0	26.5	25.6
Other			0.1	0.1	0.3	0.3	0.6	0.9	10.9	1.8
Carbamate										
Propoxur										0.2
Total amount (Kilos x 1000)	54.4	50.1	50.0	37.2	42.1	32.8	27.7	24.4	21.1	28.7
Oils (gals x 1000)	193.9	180.4	180.7	124.1	145.2	109.4	122.4	110.9	96.6	45.8

[a]Mainly *Culex fatigans* and *C. tarsalis*. Data from 19 Mosquito Abatement Districts responding to survey questionnaire.

Table 2.—Relative usage of various insecticides against *Aedes* spp. mosquitoes in the Central Valley of California during 1960-69[a].

	Percent Within Year Indicated									
Insecticide	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969
Organochlorine										
DDT & DDD	3.6	3.6	2.5	2.1	0.4	0.1			0.1	0.1
Perthane		0.9			0.4	0.3				0.3
Thiocyanate										
Lethane										0.4
Organophosphate										
Malathion	42.1	36.8	33.0	39.6	45.7	32.5	25.5	8.1	6.5	4.1
Parathion	50.3	58.2	63.4	53.5	46.0	40.0	20.4	15.2	10.7	9.9
Methyl parathion	3.9		0.4	2.1	3.2	6.4	23.9	31.1	22.8	13.1
Fenthion	0.1	0.5	0.7	2.7	4.3	20.5	29.6	45.0	52.1	65.5
Other						0.2	0.6	0.6	7.8	3.3
Carbamate										
Propoxur										3.3
Total amount (Kilos x 1000)	40.1	35.3	41.7	36.6	39.8	40.4	38.5	35.5	32.7	41.3
Oils (gals x 1000)	58.2	59.9	49.3	58.5	46.1	28.1	25.1	21.6	21.1	9.0

[a]Mainly *Aedes nigromaculis.* Data from 19 Mosquito Abatement Districts responding to survey questionnaire.

Table 3.—Relative usage of various insecticides against mosquitoes (all species) in California during 1968-73.[a]

	Percent Within Year Indicated					
Insecticide	1968	1969	1970	1971	1972	1973
Organochlorine						
DDT, Perthane	0.3	5.2	0.8	0.1		
Thiocyanate						
Lethane	3.8	1.5	0.9	0.8	1.0	3.1
Pyrethrins			0.1		0.2	0.1
Organophosphate						
Malathion	7.0	4.6	4.3	9.0	10.2	10.3
Parathion	17.1	15.9	29.9	22.3	17.0	15.5
Methyl parathion	20.9	22.6	11.7	4.7	10.6	7.2
Fenthion	43.2	44.9	45.2	49.9	46.6	45.9
Naled	3.8	0.5	0.5	1.0	1.1	2.9
EPN	3.2	0.6	1.5	0.4		
Dichlorvos	0.5	0.3	0.3	0.5	0.3	0.6
Abate	0.1	0.3	0.3	1.2	0.7	0.6
Chlorphyrifos	0.1	2.8	2.1	4.1	4.4	4.5
Carbamate						
Propoxur		0.8	2.4	6.0	7.9	9.2
Insect Growth Reg.						
Altosid						0.1
Total amount (Kilos x 1000)	83.1	108.2	90.6	53.5	41.1	49.5
Oils (gals x1000)	362.7	359.8	348.2	441.7	528.8	734.8

[a]Compiled from records in California Mosquito Control Association Yearbooks 1969-1974.

spectively, are nearly as toxic to the R as to the S strain. Sulfenylation of these carbamates has resulted in a remarkable increase in their larvicidal activity (approx. 26x over the parent compounds) thus placing them within the activity range of other effective larvicides.

The R strain was also found to be susceptible to OMS 1476, a biodegradable analog of DDT synthesized by R. L. Metcalf. Of considerable interest is the apparent absence of cross resistance to the synthetic pyrethroids cismethrin and biopermethrin. Unlike natural pyrethrins and related synthetic pyrethroids, biopermethrin is photostable and thus holds promise as a larvicide against R strains.

The IGR chemicals methoprene (= Altosid, a juvenile hormone mimic) and Dimilin (= TH-6040, an inhibitor of chitin synthesis) manifest outstanding activity against both S and R strains. However, the low level of cross resistance toward methoprene (4.9x) should be noted in view of the successful selection of *C. tarsalis* and *Musca domestica* in our laboratory for higher tolerance toward this compound.

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

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ABSTRACT

A simulated natural habitat was established in four large concrete basins (328,900 gallons total capacity when filled to a depth of 1.2 m) located at Concord, California. A total of 1,275 ripe female *Gambusia affinis* in addition to 7.8 lbs of immature and male fish was stocked between the months of May and August 1974. Water temperature ranged from 19.3°C to 26.8°C (mean 23.2°C) during these months. The fish were fed Purina Trout Chow size number 1 in a feeding schedule increasing from 0.5% of body weight/day in May to 2.3% in September (average 1.3% during the entire period). The feeding schedule supplemented the available natural food present in the basins. At the end of September the yield of *G. affinis* totaled 141 lbs (102,297 fish based upon an average of 725 fish/lb). The cost of rearing these fish based upon water and food amounted to $2.47/lb.

Concurrently with the culture program, laboratory experiments were conducted at various temperature and photoperiod ranges (12.0°C–29.5°C; 8-15 hours photoperiod) to determine the optimal breeding conditions for *G. affinis*. Dropped young were collected and counted from gravid females enclosed in breeding chambers under the various conditions. Numbers of young per female ranged from 16 to 73 with a mean of 42 (41.9) based upon 33 observations. Minimal breeding occurred at 12.8°C (12.8°C–14.5°C), but optimal results were obtained at from 21.2°C to 24.0°C. A photoperiod of 12 to 15 hours of artificial light (centered about 1200 hours) was optimal. Overcrowding increased mortality and disease incidence; approximately 20 adult fish/gallon was maximum for good breeding and disease prevention.

The success of the summer mass rearing program depends upon the capability of holding fish through the winter for the spring replenishment of breeding stock and to have fish available for field distribution prior to the summer production period. Preliminary findings from laboratory investigations indicate that temperatures of 12.8°C to 13.0°C in conjunction with a 6 hour photoperiod will produce the desired results. Although these data are based upon low numbers of fish (approximately 1,000), preparations are underway for conducting similar experiments with samples of 100,000 to 200,000 fish.

The utilization of warm water derived from the cooling systems at Pacific Gas and Electric Company installations and the development of an experimental, submerged, enclosed water heating system were described and discussed in relation to culture and overwinterholding of *Gambusia affinis*.

MOSQUITOFISH CULTURE AND EMPLOYMENT IN THE SUTTER-YUBA MOSQUITO ABATEMENT DISTRICT: A PROGRESS REPORT

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Rice is the major cash crop produced in this two-county District and about 100,000 acres are planted to rice annually. Blanket chemical control of the mosquitoes produced on this acreage has always been and will most likely continue to be too expensive for serious consideration. Physical control methods can be employed to modify only those mosquito sources in the seepage and drainage margins at the perimeters of the rice fields; so vast areas of standing water remain in the fields themselves which require some form of control methodology.

In addition to these mosquito sources are many other permanent and semipermanent waters where non-biological methods can't be justified with regard to the potential environmental degradation or to the exorbitant expenditure ncessary to chemically control mosquito production.

Sutter-Yuba Mosquito Abatement District has been actively engaged in using the mosquitofish, *Gambusia affinis* (Baird & Girard) in its overall control program since 1963. Most fish are captured in large numbers at numerous municipal sewage treatment facilities where they grow and reproduce rapidly in the nutrient-rich waters of those oxidation impoundments. Unfortunately, many of the fish in the Sacramento Valley impoundments are lost in the winter months due to anaerobic conditions which can develop. At higher elevations, more solar energy usually reaches the ponds during the same period and better overwintering survival is usually observed. As a result of the winter die-offs, not nearly enough fish can be captured at all our municipal sources to satisfy our rice stocking needs.

Mosquitofish were screened as biocontrol agents quite some time ago and after much careful consideration, which took into account economic, ecological and physical evaluation, our District now believes that this fish can provide a viable solution to the rice field mosquito problem. Some competent researchers have asserted that small and possibly endangered populations of native fish species were competitively replaced by the mosquitofish shortly after its introduction (Miller 1961, Myers 1965, and Minckley and Deacon 1968). However, in the Sacramento Valley, mosquitofish have been employed for mosquito abatement purposes since 1922 and any potential deleterious effects they may have exerted on indigenous fishes probably took place many years ago. This doesn't mean, however, that we or any other agency should ever consider stocking new sources with any biocontrol agent without adequate knowledge as to the potential ecological results. Our District has always tried to communicate and cooperate with resource agencies with regard to our biocontrol program.

Within the jurisdictional confines of our District, most of the source waters stocked with mosquitofish could be considered artificial in that they have been either constructed or extensively modified by man for domestic, recreational or agricultural purposes. Riparian and estuarine waters that may eventually receive our fish or their offspring usually contain many predatory species which undoubtedly prey upon these fish, so that few if any, are likely to survive to colonize new habitats where they could conceivably compete with endemic fish populations.

Stocking fish in rice is presently completed during the irrigation flooding of the fields in May and June. Fairly recent field experimentation has shown that 0.6 pounds of mosquitofish initially stocked per acre will reproduce to the extent that they and their offspring will be able, under typical circumstances, to consume most of the larval mosquitoes (Hoy, Kauffman and O'Berg 1972). In order to stock this District's rice almost 60,000 pounds of fish will be needed. To date, the District has been able to stock only about 10,000 acres of rice annually because of limited fish availability.

Two years ago, we tried to recapture mosquitofish from our rice fields during the fall draining prior to harvest and did plan to overwinter them for our use the following spring. This technique did not succeed, as the majority of the mosquitofish apparently resisted the water currents and were usually left high and dry in the drained rice field. Other fish species, notably carp, minnows and sunfish, were found in most recaptured fish stocks. This would indicate that all recaptured fish would have to be examined and separated before they could be placed in an overwintering site.

As a result of this experience, our District has been conducting research to learn how to best propagate and overwinter large numbers of fish in ponds of our own. Mosquitofish exhibit marked sexual dimorphism, cannibalism and numerous other unique behavioral characteristics that will probably require that cultural techniques and facilities be extensively altered from those employed in other warmwater fish culture operations. We must design and construct ponds that afford maximal protection for the offspring from their parents. We have constructed seventeen ponds that offer various degrees of shelter by way of broad littoral areas that become covered with emergent vegetation.

Winter observations have demonstrated that these fish prefer deeper water during periods of inclement weather, although they usually return to surface waters on clear, calm days. Therefore, our overwintering pond designs have a variety of depths, hopefully supplying a preferred habitat for all seasons. Many of the pond designs we have constructed thus far have been rectangular in shape and have sloped substrates to facilitate draining and harvest operations.

An attempt is being made to simulate Asian fish farms where flood irrigated croplands are employed to produce grains and fish. In these facilities, perimeter canals usually contain the adult brood stock and the flooded crop acreage serves as habitat for the fry and as a production area for forage organisms. Our modified version has channels with even greater depth for overwinter water temperature stability.

Phyto- and zooplankton production for forage purposes is presently stimulated through the use of granular inorganic fertilizers; but future cost considerations may require that we employ organic fertilizers, such as poultry manure. With respect to a supplemental feeding program, we have screened many different commercial food preparations for mosquitofish production. We are now formulating our own floating mash which contains primarily grain by-products, but has a protein content in excess of thirty percent and is only one-third to one-half as expensive to mill as the commercial feeds we evaluated. We believe that formulation refinements will result in an even better supplemental diet for our fish. To distribute this feed, which was formerly done manually, we have constructed our first mechanized broadcaster, which should reduce manpower costs significantly. Our research facility and pilot fish farm utilizes well water exclusively; but no cultural effluents requiring environmental agency monitoring result from this use as flow-through pond designs were not constructed.

In the summer of 1973, the District conducted a 120-day experiment at this facility. The purpose of the study was to quantify the beneficial effects of supplemental feeding and fertilization on fish yield.

Six ponds were incorporated in the study and each had either two or three rows of substrate ridges on which emergent vegetation was grown. All ponds were stocked with an initial fish density equal to 0.00083 pounds per cubic foot. This density converts to an average of approximately sixty pounds per surface acre. Two ponds, designated as experimental controls, did not receive fertilization or supplemental feeding treatments. Two more ponds received a supplemental food ration equal to ten percent of their initially stocked fish biomass, three times per week. The remaining two ponds received the same supplemental food ration, but in addition fertilizer treatments of 8-24-0 (N,P,K) inorganic granules were applied biweekly. Each treatment consisted of an application of fifty pounds per surface acre.

When harvest operations commenced in the fall, all fish were captured, weighed and replanted in other ponds not utilized in this study. Results of the experiment supported our experimental hypothesis that there would be a positive correlation demonstrated between the amount of supplemental treatment and the fish yield. Over the 120-day period, our control ponds provided a mean biomass increase equal to 290.8%. The two ponds receiving only supplemental feeding provided a mean biomass increase of 478.4%. The final two ponds, which received both supplemental food and fertilization treatments, provided a mean biomass increase of 587.9%. Cost evaluations for this study will be presented in the next paper given during this symposium.

Other fish-oriented studies are being conducted which deal with disease prevention and control, habitat analyses, water quality analyses, population dynamics methods, fish holding tank design, seine and trap design, fish-pesticide compatibility studies and transportation techniques and equipment design. One transportation technique the District has been investigating is that of aircraft delivery systems. Our preliminary experiments have demonstrated that mosquitofish can be successfully air-dropped into shallow rice fields with little mortality and fair accuracy. However, operational costs and logistics problems will have to be resolved if this technique is to be adopted by the District.

Experiments are being conducted with the use of pure compressed oxygen aeration systems in our transportation tanks. We have experienced problems with other aeration equipment, such as agitators and ordinary compressed air units. Medical oxygen bottles, regulators and other related equipment were recently purchased which now enable us to provide proper oxygen delivery flows resulting in dissolved oxygen saturation, regardless of the fish load being transported at the time.

Since not much oxygen is necessary to provide full dissolved oxygen saturation of the transport water, it must be circulated to effect maximum dispersal in the tank. If standard industrial airstones are used, strong currents usually develop which cause such strong circulating currents that fish fatigue can occur during trips of long duration. To circumvent this potential problem, a special elongated bubbler was constructed of porous plastic tubing.

Our first long distance run of approximately six hours with 100 pounds of fish demonstrated that this new system provides equal or increased survival when compared with the best existing aeration systems.

This report has presented a very brief view of one aspect of the biocontrol research work that is currently being investigated across the nation. However, the mosquitofish still needs much investigation before it can be used to its full capability as a mosquito control agent.

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ECONOMIC ANALYSES OF A MOSQUITOFISH PROGRAM

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We at the Sutter-Yuba Mosquito Abatement District are committed to the use of biological control agents and are expanding our biocontrol program to provide the enormous number of mosquitofish needed to satisfy our ultimate objective of planting fish in all of the rice grown in our bi-county District.

As a result, we have increased the operational scope of our fish rearing and holding facility in East Nicolaus and have actively investigated utilizing more out-of-District sources as potential mass rearing sites.

Subsidizing any type of control program is going to require the expenditure of funds, and to budget realistically, we have had to obtain cost data that would enable us to project the anticipated costs of implementing our biocontrol program.

To that end, we have completed three economic studies in the last two years which we feel provide us with needed baseline data. The data include one production study at our fish rearing and holding facility at East Nicolaus and two years of economic analyses of planting mosquitofish in our District rice.

As my colleague Bob Coykendall pointed out, the production study involved rearing mosquitofish under two different nutritional regimes, with the initial stocking rate remaining constant. The treatments selected for study and the results therefrom reflected that fish production was highest in those ponds receiving both food and fertilizer. The costs associated with that study revealed that the average cost of fish production in the first series (no food or fertilizer) was \$7.58 per pound; the second series (food only) was \$5.89 per pound; and the last series (food and fertilizer) was \$4.44 per pound. At a planting rate of 0.6 pounds per acre in rice (Hoy, Kauffman and O'Berg 1972), the cost would be \$2.66 per acre, but does not include the cost of planting the fish in the rice.

The figures at first glance appear to be inordinately high until you consider that they represent: 1) the annual fixed costs associated with the study including the amortized pond construction cost, lease cost, pond maintenance during the course of the study, etc.; and 2) the annual production costs such as food, fertilizer, electricity, travel, initial planting and harvesting (Meyer, Sneed and Eschmeyer 1973). In that light, the figures do not seem too unreasonable and will certainly be improved because of our new pond designs and an improvement in the quality of our present fish ration.

All our planting costs associated with rice have been extracted from two employee-reporting-forms that we devised specifically for data analysis. The first, a "Fish Collection Worksheet", is used to summarize physical and biological data from each separate seining endeavor, whether or not successful. The second is a "Biological Control Time-Sheet" that each District employee is required to complete each day he participates in any aspect of the biocontrol program. It provides us with an analysis of the employee's daily activities which are summarized in hours by various classifications such as travel, planting, seining, equipment preparation and cleanup, as well as the type of source that was planted.

The total planting program cost is computed from the respective or participating individual's wages per hour received in the applicable fiscal year, salary benefits calculated at 10% of gross wages and vehicle cost established at \$0.10 per mile in 1973 and \$0.12 per mile in 1974. I should also mention at this point that all our calculations including cost, were based on the surviving biomass planted and not the biomass actually seined at the harvest sites.

The dollar amount we spent in 1973 on our "rice" program totaled \$5,531.23. We planted 8,973.5 acres of rice with 4,374.4 pounds of fish for a mean planting rate of 0.49 pounds per acre. Our mean cost in 1973, therefore, was \$0.62 per acre (Table 1).

When we analyzed our 1974 planting data as we had our 1973 data, we learned that our total "rice" program cost amounted to \$3,275.00. Due to late spring rains and a concurrent reduction in the early availability of fish, we were only able to plant 6,002.8 acres of rice. The biomass planted in that acreage totaled 4,054.7 pounds and represented a mean stocking rate of 0.68 pounds per acre - up 38% from the preceding year. Our overall cost per acre last year was \$0.55. This represented an 11% reduction in cost from the 1973 figure. This reduction was attributed to better seining techniques, transportation and aeration devices that resulted in higher survival rates at the planting sites and the use of part-time labor in July, 1974. Based on the results of the 1973 data analysis previously outlined, we budgeted funds for the fiscal year 1974 – 1975 to hire a part-time crew to seine and plant fish in our District rice. Their sole responsibility was seining and planting and as a result became more proficient at their task. We have enough funds remaining from that budget item to hire another crew this year for our rice program. We expect that they will also achieve a high level of proficiency which should be reflected in our cost-per-acre analysis for 1975.

As our experience broadened in the logistics of rice field planting, we recognized the need to expand the number of out-of-District fish sources that could provide the additional biomass needed early in the year. We concluded that the benefits and/or justifications for establishing these additional sources, primarily oxidation lagoons, were two-fold. The first direct benefit would be to the respective sanitation districts, etc., by providing mosquito control in their aerobic ponds. The second benefit, relating only to our District, would be the creation of potential mass rearing and seining sites that could supplement our local sources.

The increasing importance of that particular type of source is borne out by the fact that two out-of-District oxidation sources provided 32.6% and 33.5% of the total biomass of mosquitofish planted in our District rice in 1973 and 1974, respectively. Consequently, we have already stocked or gone ahead with arrangements to stock seven additional sites this year. Data already accumulated provide

Table 1.—Cost and data comparison of planting mosquitofish in ricefields in the Sutter-Yuba Mosquito Abatement District – 1973 and 1974.

Data Summary	1973	1974	% increase/decrease
Cumulative total number of acres planted:	8973.5	6002.8	-33.1
Cumulative total number of pounds planted:	4374.4[a]	4054.7	- 7.3
Mean biomass planted per acre (pounds per acre):	0.488	0.676	+38.6
Cumulative total number of hrs. devoted to planting mosquitofish:	1100.3	682	-38.0
Total labor costs (wages only):	$4122.94	$2445.56	-40.7
Employee benefits costs (estimated at 10% of hourly wage):	$ 412.29	$ 244.56	
Cumulative total number of miles driven, chargeable to "Rice Program":	9960	4874	-51.1
Mileage costs ($0.10/mile in 1973 and $0.12/mile in 1974):	$ 996.00	$ 584.88	-41.3
Cumulative total all costs (wages, benefits and mileage):	$5531.23	$3275.00	-40.8
Mean cost per acre:	$ 0.616	$ 0.546	-11.5
Mean labor cost per hour (salaries and benefits):	$ 4.122	$ 3.945	- 4.3
Mean cost per hour (all costs):	$ 5.027	$ 4.802	- 4.5
Percent of total cost attributable to vehicles:	18.0	17.9	
Percent of total cost attributable to salaries and benefits:	82.0	82.1	

[a]The 1973 data were originally calculated in numbers rather than pounds; therefore, the 1973 total number of fish planted (2,187,210) was divided by an estimated 500 fish per pound to arrive at an estimated amount of biomass, in pounds, to compare to the 1974 data.

a good basis for calculating our projected costs of harvesting from distant sources. We will be using a cost factor of $0.55 per mile that includes salaries, benefits and vehicle cost for a four-man crew to arrive at a total cost per trip. From that, we can derive the number of pounds of fish we will have to return alive to the District to make the trip economically feasible. To make the cost projections valid, we will assume at this point that the catch per unit of effort will equal our present sources.

We expect that our future cost analyses will provide us with additional information that will: 1) document the favorable cost-benefit ratio of a biocontrol program compared to other control methods; 2) provide the necessary justification for increasing a biocontrol program budget; and 3) provide a more efficient expenditure of the tax dollar.

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PILOT PROGRAM FOR THE INTENSIVE CULTURE OF *GAMBUSIA AFFINIS* AND *TILAPIA ZILLII*: PART III – SECOND-YEAR OPERATIONS

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ABSTRACT

This paper reports upon a continuing project for the intensive culture of the mosquitofish *Gambusia affinis*. A description of the specialized facility and environment maintenance system was reported by Challet and Rohe (1974). The initial operations and production data for the first year were reported by Challet, Reynolds and Rohe (1974). Development of mass rearing techniques for *Tilapia zillii* were included in an original project as part of the Orange County Mosquito Abatement District program of developing improved methods of chironomid midge control (Legner and Medved 1972).

A description of fish rearing operations for 1974 includes production and stocking data for *Gambusia* and *Tilapia*. Operational costs are given, including ultility charges and other production costs for each species of fish. Vandalism protection and utility saving measures are described. Selection of alternate fish species to control chironomid midges in cement lined flowing channels and in still water percolation ponds is discussed. Future studies are suggested which may reduce cost and increase production of *Gambusia*, including solar heating, mechanical sorting of gravid females and a study of natural sunlight versus artificial light in propagation.

CALIFORNIA STATE DEPARTMENT OF FISH AND GAME POLICY ON THE USE OF NATIVE AND EXOTIC FISH AS BIOLOGICAL CONTROL AGENTS

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I thank the Association for this opportunity to explain the policy of the Department of Fish and Game regarding the use of fish for biological control. During the course of this presentation I shall discuss the following areas:

1. The policy of the Fish and Game Commission and the role of the Department of Fish and Game with respect to the use of fish for biological control.
2. The laws and regulations related to the importation and stocking of fish.
3. The rationale supporting the position taken by the Department relative to fish proposed for introduction.
4. The ways in which the Commission and the Department provide for the study of exotic and prohibited fishes that have potential as biological control agents.
5. The problems resulting from the introduction of undesirable animals into this State and into other parts of the country.

Requests to use native fishes for biological control normally pose few problems, and stocking permits are generally granted. However, when a fish native to one area of the State is proposed for use in another area, we must determine whether the proposed transplant will be compatible with our fisheries management programs.

Most fish proposed for biological control are nonresident, that is, they occur in another part of the United States or in another country. These are termed exotic. Because of the considerable interest in exotics, this presentation deals mainly with policy related to them.

The Department of Fish and Game is charged with the administration and enforcement of the Fish and Game Code. The Code contains laws relative to the use of fish for biological control, and directs the California Fish and Game Commission to establish regulations in this area. The Department of Fish and Game has the role of manager and protector of fish and wildlife resources. With reference to exotics, Commission policy is: "Prevent the introduction of exotic plants, fish, birds, or mammals which might prove harmful to existing species directly or indirectly". The term exotic refers to nonresident fish which have not been introduced successfully in the State so as to become a part of the resident population by natural propagation. All proposals to introduce exotic nonresident fish, birds, or mammals must be submitted to the Commission for approval.

From the Fish and Game Code:

"Section 6400. It is unlawful to place, plant, or cause to be placed or planted, in any of the waters of this State, any live fish, and fresh or salt water animal, or any aquatic plant, whether taken without or within the State, without first submitting it for inspection to, and securing the written permission of the department."

"Section 2150. The department in co-operation with the State Department of Agriculture may issue a written permit to import into or transport within this State any wild animal enumerated in or designated pursuant to Section 2118, upon determination that the animal is not detrimental or that no damage or detriment can be caused to agriculture, to native wildlife, or to the public health or safety, as a result of such importation or transportation."

From Title 14 of the California Administrative Code:

"Section 671.5 Release of Animals into the Wild. No person shall release into the wild state any bird or animal which is not native to California or which, in the opinion of the department, has not been successfully introduced prior to 1955, without receiving prior permission from the Fish and Game Commission to do so . . . "

"Section 226. Issuance of Permits to Private Persons to Plant Fish. (4) No species of fish may be planted in any water in which the planting of such fish is contrary to the fisheries management plans of the Department for that water or drainage, or in any water from which such fish might escape to other waters where such fish are not already present. All applicants shall be advised upon request of the said Departmental fisheries management plans."

Being a member of the Colorado River Wildlife Council, California must also abide by the policy and restrictions on exotic animal introduction adopted by this body. The Council is made up of states within the Colorado River drainage. One function of the Council is to prevent the establishment of undesirable species. No member state may introduce an exotic animal into the Colorado River drainage without prior approval of the other states.

You may wonder how we go about arriving at a position and subsequent recommendation to the Commission regarding a species proposed for introduction.

We begin with some very basic questions: Does the species have the capability to survive and reproduce in the wild? Will it have a detrimental impact on fish and wildlife resources? Will it be harmful to game fishes or native nongame fishes by preying on them or by competing with them directly or indirectly for food, spawning area, cover, etc.? Will it be harmful to agricultural crops? These are but a few of our considerations.

To answer these questions we gather as much objective information on the species as possible. In conjunction with an extensive literature review, we solicit information and opinions from members of the academic community, and from biologists within our Department and other agencies. It often becomes necessary to contact scientists in other states and in foreign countries. The results of these efforts usually take the form of a Department position report for presentation to the Commission and to other interested parties.

Our recommendations to the Commission vary with our findings. If the information at hand indicates that an exotic species will be harmful to fish and wildlife resources we recommend that it be prohibited in all or part of the State. A prohibited fish may not be imported, transported, or possessed, except by special permit for display at a public aquarium or for study at an institution of higher learning. When a prohibited fish has biological control potential we have recommended that the Commission permit its study at a college or university to develop nonreproducing individuals. Such a study is underway with the grass carp at the University of California, Davis. If this undertaking is successful, nonreproducing fish may be stocked only in numbers and locations as stipulated by permit from the Department.

If we find that an exotic fish poses little or apparently no threat we have recommended that a pilot study be permitted to assess its feasibility as a biological control agent. Such a study is being conducted with annual fishes by the Butte County Mosquito Abatement District.

Before a study permit is issued, an inspection is made of the study site to be certain that adequate safeguards are taken to prevent escape of the species. The terms of this type of permit are very stringent, especially when prohibited animals are involved.

The responsibility of the Department relating to the instructions of exotics is not taken lightly. There are many examples of what can happen when an undesirable exotic species is accidently or intentionally released into the wild. The starling, English sparrow, and the carp are examples of man-effected introductions that turned into widespread pests. Equally unpopular in various areas of the country are more than twenty-five different species of exotic fish that have established reproducing populations. Among these is the walking catfish. Many other exotic fish have been released into U.S. waters in recent years; however, evidence concerning natural reproduction has not yet been gathered. The grass carp, which may prove to be a greater pest than the common carp is included in this group.

Some scientists have described the impact of undesirable exotics on the natural environment as "biological pollution". Other types of pollution can be dealt with, but eradication of established exotic species is virtually impossible without causing serious harm to the ecosystem involved. The old adage of "an ounce of prevention is worth a pound of cure" is not entirely applicable; there is no cure if prevention doesn't work. We are finding this out in southern California where efforts to eradicate the prolific African clawed frog have been unsuccessful. This predacious animal is posing a serious threat to amphibians, game fishes, and native non-game fishes, so costly, time-consuming efforts will continue. This exotic animal, now prohibited, escaped from a tropical fish wholesaler's facility.

In summation: The Department of Fish and Game has a responsibility to prevent the introduction of species that might prove harmful to the fish and wildlife resources of California. However, we appreciate the need to explore the realm of biologically controlling aquatic nuisances, especially in light of the current limitations on the use of chemicals. Therefore, we will continue to support the use, or study for use, of fish for biological control purposes that will not pose a threat to the resources we are charged to protect.

TILAPIA AS BIOLOGICAL CONTROL AGENTS FOR AQUATIC WEEDS AND NOXIOUS AQUATIC INSECTS IN CALIFORNIA[1]

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INTRODUCTION.--When aquatic weeds occur in high densities, many problems and difficulties result. Aesthetic enjoyment and property values are diminished (Nichols 1974). Navigation channels and irrigation canals become blocked (Sculthorpe 1967). Large quantities of water are lost through evapotranspiration (Holm, Weldon and Blackburn 1969; Timmons 1960) and water for domestic and agricultural needs is diverted from recreational uses. Aquatic weeds compete directly with food crops such as rice, interfere with cultural activities, and lower crop production (D. E. Seaman, California Cooperative Rice Research Foundation, Inc., personal communication). In addition, aquatic weeds also provide a habitat and food source for some aquatic insects (Pennak 1953).

When the density of aquatic weeds reaches unacceptable proportions, various techniques are employed to reduce or eliminate the problem. The most common and widespread technique is by chemical control. Chemicals are easy to apply but expensive (Timmons 1966), and are frequently toxic to associated aquatic organisms as well as terrestrial organisms which use the water (Blackburn 1966). Often chemicals remain in the environment for long periods. As a result, the use of chemicals for weed control has been restricted in many areas.

Where aquatic weed control by chemicals is limited, mechanical control is often employed, but mechanical control of aquatic weeds is very costly and usually effective for only short periods (Nichols 1974). The Imperial Irrigation District (I.I.D.) in southern California, for instance, spends nearly half a million dollars annually for mechanical control of aquatic weeds in irrigation ditches (J. M. Sheldon, Water Manager, Imperial Irrigation District, Imperial, California personal communication). Aquatic weeds are removed with draglines, broken up by disking, and killed by dessication of the canals. When canals are dried, fish are also killed and tremendous quantities of water are wasted.

Aquatic weed control also can be accomplished by biological means. Many organisms have been suggested as potential biological control agents, but so far, herbivorous fish have shown the most promise (Holm, et al. 1969). Here in California, only two species of fish have been used for aquatic weed control, *Tilapia zillii* and the Mozambique mouthbrooder (*Tilapia mossambica*). Both are members of the Cichlidae family from East Africa. Most research in California has been with *T. zillii* in the heavily irrigated southern California desert and the major objective of this research is to evaluate the capability of *T. zillii* to control aquatic weeds in the irrigation systems.

RESULTS AND DISCUSSION.–To control aquatic weeds most effectively, the fish must be able to survive well in the irrigation systems and they must consume all species of weeds. If survival is poor, special management techniques must be employed, and if some weed species are ignored, then control would not be complete. Both experimental evidence and field observations have been used to evaluate these factors.

Food and Feeding--In one experiment, *T. zillii* were offered known quantities of different weed species, and the amount of weeds remaining after each feeding period was measured. The feeding rate for each weed species was determined and expressed as the grams of weeds eaten per gram of fish weight per 24 hours. When given a choice between Eurasian watermilfoil (*Myriophyllum specatum*) and sago pondweed (*Potomogeton pectinatus*), *T. zillii* consumed sago pondweed at a significantly greater rate (0.529 g/g fish weight/24 hr) than Eurasian watermilfoil (0.031 g/g fish weight/24 hr), which was nearly ignored (Table 1, unpublished data). If only Eurasian watermilfoil was available, it was consumed at a rate of 0.044 g/g fish weight/24 hr, and the fish lost weight. The feeding rate on southern naiad (*Najas quadalupensis*), however, was significantly greater than on sago pondweed when these were offered together (0.519 g/g fish weight/24 hr versus 0.016 g/g fish weight/24 hr, respectively). The weight of *Chara* sp. consumed per 24 hr was statistically similar to that of sago pondweed.

Table 1.–Feeding rate of adult *Tilapia zillii* on four species of aquatic weeds. *denotes significant differences in Student's T-test (P›0.05).

Feed Offered	Condition	Feeding rate (g of weed/g of fish/24 hr)
Sago pondweed *(Potamogeton pectinatus)*	Alone	0.324
Eurasian watermilfoil *(Myriophyllum spicatum)*	Alone	0.044
Sago pondweed	Together	0.529*
Eurasian watermilfoil		0.031
Sago pondweed	Together	0.016*
Southern naiad *(Najas quadalupensis)*		0.519
Sago pondweed	Together	0.227
Chara sp.		0.490

From the results of this experiment, I predicted that southern naiad, sago pondweed, and *Chara* sp. could be eliminated from an area by *T. zillii*, but Eurasian watermilfoil would be ignored. A monoculture of that species would result and the weed problem still exist. In a concurrent field experiment, however, *T. zillii* were stocked in a section of

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Table 2.—Characteristics of the weed population in Flax Canal, Imperial Irrigation District, 1974. Area above test section was cleared mechanically on approximately 15 October 1974.

	June 11, 1974				October 30, 1974			
Location	Percent coverage	Biomass (g/sq M)	Species present	Condition	Percent coverage	Biomass (g/sq M)	Species present	Condition
Above test location	17.9	1236	Eurasian water-milfoil	Young	29.1	2280	Erasian water-milfoil; sago pondweed; southern naiad	Lush
Test section	27.0	861	Eurasian water-milfoil; sago pondweed(trace)	Young	3.8	368	Eurasian water-milfoil	Poor
Below test section	8.2	299	Eurasian water-milfoil	Young, poor	22.3	1724	Eurasian water-milfoil; sago pondweed (trace); southern naiad (trace)	Lush (in parts)

the Flax Canal, I.I.D., at a density of 108.6 kg (4,740 fish) /ha (97 lb.[1,920 fish]/acre) on June 1, 1974. By mid-October 1974, the control section was cleared mechanically because the lush weed infestation which included all common weed species had restricted water flow to 20 cfs less than capacity (Table 2, unpublished data). At nearly the same time, however, very poor stands of Eurasian watermilfoil covered only 4% of the test section. Actually, weed control in the test section may have been effective as early as mid-August. Apparently, *T. zillii* can reduce or contain Eurasian watermilfoil in canals through constant "harassment" while foraging for available food material.

In another field experiment, a mixed population of *T. zillii* and *T. mossambica* effectively controlled filamentous algae and submerged aquatic weeds in a rice pond. Similarly, *T. mossambica* populations in 4 enclosures demonstrated their ability to control a dense stand of filamentous algae. The extent of immediate control was directly proportional to the fish density, but eventually, control was effective in all enclosures regardless of fish density. Other authors have reported similar results (Sills 1970; Swingle 1957; Shell 1962; Avault, Smitherman and Shell 1968).

Preliminary analyses of food habits based on stomach content observations indicate that *T. zillii* adults consume few aquatic invertebrates. These were probably incidental to weed ingestion. Juvenile *T. zillii* may be more omnivorous. Pelzman (1973) found limited evidence that mosquito and midge larvae were eaten by *T. zillii.* Legner and Medved (1972) (1973) reported that *T. zillii* and *T. mossambica* were effective in reducing mosquito and midge populations, mainly through the disruption of benthic areas while searching for food after weeds were consumed. In the Imperial Valley, I have also observed this habit of substrate disruption. It appears, therefore, that adult *Tilapia* are effective in reducing mosquito and midge populations indirectly by elimination of their habitat and by disruption of the substrate.

Temperature Tolerance--The ability of *T. zillii* to survive a wide range of water temperatures was observed experimentally in an environmentally controlled chamber (Table 3, unpublished data). There are few habitats in California with water too warm for *T. zillii* since water temperatures between 16° and 32°C are favorable for good living conditions. Spawning occurs when water temperatures exceed 20°C. When water temperatures decline to 13°C, however, *T. zillii* become letharigc and vulnerable to predation. Water temperatures less than 10°C are fatal to approximately half the population, and no *T. zillii* survive even brief exposure to water temperatures of 7.5°C. The lower lethal temperature tolerance level of *T. mossambica* is about 12°C (Hoover 1971; Legner and Medved 1973). Field observations support these data and few overwintering populations of *T. zillii* or *T. mossambica* occur in California. Thus, to be used effectively as biological control agents for aquatic weeds or aquatic insects, these *Tilapia* would normally have to be restocked annually, just as any other herbicide or insecticide would require regular application.

Political Restrictions--*T. zillii* cannot be introduced into the Colorado River drainage because of an interstate agreement with states bordering that river which prohibits uni-

Table 3.—Effects of water temperature on *T. zillii.*

Water Temperature		Response by *Tilapia zillii*
°C	°F	
40	104	Approximate upper lethal tolerance level
16 - 32	61 - 90	Favorable living conditions
>20	>68	Spawning
13 - 16	55 - 61	Reduced feeding
10 - 13	50 - 55	Lethargic - some deaths
10	50	Most deaths (vary with acclimatization, exposure time, and rate of change)
7.5	46	Lower lethal tolerance level

lateral faunal introductions. Pelzman (1973) recommended that *T. zillii* be prohibited from introduction into central and northern California because of fear of detrimental effects on established species. Subsequently, the introduction of *T. zillii* outside of the Imperial Irrigation District and the Coachella Valley County Water District has been closely regulated by the California Fish and Game Department. Consequently, the practical application of *Tilapia* for weed or insect control in California has not been extensive.

CONCLUSIONS.–*T. zillii* and *T. mossambica* are good potential biological herbicides. Their potential as biological insecticides, however, appears to be limited to the indirect effects of incidental ingestion of organisms, elimination of habitat, and disruption of the substrate. When these are considered together, however, *Tilapia* may be quite effective as a biological control agent for both aquatic weeds and aquatic insects. Intensive use, however, will be restricted largely to southern California, will require annual application, and will depend on the attitude of the State of California Fish and Game Department.

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THE USE OF FISH FOR THE BIOLOGICAL CONTROL OF AQUATIC VEGETATION

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INTRODUCTION.–Dense stands of submersed aquatic vegetation offer excellent conditions for mosquito infestations. They protect floating egg rafts by minimizing the agitating effects of wind and wave actions, and they reduce the velocity of flowing water which can result in stagnant areas on the water surface. Mosquito larvae also find protection from mosquitofish (*Gambusia affinis affinis* Baird and Girard) and other aquatic predators in dense vegetation (Goma 1966). Control of aquatic weed growth is important in mosquito abatement programs. Current control procedures include: chemical, mechanical and biological.

Chemical and Mechanical--Chemical and mechanical control measures are often physically or economically impractical. Both usually provide temporary relief and continuous or follow-up applications are often necessary. The use of many aquatic herbicides is currently subjected to severe restrictions in domestic and agricultural water systems. Their use is also discouraged by the recent scarcity of these herbicides on the market and the high cost of those that are available.

Biological Control--Biological control of aquatic weeds is being considered with increasing interest. Mechanical and chemical treatments were more desirable in the past because they achieved immediate results. However, due to the rising concern over contamination of water supplies and the rigid restrictions imposed upon pesticide use, more research is being conducted to find alternatives to chemical control. Certain plant pathogens (Yeo and Fisher 1970), snails (Seaman and Porterfield 1964), crayfish (Dean 1968), aquatic mammals (Gillespie 1962), competitive plants (Yeo and Fisher 1970), and fish (Sills 1970) have all been tested with varying degrees of success. Many of these organisms have limitations and presently fish and competitive plants possess the most potential for aquatic weed control in California. For the purposes of this paper, only herbivorous fishes will be discussed.

Phytophagous fishes show promise for broad spectrum aquatic weed control. In general, they are easily acclimated to different locations, and there are species which can occupy many aquatic situations. Species in the families Cichlidae and Cyprinidae are most often considered for control of aquatic plant growth. The Cichlids *Tilapia mossambica* Peters and *Tilapia zillii* Gerv. are used successfully in several states for weed control. They are tropical species and cannot survive for extended periods in water below 50°F. This factor limits their use in California where year-round weed control is desired. Both species are very prolific and often spawn several times during the summer period. Also, *Tilapia* are territorial and will aggressively defend their nesting sites, even against other species. Therefore, it is likely that their reproductive potential and territorial nature could be quite damaging to existing fish species. Current legislation in California restricts *Tilapia zillii* to the region south of the Tehachapi Mountains.

The common and Israeli strains of carp (*Cyprinus carpio* L.) and the white amur (*Ctenopharyngodon idella* Val.) are cyprinids which have been used for aquatic weed control extensively in Europe, Asia, and the United States. Carp reduce plant growth by uprooting vegetation while foraging in the bottom mud for insects. They also increase the turbidity by stirring up bottom deposits causing a reduction in the light penetration necessary for plant growth (Sills 1970). The Israeli strain is more herbivorous and does not roil the bottom as much as the European variety. Under certain environmental conditions about 450 pounds of fish per acre of either variety are necessary for adequate control of aquatic weed infestations. Much lower densities of carp have proved to be detrimental to native fish populations; therefore, they should not be used for weed control in areas where such pre-existing populations have recreational or esthetic value.

WHITE AMUR.–General Information--The white amur is a cyprinid endemic to rivers of northeastern China and southeastern Russia. Due to its rapid rate of growth, extensive consumption of higher aquatics, and wide range of temperature tolerance, this fish is potentially valuable in programs for management of aquatic weeds. It is currently used in eastern Europe, Russia, India, Malaysia, Japan, Taiwan, Israel and the United States (Greenfield 1971).

The amur was researched extensively in Alabama and Arkansas and it is now widely distributed throughout Arkansas. No detrimental effects have been noted when the amur was stocked with other fish species (Kilgen and Smitherman 1971). Areas choked with weeds often prevent access to fishing and make boat navigation impossible. Vegetation also can encroach into the spawning substrate of bass and other warmwater game fish which may result in lowered production. Utilization of the amur in such areas has actually improved fisheries by reducing noxious weed growth.

The white amur is almost exclusively herbivorous. For the first 2 days after hatching it feeds endogenously living off yolk supplies, but at the end of 4 days it feeds exogenously ingesting primarily phytoplankton. Feeding shifts to zooplankton with further growth. Macrophytic material is first eaten when the fish are 17 to 18 mm long, but chironomid larvae and other animal forms are predominant in the diet. The amurs are almost exclusively phytophagous at 30 mm and animal content in the diet is negligible (Michewicz, Sutton and Blackburn 1972). Thus, the amur ceases to compete directly with game fishes while at a fairly small size. In experiments conducted in Florida, amurs stocked in ponds devoid of vegetation lost weight despite an abundance of zooplankton, cladocera, copepoda, rotifera, and insect larvae (Michewicz, Sutton and Blackburn 1972).

Growth of the amur is related directly to the water temperature, length of the growing season, and amount of food available. Fish grow up to 6 pounds (2.7 kg) in one year in Alabama lakes (Greenfield 1971). In studies conducted at Davis, amurs stocked at an average weight of 25 g gained an average of 0.7 kg in 16 months.

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Amur feeding habits are directly affected by temperature. Feeding is very sporadic at temperatures below 12°C, but at temperatures above 20°C, intensive feeding occurs and food selectivity is minimized. The optimum temperature for feeding and growth of one-year-old fish is about 33°C (Opuszynski 1972). American elodea (*Elodea canadensis* Michx.), common coontail (*Ceratophyllum demersum* L.), sago pondweed (*Potamogeton pectinatus* L.), and Eurasian watermilfoil (*Myriophyllum spicatum* L.) are readily consumed by this fish (Verigin, Viet and Dong 1963). These species of weeds commonly infest California water systems. Filamentous algae, emersed vegetation and even riparian plant species are consumed when other more preferred species are not available (Opuszynski 1972). Hickling (1966) states that the amur digests only about 50% of the total amount of food ingested. Grazed material is cut into small fragments by the large set of finely serrated pharyngeal teeth that grind the foliage against a horny pad located on the roof of the pharynx. The shredded edges of the food material expose ruptured cells for digestion within the gut. Amurs do not possess cellulase, an enzyme which digests plant cell walls, nor do they seem to possess microflora in the digestive tract. Therefore, a very large amount of material must be ingested for the amur to assimilate adequate nutrients. The rest of the plant material passes out of the gut undigested. This finely ground material adds to the food chain of fish and other aquatic organisms.

The amur is prohibited in many states including California due to the apprehension that this fish, if released indiscriminately, may reproduce and establish itself permanently as a threat to existing fisheries. Natural spawning has only taken place in four locations outside of its natural habitat: the Akungtein and Washantou reservoirs in Taiwan, Nivka Fish Farm in Russia, and the Tone River in Japan (Burress 1972). Environmental parameters reported to be requisites for successful natural spawning include: sand or gravel substrate, a water velocity between 3 to 6 feet per second (Greenfield 1971), a 2 to 4 foot rise in water level over a short period (Bailey 1972), and water temperatures greater than 68°F (Fishery Ministry of USSR 1970). Chances of these requirements being satisfied in California water systems are very remote. This does not mean that precaution and care should not be implemented if this fish is to be released in the future, but rather, that chances of an environmental mishap occuring due to accidental release of this fish are minimal.

When on a proper diet white amurs mature in four years in Arkansas (Bailey and Boyd 1970), and it is probable that maturation would be similar in California. Eggs can be successfully hand-stripped from these fish following hormone injections. Eggs obtained and fertilized artificially exhibit high viability and large numbers of fry can be produced with minimal manpower and equipment. Hence, propagation of this fish for weed-choked areas would be economically feasible. However, before any stocking can be carried out in California, further research must be performed to determine the environmental impact the amur may have on aquatic communities.

Past Research on the Amur at Davis--In May of 1973, 248 one-year-old amurs were stocked in a 1.5 acre reservoir. Eurasian watermilfoil was very dense in ¼ of the total area and was the dominant plant species. Horned pondweed (*Zannichellia palustris* L.) and curlyleaf pondweed (*Potamogeton crispus* L.) were also present but comprised less than 1% of the total plant biomass. Throughout the summer of 1973 there was little evidence that grazing was effectively controlling the vegetation. The weeds died back the following winter and regrew in 1974. The new growth did not reach the surface but shoots could be seen in deep water. By mid-June the vegetation was grazed entirely except for sparse stubble that remained in the bottom mud.

Another smaller reservoir, with a surface area of 0.5 acre and a depth of 4 feet, was stocked with 86 one-year-old amurs in May of 1973. Eurasian watermilfoil was the dominant plant species. There was no evidence of grazing throughout 1973 nor by July of 1974, presumably because the shallow depth of this pond allowed greater solar absorption which enable the vegetation to survive the winter temperatures. This suggested that to obtain adequate control with amurs the vegetation must be eliminated (chemically, mechanically, or naturally) before the amurs are stocked. The fish can then prevent the formation of nuisance stands by grazing the young new growth. Communication with personnel in the Arkansas Department of Fish and Game substantiated the fact that this grazing characteristic was also observed in their studies (William Bailey personal communication). This condition of over-wintering aquatic vegetation does not occur in many areas in California.

In July of 1974, 20 two-year-old amurs were stocked in another 0.5 acre reservoir which had a perennial infestation of *Cladophora glomerata* (L.) Kutz. Large floating mats of the algae were present prior to stocking, and the amurs were observed feeding on the algae shortly after their introduction. The mats disappeared in 10 days and the reservoir remained clear during the rest of the summer when *Cladophora* infestations have been heaviest.

Current Research on the Amur at Davis--Non-reproducing amur populations must be developed and the effects of their introduction on the aquatic environment must be ascertained before stocking is possible in California waters. Both of these aspects are currently being researched at the Aquatic Pest Control Research Facility at the University of California at Davis.

A few of our river systems may offer conditions that approximate those required for the natural spawning of the amur. Although this possibility may be remote, precautions must be taken to insure that this fish does not establish itself in California waters. Our research involves both long-term and short-term studies to develop non-reproducing fish.

Tilapia zillii were used as test fish in place of the amur for the short-term experiment. *Tilapia* mature in three months and will spawn continually if the water temperature is suitable. It is possible to treat young sexually undifferentiated fry, raise them to maturity, and examine them histologically for destruction of the sex germ cells within a period of three to four months. It would take four years to perform similar work using the amur. We have treated groups of *Tilapia* with hormones, chemosterilants, and acute and chronic exposures of radiation. Results of the trials are not yet available.

A cooperative research program is being conducted with the University to develop a sterile or monosex population of amurs. This is a long-term project due to the slow development of this species. Varying doses of chemosterilants and testosterone hormone are being given to groups of amur fry in their food supply.

The influence of flowing water on amur stocking rates and the possible interactions between this species and game fish are two studies which are currently being conducted.

The different number of amurs necessary to control aquatic weed infestations has been reported by researchers in many parts of the world (Konradt 1968; Sills 1970; Singh et al. 1969). These rates were formulated for static systems and it is likely that adjustments may be necessary in flowing water. The metabolic rate of the fish and several physical characteristics of aquatic plants are altered in moving water and may directly affect fish stocking rates necessary to attain weed control. Aquatic plant infestations are common in flowing systems in California and a knowledge of the number of fish necessary to either reduce or eliminate these stands is essential.

In August of 1974 studies were begun in four outdoor experimental canals at the Aquatic Pest Control Research Laboratory. Flow velocities of 0, 0.7, and 1.1 feet per second were tested on groups of five two-year-old amurs in each canal. Weighed amounts of sago pondweed were planted in trays and placed in the canals. The amount of vegetation consumed by the fish was determined at the conclusion of each trial. The experiment will be completed in the spring (1975).

A study to determine the possible competition between amurs and a game fish the common bluegill (*Lepomis macrochirus* Raf.), was initiated during the fall of 1974. The bluegill was chosen because it is cosmopolitan in California water systems and due to its prolific nature, any effect of the amur on its reproduction can be clearly determined. Two 0.5 acre earth-lined ponds, which have been infested annually with aquatic weeds, were each divided into four fish-proof quadrants of equal surface area. Ten adult bluegills (80 per acre) and 0, 4, 8 or 16 (0, 32, 64 or 128 per acre, respectively) two-year-old amurs were released into each quadrant. Sills (1970) observed that 20 sixteen-inch amurs per acre partially controlled *Chara* and *Potamogeton* spp. and that 40 completely eliminated them. The increasing stocking rates were used to establish a gradient from zero to total control of the vegetation within the ponds. Reproduction and growth of the bluegills will be monitored to determine possible direct (predation or food competition) or indirect (removal of cover, exposure of food organisms, siltation of bluegill nests, etc.) interactions with the amur. Preliminary results will be forthcoming next fall and the termination date for the experiment is the fall of 1976.

Future Research at Davis--In the spring of 1975 we will attempt to develop an all-male population of amurs with which we may do controlled research outside of the facility. Bailey and Boyd (1970) state that three-year-old females have been artificially spawned in Arkansas. Males generally mature more rapidly than females (Hickling 1967) and it is very likely that a large portion of our three-year-old males will be mature. Mature males can be differentiated from females by their smaller size, larger pectoral fins and the presence of tubercles on the surface of the pectoral fins (Michewicz et al. 1972). Although these features can generally be discerned without the administration of pituitary hormone injections, we will inject all the fish in order to obtain sperm as positive proof of the sex.

We anticipate using these male fish to determine their effects on waterfowl habitat. Waterfowl utilize aquatic plants for food and elimination of vegetation or a shift in plant species could greatly affect this wildlife resource. Next summer we plan to introduce male amurs at varying stocking rates into isolated areas of waterfowl habitat. All vegetation within the sites, both submersed and emersed, will be mapped and monitored for disappearance and/or replacement. This project is in the preliminary stages of planning and final approval from California Department of Fish and Game Commission will be necessary before it can be initiated.

SUMMARY.–The white amur appears to be one of the most promising agents for the biological control of aquatic vegetation in water storage and transport systems in California. It has been reported that stocking rates of only 40 fish per acre can keep reservoirs totally clear of weeds. Once water systems are stocked, the cost of weed management should be minimal for a number of years. Before the amur can be stocked into California waters, a non-reproducing population must be developed and the impact of the fish on the aquatic environment must be assessed. These phases of research currently are being conducted by ARS and state personnel at the University of California at Davis.

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THE NEW LOOK IN MOSQUITO AND VIRUS SURVEILLANCE

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ABSTRACT

An organized system of larval and adult mosquito occurrence surveillance has been operating in its present form since 1953. The program emphasizes *Culex tarsalis*, the principal vector of western equine encephalitis and St. Louis encephalitis. Larval occurrence is reported to the California Department of Health during the spring and early summer months. Adult occurrence reporting begins some weeks later than larval occurrence reporting, and continues through the summer into early fall. Larval surveillance is based upon dipping and the American model light trap is the standard for the adult program. Larvae from natural, agricultural, industrial, and domestic sources are reported, but only the results obtained from the catches of 300-plus urban light traps are requested.

Both aspects of the mosquito surveillance system have suffered from a lack of continued direction. The Entomology Committee of the California Mosquito Control Association is presently studying ways to revise the larval program. Concurrently, the Vector Control Section is attempting to reorganize the adult program. For the immediate future, compliance with standards for light trap placement and operation is being investigated. Better coverage of the state is being encouraged. Under consideration as means of making the currently-available data more useful are: including the several hundred rural and suburban traps now operated statewide but not reported (defining their locations so as to distinguish them from the urban traps); extending the reporting season in Southern California; and providing better data handling. In the next few years, other sampling devices and methods may be evaluated and incorporated into the program as warranted.

The mosquito-borne virus surveillance system has operated in its present form since 1969. Mosquitoes collected with the CDC miniature light trap using CO_2 from dry ice as the primary attractant are sorted to species, pooled, and submitted to the Viral and Rickettsial Diseases Laboratory of the California Department of Health for analysis. Most of the field work is performed by Vector Control Section personnel, although some mosquito control agencies have provided a great deal of assistance. The present laboratory capability allows about 2000 pools to be processed annually. Currently being studied are means of making the results of the effort more useful. For the short term, timing and location sampling priorities are being established with the assistance of the University of California School of Public Health. For the future, possibilities are being investigated of including the data, together with the mosquito occurrence data, in a statistical system that will increase their productive value.

SURVEILLANCE FOR ARTHROPOD-BORNE VIRUSES AND DISEASE BY THE CALIFORNIA STATE DEPARTMENT OF HEALTH, 1974

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Surveillance for mosquito-borne encephalitis during 1974 again confirmed the persistance of western encephalitis (WEE) and St. Louis encephalitis (SLE) viruses in mosquitoes, but they were detected much less frequently than in previous survey years. Only 4 strains of WEE virus were isolated from sites in Riverside County, and only 2 strains of SLE virus from Imperial County. However, high isolation rates from selected foci in Imperial County were reported by Dr. Telford Work, UCLA. There were 29 strains of Turlock virus isolated from Colusa, Imperial, Kern, Placer, Riverside, San Bernardino, Shasta, Solano, and Sutter Counties, and from Mojave and Yuma Counties in Arizona. Nine strains of Hart Park virus were isolated from Kern, Placer, Solano, Stanislaus, Tehama and Yuba Counties. All viral isolates were from *Culex tarsalis* except for 2 isolates of Turlock virus from *Culex erythrothorax* (Table 1). A total of 67,299 mosquitoes in 1,690 pools was collected and tested from April 10 through November 22 from study sites in 26 California counties and from Mojave and Yuma Counties in Arizona (Tables 2-4). A limited budget dictated this smaller effort as compared with 1973, when 4,838 pools were tested (75 strains of SLE virus and 97 strains of WEE virus isolated) or 1972 when 6,336 pools were tested (64 strains of SLE virus and 42 strains of WEE virus isolated).

The apparently low level of WEE and SLE virus activity during 1974 was reflected in unusually low incidence of human and equine arbovirus disease. Although the usual extensive screening program of suspect encephalitis cases was carried out statewide (643 persons tested serologically for WEE and SLE by the Viral and Rickettsial Disease Laboratory and County Public Health Laboratories) (Table 5), for the first time since specific laboratory tests were developed in the 1940's and accurate records have been kept, there were no proved human cases of WEE or SLE acquired in California. One case of SLE was confirmed in an 87 year-old woman in Bakersfield, Kern County. She had arrived for a visit on August 23, having left her home in Alvin, Brazori County, Texas, on August 20. Mosquito exposure in Texas was her presumptive source of infection. Onset of illness was August 28, with fever, earache, and "cold" symptoms. She was hospitalized when she developed fever to 104°F, inability to walk, then coma. The cerebrospinal fluid had 68 cells (predominantly lymphocytes) and 70 mgm% protein. The complement-fixation test for SLE showed a rising antibody titer from 1:4 to 1:32. The indirect fluorescent antibody test showed SLE antibody titers of 1:256, and the hemagglutination-inhibition test showed titers of 1:640 in both the acute- and convalescent-phase sera. The mouse neutralization index was 2.8 in the acute-phase serum and 3.3 in the convalescent-phase serum. The patient recovered completely and returned home to Texas.

Only 2 cases of WEE in equines had laboratory confirmation during the year: a 6 year-old horse from Bakersfield, Kern County, with onset July 13 (presumptive positive); and a 1 year-old horse from Temecula, Riverside County, with onset August 31. There were 61 other suspect equine cases reported to the Department, but laboratory proof of the etiology could not be obtained (Table 6).

The other arbovirus disease of significance during 1974 was Colorado tick fever: 14 cases were confirmed in persons exposed to tick bite in Alpine, Lassen, Modoc, Mono, Placer, and Plumas Counties and in Colorado. This brings the total proved cases since record-keeping began in 1954 to 179. It is undoubtedly under-reported, and may be the commonest vector-borne infectious disease in California.

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Table 1.—Viral isolates from mosquito pools, by the Viral and Rickettsial Disease Laboratory, California State Department of Health, 1974.

Identifying Number	County	Place	Date Collected	Species	Number in Pool	Isolate
V5-5515	Imperial	Calexico	May 22	*C. tarsalis*	50	Turlock
V5-5536	Imperial	Westmoreland	May 22	*C. tarsalis*	50	Turlock
V5-9337	San Bernardino	Needles	May 31	*C. tarsalis*	50	Turlock
V4-1576	Kern	Bakersfield	July 3	*C. tarsalis*	50	Turlock
V2-2118	Sutter	Sutter	July 9	*C. tarsalis*	29	Turlock
V2-2119	Colusa	Colusa	July 9	*C. tarsalis*	50	Turlock
V5-5722	Imperial	Westmoreland	June 18	*C. tarsalis*	50	Turlock
V5-5724	Imperial	Westmoreland	June 18	*C. tarsalis*	50	SLE
V2-2171	Stanislaus	Newman	July 17	*C. tarsalis*	50	Hart Park
V2-2172	Stanislaus	Newman	July 17	*C. tarsalis*	50	Hart Park
V3-0014	Solano	Tremont cemetery	July 29	*C. tarsalis*	50	Turlock
V3-0005	Solano	Tremont cemetery	July 29	*C. tarsalis*	50	Hart Park
V3-0007	Solano	Tremont cemetery	July 29	*C. tarsalis*	50	Turlock
V5-7523	Riverside	Blythe	July 24-25	*C. tarsalis*	50	WEE
V5-7524	Riverside	Blythe	July 24-25	*C. tarsalis*	7	Turlock
V5-7525	Riverside	Blythe	July 24-25	*C. erythrothorax*	17	Turlock
V5-7540	Riverside	Blythe	July 24-25	*C. tarsalis*	6	Turlock
V5-7543	Riverside	Blythe	July 24-25	*C. erythrothorax*	50	Turlock
V5-7545	Riverside	Blythe	July 24-25	*C. tarsalis*	50	WEE
V5-7547	Riverside	Blythe	July 24-25	*C. tarsalis*	50	WEE
V5-7548	Riverside	Blythe	July 24-25	*C. tarsalis*	50	WEE
V5-9399	Mojave, Arizona	Bermuda City	June 26	*C. tarsalis*	50	Turlock
V5-5820	Imperial	Calexico	July 24	*C. tarsalis*	50	SLE
V5-5823	Imperial	Calexico	July 24	*C. tarsalis*	25	Turlock
V5-5833	Yuma, Arizona	Yuma	July 25	*C. tarsalis*	50	Turlock
V3-0018	Solano	Tremont cemetery	August 5	*C. tarsalis*	10	Hart Park
V5-5871	Yuma, Arizona	Yuma	July 25	*C. tarsalis*	50	Turlock
V5-5833	Imperial	Winterhaven	July 25	*C. tarsalis*	50	Turlock
V5-5892	Imperial	Winterhaven	July 25	*C. tarsalis*	50	Turlock
V3-0016B	Solano	Tremont cemetery	August 8	*C. tarsalis*	50	Turlock
V3-0020	Solano	Tremont cemetery	August 8	*C. tarsalis*	50	Hart Park
V2-2242	Placer	Lincoln	August 6	*C. tarsalis*	50	Turlock
V2-2255	Placer	Lincoln	August 7	*C. tarsalis*	53	Hart Park
V4-1592	Kern	Bakersfield	August 7	*C. tarsalis*	50	Turlock
V4-1594	Kern	Bakersfield	August 7	*C. tarsalis*	75	Turlock
V4-1597	Kern	Bakersfield	August 7	*C. tarsalis*	66	Hart Park
V4-1601	Kern	Maricopa	August 7	*C. tarsalis*	72	Turlock
V6-1503	Yuba	Marysville	August 12	*C. tarsalis*	19	Hart Park
V1-1134	Shasta	Anderson	August 7	*C. tarsalis*	50	Turlock
V6-1160	Yuma, Arizona	Yuma Test Station	August 5	*C. tarsalis*	50	Turlock
V6-1161	Yuma, Arizona	Yuma Test Station	August 5	*C. tarsalis*	50	Turlock
V1-1239	Tehama	Woodson Bridge	August 5	*C. tarsalis*	50	Hart Park
V5-9451	San Bernardino	Needles	September 26	*C. tarsalis*	50	Turlock
V5-8229	Riverside	Macca	October 14-15	*C. tarsalis*	50	Turlock

Table 2.—Number of mosquitoes (pools) tested, by species and month, by the Viral and Rickettsial Disease Laboratory, California State Department of Health, 1974.

Species	April	May	June	July	August	September	October	November	TOTALS
Culex									
tarsalis	1,090 (36)	11,312 (242)	7,494 (177)	15,277 (352)	12,869 (321)	5,826 (135)	1,335 (39)	54 (5)	55,257 (1,307)
erythro-thorax	779 (20)	1,565 (41)	1,366 (35)	1,381 (34)	1,277 (32)	454 (16)	746 (20)	104 (4)	7,672 (202)
peus	4 (1)	8 (3)	1 (1)		252 (13)	17 (2)			282 (20)
pipiens	3 (2)	405 (13)	43 (3)	25 (4)	28 (4)		6 (1)		510 (27)
Aedes									
melanimon					408 (9)				408 (9)
vexans			752 (17)		254 (7)				1,006 (24)
dorsalis	11 (4)	110 (8)	129 (9)	924 (26)	1 (1)				1,175 (48)
squamiger		2 (1)							2 (1)
Anopheles									
freeborni		53 (4)		8 (1)					61 (5)
occidentalis				50 (1)					50 (1)
franciscanus				2 (2)					2 (2)
punctipennis					140 (4)				140 (4)
Culiseta									
incidens	6 (2)	3 (3)							9 (5)
inornata	14 (7)	3 (3)		590 (16)		1 (1)	115 (7)		723 (34)
particeps		2 (1)							2 (1)
TOTALS	1,907 (72)	13,463 (319)	9,785 (242)	18,257 (436)	15,229 (391)	6,298 (154)	2,202 (67)	158 (9)	67,299 (1,690)

Table 3.–Number of mosquitoes (pools) tested, by county and month, by the Viral and Rickettsial Disease Laboratory, California State Department of Health, 1974.

County	April	May	June	July	August	September	October	November	TOTALS
Colusa				4,419 (94)					4,419 (94)
Contra Costa					513 (11)				513 (11)
Fresno				25 (1)		206 (4)			213 (5)
Imperial	588 (20)	7,428 (161)	2,465 (53)	1,094 (29)	296 (13)		171 (9)	26 (2)	12,068 (287)
Kern			41 (3)	548 (13)	1,483 (30)	2,461 (51)			4,533 (97)
Lake				70 (2)					70 (2)
Los Angeles				167 (5)	13 (4)	116 (4)			296 (13)
Madera				14 (2)		704 (14)			718 (16)
Merced					410 (9)				410 (9)
Orange				95 (2)	76 (4)	7 (1)			178 (7)
Placer				346 (9)	2,065 (46)				2,411 (55)
Riverside	35 (3)	489 (13)	243 (22)	749 (37)	289 (19)	929 (33)	1,039 (22)		3,773 (149)
Sacramento				53 (2)					53 (2)
San Bernardino	552 (18)	2,091 (48)	116 (7)		830 (22)	266 (8)	135 (10)		3,990 (113)
San Diego	533 (19)	450 (3)	634 (20)	476 (15)	526 (18)	301 (10)	550 (13)		3,470 (125)
San Joaquin			33 (2)	580 (13)					613 (15)
Shasta			36 (1)		1,194 (32)				1,230 (33)
Siskiyou				2,677 (65)					2,677 (65)
Solano				606 (13)	1,099 (25)				1,705 (38)
Stanislaus			314 (11)	1,118 (25)	333 (8)				1,765 (44)
Sutter				1,089 (24)	414 (10)				1,503 (34)
Tehama					1,568 (36)	1,193 (24)			2,761 (60)
Tulare			52 (3)	37 (1)	148 (4)				237 (8)
Ventura					282 (13)	9 (2)			291 (15)
Yolo				398 (9)	275 (8)				673 (17)
Yuba					261 (6)				261 (6)
Mojave, Arizona	50 (4)	752 (18)	1,444 (29)		306 (9)	106 (3)	144 (6)		2,802 (69)
Yuma, Arizona	149 (8)	2,253 (49)	4,407 (91)	3,696 (75)	2,848 (64)		163 (7)	132 (7)	13,648 (301)
TOTALS	1,907 (72)	13,463 (319)	9,785 (242)	18,257 (436)	15,229 (391)	6,298 (154)	2,202 (67)	158 (9)	67,299 (1,690)

Table 4.–Number of mosquitoes (pools) tested by the Viral and Rickettsial Disease Laboratory, California State Department of Health, by county and species, 1974.

County	*Culex*				*Aedes*				*Anopheles*				*Culiseta*			Totals
	tarsalis	*erythrothorax*	*peus*	*pipiens*	*melanimon*	*vexans*	*dorsalis*	*squamiger*	*freeborni*	*occidentalis*	*franciscanus*	*punctipennis*	*incidens*	*inornata*	*particeps*	
Colusa	4,419 (94)															4,419 (94)
Contra Costa	272 (6)				241 (5)											513 (11)
Fresno	231 (5)															231 (5)
Imperial	10,198 (228)	1,247 (31)		426 (15)			193 (10)							4 (3)		12,068 (287)
Kern	4,506 (96)			27 (1)												4,533 (97)
Lake	20 (1)									50 (1)						70 (2)
Los Angeles	30 (6)	266 (7)														296 (13)
Madera	710 (15)			8 (1)												718 (16)
Merced	410 (9)															410 (9)
Orange	78 (5)	100 (2)														178 (7)
Placer	2,411 (55)															2,411 (55)
Riverside	3,171 (101)	445 (20)	13 (3)	20 (6)		2 (2)	71 (12)				2 (2)			49 (3)		3,773 (149)
Sacramento	53 (2)															53 (2)
San Bernardino	3,822 (95)	138 (10)					8 (2)							22 (6)		3,990 (113)
San Diego	144 (27)	3,173 (74)	50 (6)	29 (4)			2 (1)	2 (1)	53 (4)				9 (5)	6 (2)	2 (1)	3,470 (125)
San Joaquin	613 (15)															613 (15)
Shasta	955 (23)	23 (1)	110 (5)			106 (3)						36 (1)				1,230 (33)
Siskiyou	1,181 (27)						898 (21)		8 (1)					590 (16)		2,677 (65)
Solano	1,705 (38)															1,705 (38)
Stanislaus	1,765 (44)															1,765 (44)
Sutter	1,503 (34)															1,503 (34)
Tehama	2,267 (46)		75 (3)		167 (4)	148 (4)						104 (3)				2,761 (60)
Tulare	237 (8)															237 (8)
Ventura	257 (12)		34 (3)													291 (15)
Yolo	673 (17)															673 (17)
Yuba	261 (6)															261 (6)
Mojave, Arizona	2,694 (62)	56 (3)					1 (1)							51 (3)		2,802 (69)
Yuma, Arizona	10,671 (230)	2,224 (54)				750 (15)	2 (1)							1 (1)		13,648 (301)
TOTALS	55,257 (1,307)	7,672 (202)	282 (20)	510 (27)	408 (9)	1,006 (24)	1,175 (48)	2 (1)	61 (5)	50 (1)	2 (2)	140 (4)	9 (5)	723 (34)	2 (1)	67,299 (1,690

Table 5.—Humans tested serologically for mosquito-borne arboviruses 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, California, 1974.

County	Total	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	unknown
Alameda	22	1	-	-	-	3	4	-	1	3	1	-	1	8
Butte	10	-	-	-	-	1	2	1	1	1	-	-	-	4
Colusa	1	-	-	-	-	-	-	-	-	-	-	1	-	-
Contra Costa	18	-	-	-	-	-	6	3	2	2	3	-	-	2
El Dorado	1	-	-	-	-	-	-	-	-	1	-	-	-	-
Fresno*	64	-	2	-	-	1	7	16	7	9	13	9	-	-
Imperial	1	-	-	-	-	-	-	-	-	1	-	-	-	-
Inyo	2	-	-	-	-	-	1	-	-	1	-	-	-	-
Kern	44	-	-	-	-	3	4	3	16	10	3	1	-	4
Kings	5	-	-	-	-	-	-	2	1	1	-	-	-	1
Lassen	2	-	-	-	-	-	-	-	1	1	-	-	-	-
Los Angeles**	58	-	1	-	-	1	-	12	13	8	12	5	5	1
Madera	2	-	-	-	-	-	1	-	-	-	-	-	-	1
Marin	12	-	-	-	-	1	2	2	-	3	1	-	-	3
Mariposa	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Mendocino	1	-	-	-	-	-	-	1	-	-	-	-	-	-
Merced	1	-	-	-	-	-	-	-	-	1	-	-	-	-
Monterey	7	-	-	-	-	-	1	2	3	-	1	-	-	-
Napa	5	-	-	-	-	-	1	1	-	-	-	-	-	3
Nevada	1	-	-	-	1	-	-	-	-	-	-	-	-	-
Placer	12	-	-	-	-	2	1	2	1	1	4	-	-	1
Orange***	10	-	-	1	1	1	1	3	1	1	1	-	-	-
Riverside	9	-	-	-	-	1	-	3	-	3	1	-	-	1
Sacramento****	36	-	-	-	1	-	3	4	12	4	4	7	1	-
San Bernardino	15	-	-	-	-	1	2	-	6	2	2	-	-	2
San Diego*****	69	-	-	-	2	14	7	11	13	7	10	1	-	4
San Francisco	50	-	-	-	1	8	6	6	11	10	3	2	-	3
San Joaquin	25	-	-	-	-	1	3	2	6	7	3	1	-	2
San Luis Obispo	2	-	-	-	-	-	-	-	-	1	-	-	-	1
San Mateo	41	-	-	-	-	5	5	9	12	4	5	-	-	1
Santa Barbara	25	-	-	-	-	-	2	9	2	5	7	-	-	-
Santa Clara	19	-	-	1	1	7	5	-	1	-	2	-	-	2
Santa Cruz	7	-	-	-	-	-	2	1	2	-	1	-	-	1
Shasta	3	-	-	-	-	-	-	-	-	1	1	-	-	1
Solano	10	-	-	-	-	2	3	3	-	1	1	-	-	-
Sonoma	13	-	-	-	-	1	2	2	3	1	-	-	-	4
Stanislaus	15	-	-	-	-	1	2	2	2	3	3	-	-	2
Sutter	4	-	-	-	1	-	-	-	1	-	-	-	-	2
Tehama	3	-	-	-	-	-	2	-	-	1	-	-	-	-
Tulare	4	-	-	-	-	-	-	-	1	1	1	-	-	1
Tuolumne	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Ventura	2	-	-	-	-	-	-	-	-	-	-	-	-	2
Yolo	9	-	-	-	-	2	1	2	1	2	-	-	-	1
Yuba	1	-	-	-	-	-	-	-	1	-	-	-	-	-
Total	643	1	3	2	8	56	76	102	121	97	83	27	7	60

* Tested by County Health Department Laboratory (includes 2 patients tested by State VRDL).
** Tested by County Health Department Laboratory (includes 2 patients tested by State VRDL).
*** Tested by County Health Department Laboratory (includes 1 patient tested by State VRDL).
**** Tested by County Health Department Laboratory (includes 2 patients tested by State VRDL).
***** Tested by County Health Department Laboratory (includes 1 patient tested by State VRDL).

Table 6.–Suspected clinical cases of arbovirus encephalitis in equines by county and month, for California 1974.

County	Month of Onset: Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Undetermined[a] or Not Tested	Totals[b]
Totals	4	1	0	2	6	6	11	11	5	5	1	3	8	63
Alameda	-	-	-	1	-	-	4	-	-	-	-	-	-	5
Butte	-	-	-	-	-	-	-	-	1	-	-	-	-	1
Fresno	-	-	-	-	-	-	1	1	-	1	-	1	1	5
Glenn	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Humboldt	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Imperial	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Kern	-	-	-	-	-	-	1[c]	1	-	-	-	-	-	2
Kings	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Los Angeles	1	-	-	-	-	-	-	-	1	1	-	-	1	4
Madera	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Mendocino	-	-	-	1	1	-	-	-	-	-	-	-	-	2
Merced	-	-	-	-	1	-	1	-	-	-	-	-	-	2
Monterey	-	-	-	-	-	-	-	1	-	-	-	1	-	2
Orange	-	1	-	-	-	-	-	-	-	-	-	-	-	1
Placer	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Riverside	-	-	-	-	1	1	-	1[d]	-	-	-	-	-	3
Sacramento	-	-	-	-	-	-	-	1	-	1	-	-	-	2
San Bernardino	1	-	-	-	-	1	-	1	-	-	-	-	1	4
San Diego	1	-	-	-	-	-	-	-	-	-	-	-	-	1
San Joaquin	-	-	-	-	-	-	1	1	-	-	-	-	-	2
San Luis Obispo	-	-	-	-	-	1	2	-	-	-	1	-	2	6
Santa Clara	1	-	-	-	-	-	-	-	-	1	-	-	-	2
Shasta	-	-	-	-	-	-	-	-	1	-	-	-	1	2
Solano	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Sonoma	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Tehama	-	-	-	-	-	-	-	1	-	-	-	-	1	2
Tulare	-	-	-	-	-	2	1	-	1	-	-	-	-	4
Tuolumne	-	-	-	-	-	-	-	-	1	-	-	-	-	1
Ventura	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Yolo	-	-	-	-	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 case of a presumptive positive WEE in a six-year-old pony from Kern County; onset July 13: hemagglutination test (HI) 1:320-1:640-1:320; neutralization test (NI) 5.5-6.2-6.4; indirect fluorescent antibody test (IFA) 1:128-1:128-1:128 (complement-fixation test nonspecific, unsatisfactory).

[d]One case from Riverside County confirmed serologically for WEE: a one-year-old colt with onset of disease on August 31, 1974, with no history of vaccination; rising CF antibody titers on blood specimens obtained on August 31, September 3 and September 23 were (1:8, 1:32 and 1:128 respectively; HAI titers 1:640 and 1:640 on 1st 2 samples; IFA titers 1:64, 1:256, and 1:1024; neutralization index titers 2.4, 3.2, and 5.4.

COMPARISON OF TWO CHLORPYRIFOS FORMULATIONS AND TWO ULV AEROSOL GENERATORS AGAINST CAGED MOSQUITOES

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ABSTRACT

Twenty-six ULV field tests were conducted at a military reservation in Sonoma, California to determine if a one pound per gallon chlorpyrifcs formulation dispersed at 8 ounces per minute was more effective than the technical grade material dispersed at 1-1/3 ounces per minute. Results indicate that both formulations were equal in effectiveness in achieving swath widths (?90% mortality) of 500 feet against caged *Culex pipiens* in daytime tests. Aerosol droplet size determinations by the gravitational settling techniques indicated the VMD for the 1-1/3 ounces per minute was 8.9 μm compared to 12.9 μm for the higher flow rate when dispersed from the Navy ULV converted cold fogger.

A second objective of the tests was to compare swath width efficacy of the Navy ULV converted military cold fogger to the Micro-Gen® LS2-15. Results for both formulations were essentially the same for both units.

NONTHERMAL AEROSOL APPLICATIONS OF CHLORPYRIFOS IN AN URBAN AREA

Kenneth R. Townzen[1] and Kenneth G. Whitesell[2]

INTRODUCTION.–The village of Colusa (population 3,825) has long been periodically infested with adult mosquitoes (*Anopheles freeborni* and *Culex tarsalis*) from neighboring extensive rice fields. In 1967, a large scale experimental study was made to evaluate wide swath, low-volume aerial larviciding with chlorpyrifos. The work was described in a series of papers by Womeldorf and Whitesell (1972); Akesson et al. (1972); Washino et al. (1972); and Atkins (1972). The applications killed larvae, but reinfestation from surrounding uncontrolled rice fields quickly replaced high adult populations in town. Therefore, a decision was made to try the increasingly popular nonthermal aerosol generator (coldfogger). Construction of the first "Colusa modified" low-volume, nonthermal aerosol machine was completed in 1970, and a second one was built during the winter of 1971 (Whitesell 1973). Experimental applications of synergized pyrethrins and chlorpyrifos were made to determine effective swath widths with various formulations. In 1972, experimental applications were conducted in the urban areas of Colusa (Womeldorf et al. 1973). The test results indicated that penetration of shrubbery and other obstacles could be obtained.

Based on the 1972 experience, modified operational applications were made in 1973 and 1974. Two applications were evaluated in 1973 and two in 1974. The results are presented in this paper.

METHODS AND MATERIALS.–Equipment.--Crosley engine-powered Roots and Schwitzer blowers equipped with Afa nozzles, with the orifices reduced to 1/32 inch (Whitesel 1973).

Chemical.--Dow Mosquito Fogging Concentrate® containing 6 lb/gal (719 gm/l) chlorpyrifos, was diluted with Occidental Chemical Super 94 Spray Oil® to 0.5 lb/gal (60 gm/l). The flow rate for each machine was 15 fl oz/min (440 ml/min) and the vehicle speed was 5 mph (8 km/hr). Each machine was calibrated before each operation.

Procedures.--A weather station set up at the Colusa County Airport two miles (3.2 km) south of town monitored air temperatures at 32.8 ft (10 meters) and 9.8 ft (3 meters) above ground, and wind velocity at 6.6 ft (2 meters above ground). The applications were made only when an inversion was apparent and wind velocity less than 6 mph (9.6 km/hr). If these conditions were not met, the application was cancelled. Inversion, satisfactory wind velocity, sunset, appearance of first *Anopheles* spp., and increased humidity normally occurred within a short period of time (Figure 1). Stability ratios (SR) as described by Christensen et al. (1972) were calculated for each test.

Three vehicle-mounted coldfoggers were used in the applications. Two were driven through the town and one through the outlying areas. The in-town vehicles were driven through adjacent streets and alleys in tandem. Total time to complete the applications in the town and outlying area was two hours and 30 minutes. The swath width in town was 165 ft (50.3 meters), determined by the street-alley spacing; no swath width determinations were made for the outlying areas. Evaluation was done in the urban areas, using caged *Culex pipiens quinquefasciatus* from a known susceptible laboratory colony in disposable cages (Townzen and Natvig 1973). These were placed in various locations (open and concealed) in two adjacent swaths to serve as air samplers. Figure 2 and Table 1 indicate the location and the types of sites. The caged mosquitoes were collected two to eight hours after the application. Mortality counts were made 12 hours and 24 or 36 hours after the application.

RESULTS AND DISCUSSION.–At the final count, 100% mortality was observed in all but six of the 71 test cages (Table 2). Mortalities of less than 100% were observed at two locations: one at ground level on the downwind side of a fence, and one in shrubbery on the downwind side of a house. The consistently high mortalities at the final count do not permit critical evaluation of mortality as a function of SR. Analysis of the mortality at 12 hours indicates a direct correlation between SR and percent kill. Average mortality per test charted against SR is shown in Figure 3.

CONCLUSIONS.–Penetration of shrubbery was evident in the four nonthermal aerosol applications of chlorpyrifos in the village of Colusa. Mortality of caged mosquitoes as a

Table 1.–Description of cage placement.

Station:	Site 1
1	High stake, open
2a	High stake, near fence
2b	Ground-level, against fence
3a	High stake, open
3b	Tree fork, with dense ivy
4	High stake, in shrubbery
5a	High stake, open
5b	Ground-level, open
Station:	**Site 2**
1a	High stake, near fence
1b	Ground-level, against fence
2	High stake, open
3a	High stake, open
3b	Ground-level, in groundcover
4	High stake, open
5b	Dense shrubbery
6a	High stake, open
6b	Dense Shrubbery against house
7a	High stake over shrubbery
7b	Dense shrubbery
check 1	- 2 miles up-wind, open.
check 2	- 2 miles up-wind, open.

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measurement of penetration appeared to be directly correlated with atmospheric stability expressed as the stability ratio. The SR may prove useful for determining when a coldfogger application will be successful. An SR greater than 1 appears necessary, and little difference in mortality can be expected beyond SR 2 or 3. SR, or a similar measurement, should be regarded as an important appraisal factor in determining when to make aerosol applications.

During the 1975 mosquito season, further tests are planned to ascertain the effectiveness of applications of chlorpyrifos at rates of 0.5 lb/gal (60 gm/l) and 0.25 lb/gal (30 gm/l) against naturally occurring populations when applied at various stability ratios. These tests may show whether applications of 0.25 lb/gal (30 gm/l) chlorpyrifos are effective when the SR is favorable.

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Table 2.–Observed mortality of caged *Culex pipiens quinquefasciatus*, expressed in percentages, following experimental nonthermal aerosol application of chlorpyrifos at Colusa, California. Date of application and stability ratio.

Site 1	8-29-73			9-12-73		7-1-74		9-12-74	
	.93			.42		.75		37.7	
Station	12 hr	36 hr		12 hr	24 hr	12 hr	24 hr	12 hr	24 hr
1	100			100		100		100	
2a	100			100		95	100	100	
2b	55	100		100		73	88	100	
3a	100			100		N.C.[1]		N.C.	
3b	100			100		89	100	100	
4	80	84		80	93	57	76	100	
5a	100			100		88	100	100	
5b	86	100		94	100	93	100	100	
Site 2									
1a	100			100		93	100	100	
1b	95	100		92	100	74	96	100	
2	100			100		100		100	
3a	100			100		100		100	
3b	71	100	100	100		100		N. C.	
4	100			100		100		100	
5a	100			100		92	100	100	
5b	N.C.			N.C.		53	93	100	
6a	100			100		100		100	
6b	96	100		92	100	83	100	100	
7a	N.C.			N.C.		100		100	
7b	N.C.			88	100	N.C.		100	
Check 1	13	19[2]		0	0	0	3	0	
Check 2	11	19		0	0	0	0	0	

[1]No cage set out, or destroyed.

[2]Corrections for the appreciable control mortalities have been made using Abbott's formula.

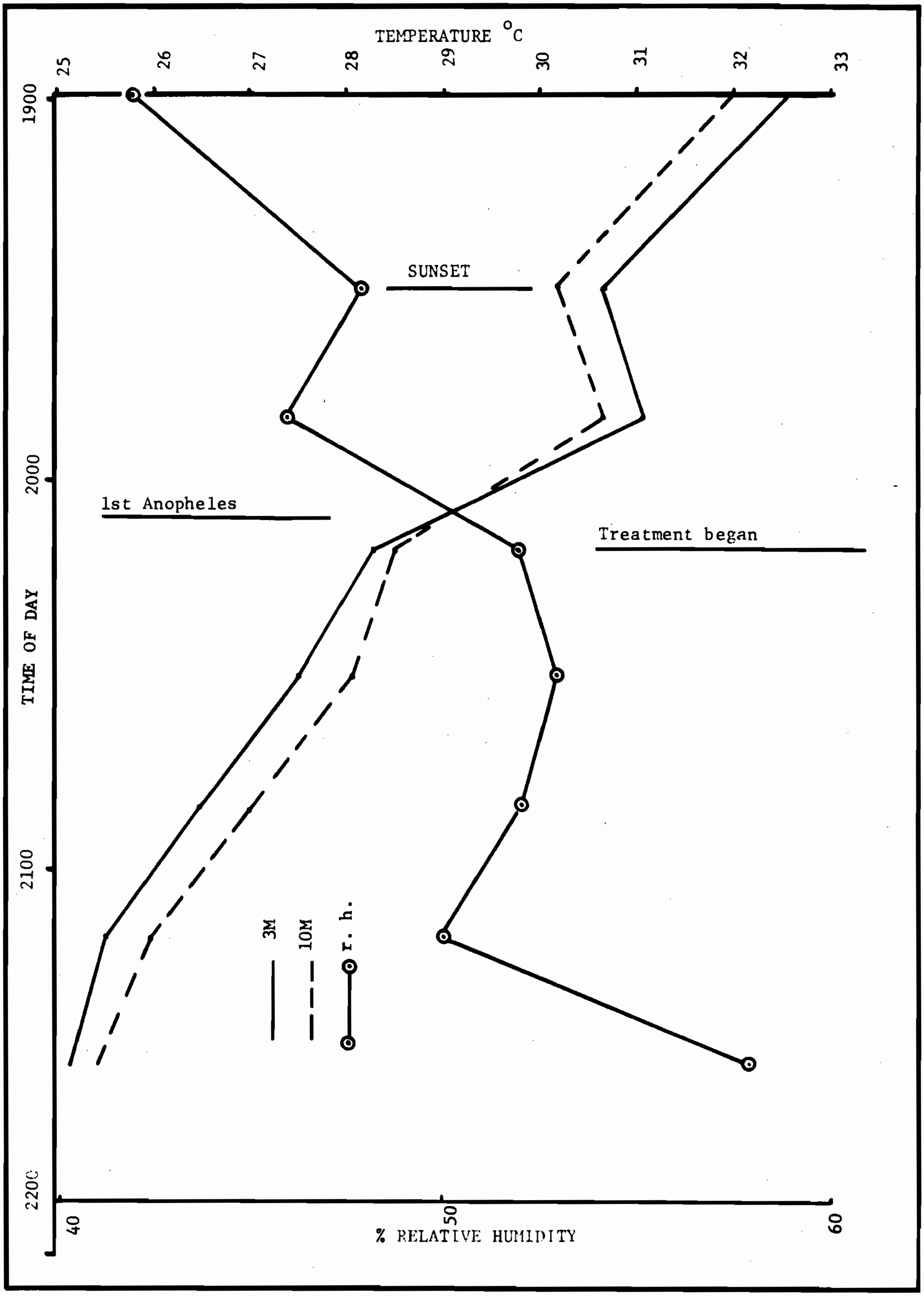

Figure 1.–Weather data, Colusa Airport, August 29, 1973.

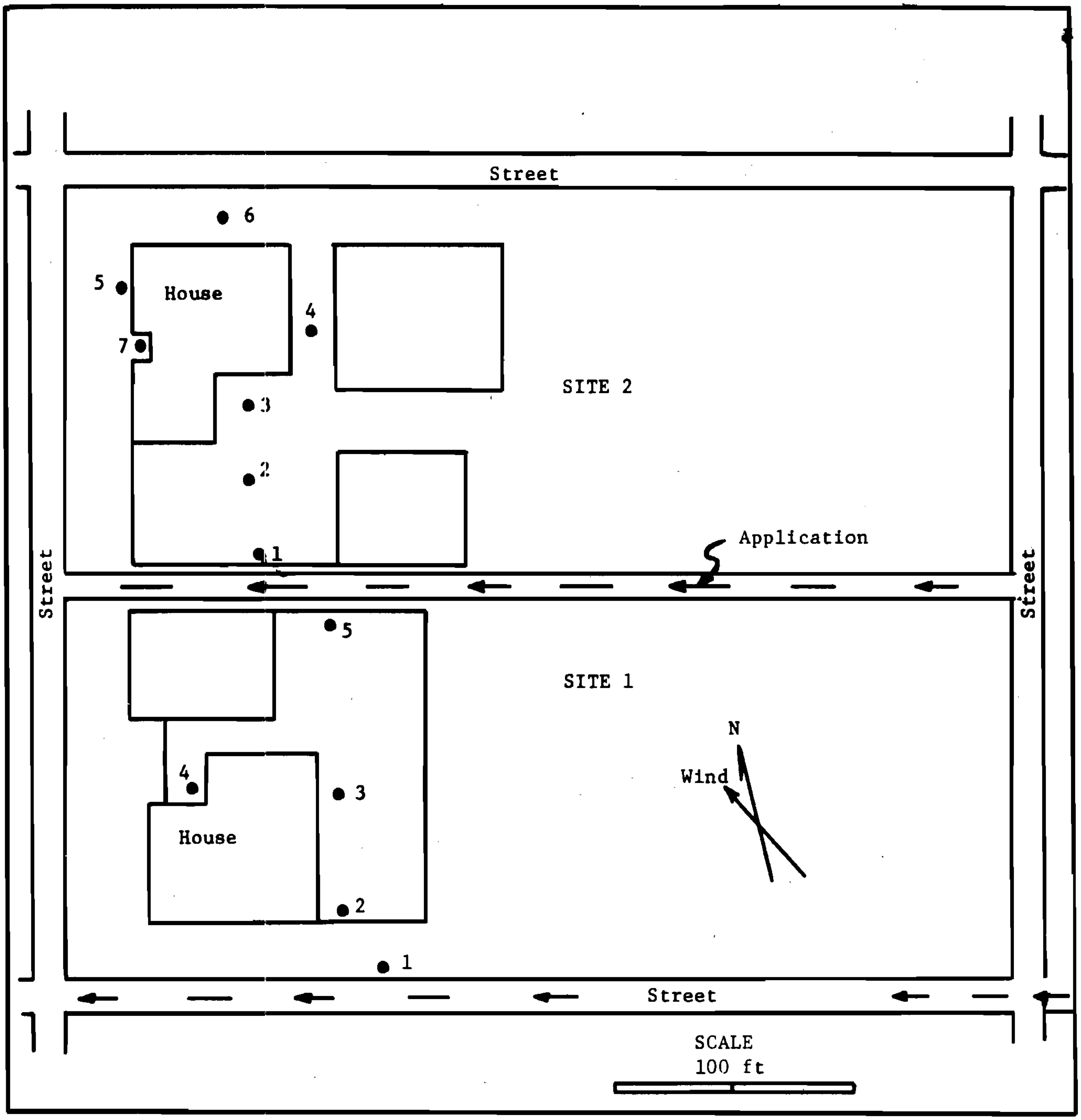

Figure 2.–Cage placement, Colusa coldfogger tests, Colusa, California.

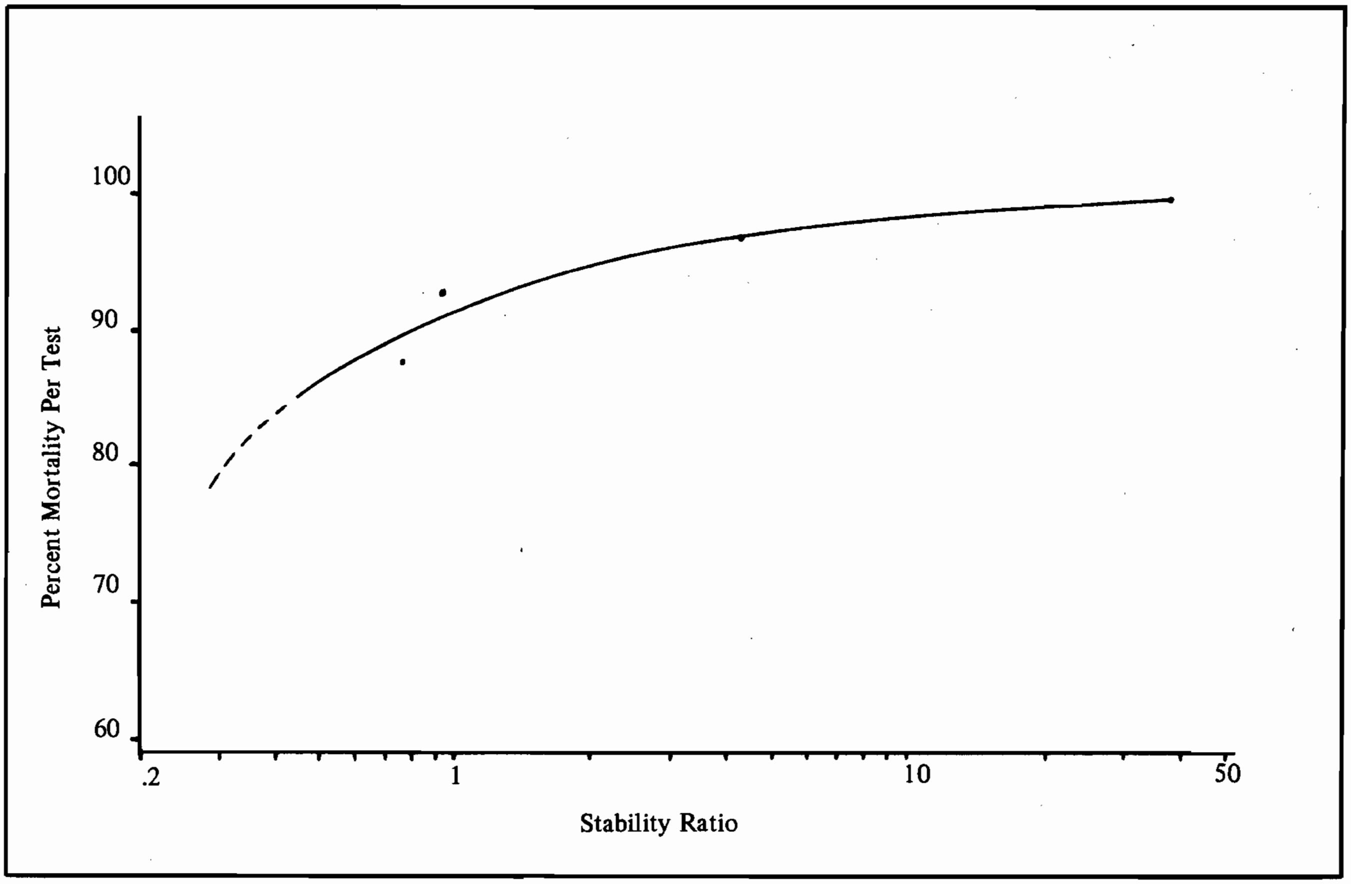

Figure 3.—Average mortality of mosquitoes in cages for each test, versus stability ratio.

STUDIES ON A NEW MOSQUITO LARVICIDE CELAMERCK S-2957

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ABSTRACT

Until OP multiresistance had appeared in *Culex pipiens quinquefasciatus* in September, 1974, a new organophosphate received through WHO [Celamerck S-2957, O-(2,5-dichloro-4-methylthiophenyl 0,0-diethyl phosphorothioate)] had shown good activity on all strains of this species. Because of its reputed prolonged stability, we carried out extensive tests on the persistence of the compound in the greenhouse, and also small-scale field trials at Riverside, Coachella Valley and San Joaquin Valley. The compound showed outstanding stability in water, exceeding that of chlorpyrifos, and good activity at 0.05 to 0.1 lb/A against non-multiresistant mosquitoes (*C. quinquefasciatus*, *C. peus*). It was, however, judged inadequate against *A. nigromaculis*.

OVERCROWDING FACTORS OF MOSQUITO LARVAE THEIR POTENTIAL FOR MOSQUITO CONTROL

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Overcrowding of mosquito larvae induces various responses resulting in retarded development, reduced size, and morphological aberrations in larvae, and mortality of the immature stages. During the course of investigations on the intra-specific competition of mosquito larvae, Moore and Fisher (1969) showed that *Aedes aegypti* (L.) produced a heat-stable, steam-distillable metabolite which increased the period of larval development. Independently, Ikeshoji (1965) showed that, by giving a limited amount of food to larvae of *Culex pipiens fatigans* Wiedemann, the amount of food provided and the larval density in the rearing units influenced the size of emerging adult mosquitoes and the number of follicles in the females. Subsequently, Ikeshoji and Mulla (1970 a,b) found that third-instar larvae of *Culex pipiens quinquefasciatus* Say, raised under overcrowded conditions with a larval density of 20-27 larvae/cm^2 or 5-7 larvae/ml elaborated chemical factors which were toxic to first-instar larvae and produced growth-retarding effects in younger larvae. The overcrowding factors of *C. p. quinquefasciatus* also showed considerable activity against first-instar larvae of *Culex tarsalis* Coquillett, *A. aegypti*, and *Anopheles albimanus* Wiedemann.

Isolation and identification studies on the overcrowding factors resulted in obtaining two active fractions of organic compounds from an overcrowded culture of larvae of *C. p. quinquefasciatus* (Ikeshoji and Mulla 1974a). The major fraction contained two straight-chain alkanes, heptadecane and octadecane, and two branched-chain alkanes, 7-methyl= octadecane and 8-methylnonadecane. The minor fraction consisted of 2-methyl- and 2-ethyl-substituted long-chain fatty acids, the detailed structures of which are still unknown due to the extremely minute quantities of the acids present in the overcrowded culture.

To confirm the larvicidal activity of substituted fatty acids, a number of branched-chain fatty acids with various structural types were evaluated against mosquito larvae (Ikeshoji and Mulla 1974b, Hwang et al. 1974a, Mulla 1974). It was found that branched-chain fatty acids, in general, exhibited good biological activity against mosquito larvae whereas straight-chain fatty acids showed little or no activity. For systematic studies on the structure-activity relationships of substituted fatty acids against mosquito larvae, 20 2-ethyl-, 2-butyl-, and 2-hexyl-substituted fatty acids were synthesized, and their activity against mosquito larvae was investigated (Hwang et al. 1974b, Hwang 1974). The level of biological activity of these acids was found to be dependent upon the total number of carbon atoms in the acids.

To facilitate studies on the effects of structural modifications on the activity of these branched-chain fatty acids, their methyl esters were synthesized and evaluated for their activity against first-instar larvae of *C. p. quinquefasciatus*.

Branched-chain alkanes were also found to be much more active than their straight-chain analogs (Ikeshoji and Mulla 1974a, Mulla 1974). To complement this finding, naturally occurring 7-methyloctadecane and 8-methylnonadecane and their structural isomers were synthesized and evaluated against first- and fourth-instar larvae of *C. p. quinquefasciatus*.

BRANCHED-CHAIN FATTY ACID METHYL ESTERS. —Methyl esters of 2-ethyl-, 2-butyl-, and 2-hexyl-substituted fatty acids were prepared by reacting these fatty acids with either diazomethane or 1-methyl-3-p-tolytriazene in ether. The crude methyl esters were purified by vacuum distillation and bioassayed. The 2-alkyl-substituted fatty acids used as starting materials for preparing the methyl esters consisted of seven 2-ethyl-, seven 2-butyl-, and six 2-hexyl-substituted fatty acids having even numbers of carbon atoms and a total number of carbon atoms from 12 to 24.

In comparing the biological activity of the branched-chain fatty acids with their methyl esters, it was found that, in every case, the esters were more active than their corresponding acids. The increase of activity by esterification was more distinct in the less active acids than in the more active acids.

Among methyl esters of 2-ethyl-substituted fatty acids, methyl 2-ethyloctadecanoate exhibited the greatest activity. By esterification, the activity of 2-ethyloctadecanoic acid increased from LC_{50} 2.2 to 1.5 ppm whereas the activity of 2-ethyleicosanoic acid increased from LC_{50} >25 to 10 ppm. Except for methyl 2-ethyldocosanoate, all methyl esters of 2-ethyl-substituted fatty acids tested showed good activity.

Methyl esters of 2-butyl-substituted fatty acids were also more active than their corresponding acids. Especially methyl 2-butyltetradecanoate, methyl 2-butylhexadecanoate, and methyl 2-butyloctadecanoate showed good activity. By transforming 2-butyltetradecanoic acid to methyl 2-butyltetradecanoate, the activity increased from LC_{50} 6.0 to 0.6 ppm. By esterification of 2-butyloctadecanoic acid to its methyl ester, the activity greatly increased from LC_{50} >50.0 to 0.5 ppm.

Methyl esters of 2-hexyl-substituted fatty acids were also better larvicides than their parent acids. All esters, from methyl 2-hexyloctanoate to methyl 2-hexyloctadecanoate, exhibited considerable activity. The LC_{50} of 2-hexyloctanoic acid decreased from 4.8 to 2.6 ppm when it was esterified, while a greater increase in activity was obtained when 2-hexylhexadecanoic acid (LC_{50} 40.0 ppm) was esterified to its methyl ester (LC_{50} 1.5 ppm).

It is therefore concluded that the biological activity of 2-alkyl-substituted fatty acids can be enhanced by esterification of the acids to their methyl esters. In some cases, the activity increased considerably.

BRANCHED-CHAIN ALKANES.—7-Methyloctadecane, 8-methylnonadecane, and their structural isomers were synthesized by the Grignard reaction of alkylmagnesium bromides with methyl ketones, dehydration of the resulting tertiary alcohols, and subsequent hydrogenation of substituted alkenes thus obtained. They included 2-, 3-, 4-, 5-, 6-, 7-, 8-, and 9-methyloctadecanes and 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, and 10-methylnonadecanes.

The biological studies showed that naturally occurring 7-methyloctadecane exhibited some activity; however, other synthesized branched-chain alkanes, such as 2-, 3-, and 8-methyloctadecanes were more active than the natural product. Naturally occurring 8-methylnonadecane was less active than some of its isomers, such as 2-, 4-, 5-, 7-, and 9-methylnonadecanes. Among these branched-chain alkanes, 4-, 7-, and 9-methylnonadecanes were particularly effective with LC_{50} in the range of 1-2 ppm. These compounds are more active against first-instar larvae than fourth-instars. It is thus apparent that the position of the methyl substituent in the alkane carbon chain greatly influences the biological activity of the branched-chain alkanes.

For comparison, straight-chain alkanes, from undecane to eicosane, were also evaluated for their biological activity against mosquito larvae. The bioassays showed that straight-chain alkanes generally exhibited lower levels of activity than methyloctadecanes and methyl=nonadecanes. Some of these straight-chain alkanes, such as undecane, pentadecane, and heptadecane, however, showed some activity. Heptadecane and octadecane were previously reported to be found in the overcrowded culture of mosquito larvae.

In comparing the biological activity of straight-chain and branched-chain alkanes, it is reasonable to conclude that the methyl branching at various positions on the alkane carbon chain invests significant increase in biological activity.

GROWTH RETARDING.—In addition to the toxic effects, branched-chain alkanes also manifested growth-retarding activity under sublethal concentrations against mosquito larvae. First-instar larvae of *C. p. quinquefasciatus* treated with 0.5 and 1.0 ppm of 7-methylnonadecane emerged into adults in 21 and 28 days, respectively, after treatment. In contrast, untreated larvae completed adult emergence within 14 days. The growth and development of mosquito larvae were thus drastically delayed by the branched-chain alkane, and the results of the biological studies therefore confirmed the previous findings that the isolated active fraction of larval cultures manifested toxicity as well as growth-retarding activity.

POTENTIAL FOR MOSQUITO CONTROL.—The overcrowding factors of mosquito larvae are toxic to younger larvae, and therefore independently or in combination with other insecticides, such as juvenile hormone analogs, they have good potential in controlling the immature stages of mosquitoes. The activity of the overcrowding factors can be greatly increased by formulation with organic solvents and surface active agents and by appropriate combination of different types of the overcrowding factors which show synergic effect to one another. Large-scale production of these compounds is feasible due to the fact that the synthetic procedures are simple and straightforward and the yields of the synthetic products are excellent. Although these compounds are presumably biodegradable in the environment, they are reasonably stable under experimental and field conditions because of lack of vulnerable functional groups in their molecules. Furthermore, as proposed by Mulla (1974), the growth-retarding features of the overcrowding factors would render these potentially useful controlling floodwater mosquitoes if development of larvae can be delayed until temporary water disappears.

The overcrowding factors apparently possess little or no toxicity to birds and mammals. Additionally, they are considered to be highly specific against mosquito larvae, thus showing little or no adverse effects on nontarget organisms in aquatic habitats. These compounds therefore offer a good potential for specific and safe control of mosquito populations.

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EVALUATION OF NEW CHEMICALS AS MOSQUITO CONTROL AGENTS

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TH6040 [1-(4-chlorophenyl)-3-(2, 6-difluorobenzyl)-urea], which showed promise in 1973, was extensively evaluated as a mosquito control agent under operational conditions during 1974 (Schaefer et al. 1975). Simultaneous studies of its effects on target and nontarget aquatic organisms were made following aircraft applications (Miura and Takahashi 1975). A dose of 0.025 lb AI/acre successfully controlled mixed populations of *Aedes nigromaculis* (Ludlow) and *Aedes melanimon* Dyar. This dosage was operationally effective against organophosphorus-resistant (OP-R) strains of these species in both the Sacramento and San Joaquin Valleys. TH6040 also appears to provide a means of controlling OP-R strains of *Culex tarsalis* Coquillett. No long-term undesirable effects on nontarget aquatic organisms resulted from applications of TH6040 that were operationally effective against mosquitoes (Miura and Takahashi 1975). Samples of treated water, soil, and vegetation were collected and are now being analyzed for TH6040 residues. Sufficient data were obtained to prove the operational efficacy of TH6040 as a mosquito control agent.

Our previous studies on Altosid® formulation (Schaefer et al. 1974) were continued during 1974. Numerous formulations were compared in laboratory and in small pond tests and the formulation that appeared to be superior, a charcoal-base suspension, was then evaluated in aircraft trials. A summary of trials with this formulation (Altosid 10F) is shown in Table 1. Unfortunately the 10F formulation does not appear to provide operational efficacy on *Culex* spp. nor is it superior to the commercial Altosid SR-10 formulation against *Aedes* sp. Further work to find a formulation of Altosid that will be useful for controlling *Culex* spp. is continuing.

Hercules 24108 [3 butyn-2yl-N-(p-chlorophenyl) carbamate], which showed relatively low activity in 1973 tests (Schaefer et al. 1974), was further evaluated during 1974, because of its projected low cost and because it is a carbamate which causes inhibition of pupal development following larval exposure. Two pasture plots (1½ acre each), containing mixed populations of 3rd and 4th stage *A. nigromaculis* and *A. melanimon* were sprayed by handcan at rates of 0.5 and 0.75 lb AI/acre using a 50% WP formulation of Hercules 24108. The 0.5 lb rate yielded a final mortality of 75% and that for the 0.75 lb rate was 100%. Observations and population collections and counts of aquatic, nontarget organisms showed no deleterious effects even at the high rate (Table 2).

A new microencapsulated formulation of Stauffer R-20458 [1-(4 ethylphenoxy)-6, 7-epoxy-3, 7 dimethyl-2-octene], which contained 2 lbs AI/gal and denoted as R20458-25, was evaluated during 1974. Results in 1 m^2 outside ponds showed that this formulation was superior to previous formulations of R20458 that we had evaluated; therefore, larger scale tests against field populations of mosquitoes were conducted. A 10-acre alfalfa field, which contained 1st to 4th stage *A. nigromaculis* (up to 50/dip) and a 40-acre pasture having large numbers of 3rd and 4th stage *A. nigromaculis* larvae were both treated with 0.1 lb AI/acre of R20458-25 in 1 gal of water/acre by aircraft. Final *Aedes* mortality was approximately 80% on the alfalfa field and 50% on the pasture. There was no apparent adverse effect on nontarget organisms (Table 3), but higher rates will be necessary to obtain operational efficacy against mosquitoes.

A report of the biological activity of di-t-butyl substituted phenols (RE17565, RE17937, and RE18286) was presented last year (Schaefer et al. 1974). While these compounds offer promise as mosquito control agents and appear to be relatively safe to nontarget organisms, commercial development is not considered to be justified by the company involved because of the limited market for mosquitocides. This is another example of active and apparently safe compounds being dropped from our program due to lack of commercial interest.

While we continue to test new organophosphorus compounds that are received, virtually all of these have shown

Table 1.–Summary of 1974 aircraft trials with Altosid 10F.

Test No.	Date (1974)	No. Acres	Rate (lb AI/acre)	Species	% Control
74-15	6/19	20	1/10	*Culex tarsalis*	60 - 80 % for 6 days
74-16	6/19	35	1/50	*Aedes nigromaculis*	100%
74-20	7/3	30	1/80	*Aedes nigromaculis*	75 - 90%
74-22	7/6	30	1/60	*Aedes nigromaculis*	∿ 95%
74-24	7/9	40	1/70	*Aedes nigromaculis*	100%
74-29	7/20	40	1/60	*Aedes nigromaculis*	∿ 70%
74-30	7/25	77	1/60	*Aedes nigromaculis*	∿ 99%
74-45	9/27	5	3/10	*C. p. quinquefasciatus* *C. peus*	100% for 3 days [a]

[a]The water source was drained on the 4th day after treatment.

Table 2.—Side effects of H24108 on nontarget organisms applied at 0.75 lb/acre against pasture mosquitoes, (Treated July 16, 1974).

Date	July 16	17	18	19	20
Organisms	No. of organisms in water (4,500 ml) collected immediately before treatment and held in the laboratory for daily counts.				
Copepods	97	121	116	179	186
Seed shrimp	3	3	3	3	3
Mayfly N[a]	8	7	7	7	7
Corixid N	7	7	7	7	7
Shore fly L[b]	3	2	2	2	2
	No. of organisms in water (4,500 ml) collected immediately after treatment and held in the laboratory for daily counts.				
Copepods	155	154	183	267	276
Seed shrimp	1	1	1	1	1
Mayfly N	2	2	2	2	2
Corixid N	5	5	5	5	5
Beetle L	1	1	1	1	1
	No. of organisms in daily collections of field water (4,500 ml)[c]				
Copepods		114	183		
Water fleas		2	10		
Seed shrimp		1	1		
Mayfly N		29	128		
Corixid N		3	2		
Beetle L		3	3		

[a] N = Nymphs

[b] L = Larvae

[c] Water in the treated area dried up by July 19, 1974.

Table 3.—Side effects of Stauffer R20458-25 on nontarget organisms applied at 0.1 lb/acre against pasture mosquitoes, September 24, 1974.

Organism	September 24	25	27	30	Oct. 2
	No. of organisms in water (2,250 ml) collected immediately prior to treatment and held in the laboratory.				
Water fleas	108	157	244	425	440
Copepods	87	49	46	246	521
Damsel fly N[a]	3	3	3	3	3
Shore fly L[b]	2	2	2	2	2
Chironomid L[c]	6	6	6	6	10
	No. of organisms in water (2,250 ml) collected immediately after treatment and held in the laboratory.				
Water fleas	93	124	131	125	9
Copepods	127	53	56	165	542
Seed shrimp	1	1	1	1	1
Shore fly L	2	2	4	0	0

[a] N = Nymphs

[b] L = Larvae

[c] Chironomid larvae: *Chironomus stigmaterus Goeldichironomus holoprasinus.*

a high potential for cross-resistance, as evidenced by plots of mortality versus concentration for S- and R-strains of *C. tarsalis.* The LC_{50}'s often do not differ greatly between strains but divergence of the ldp lines at high test concentrations is indicative of cross-resistance. An example of this is shown for N-2596 (O-ethyl S-(p-chlorophenyl) ethylphosphonodithioate) in Figure 1.

In the past several years different types of compounds that have demonstrated unique biological activity have been evaluated and have shown promise as mosquito control agents. Altosid has advanced from the level of high activity in laboratory tests to a commercial product. It now appears that TH6040 will follow the same course. However, few compounds show this degree of promise so it is imperative that we use new, approved compounds wisely in order to delay development of resistance.

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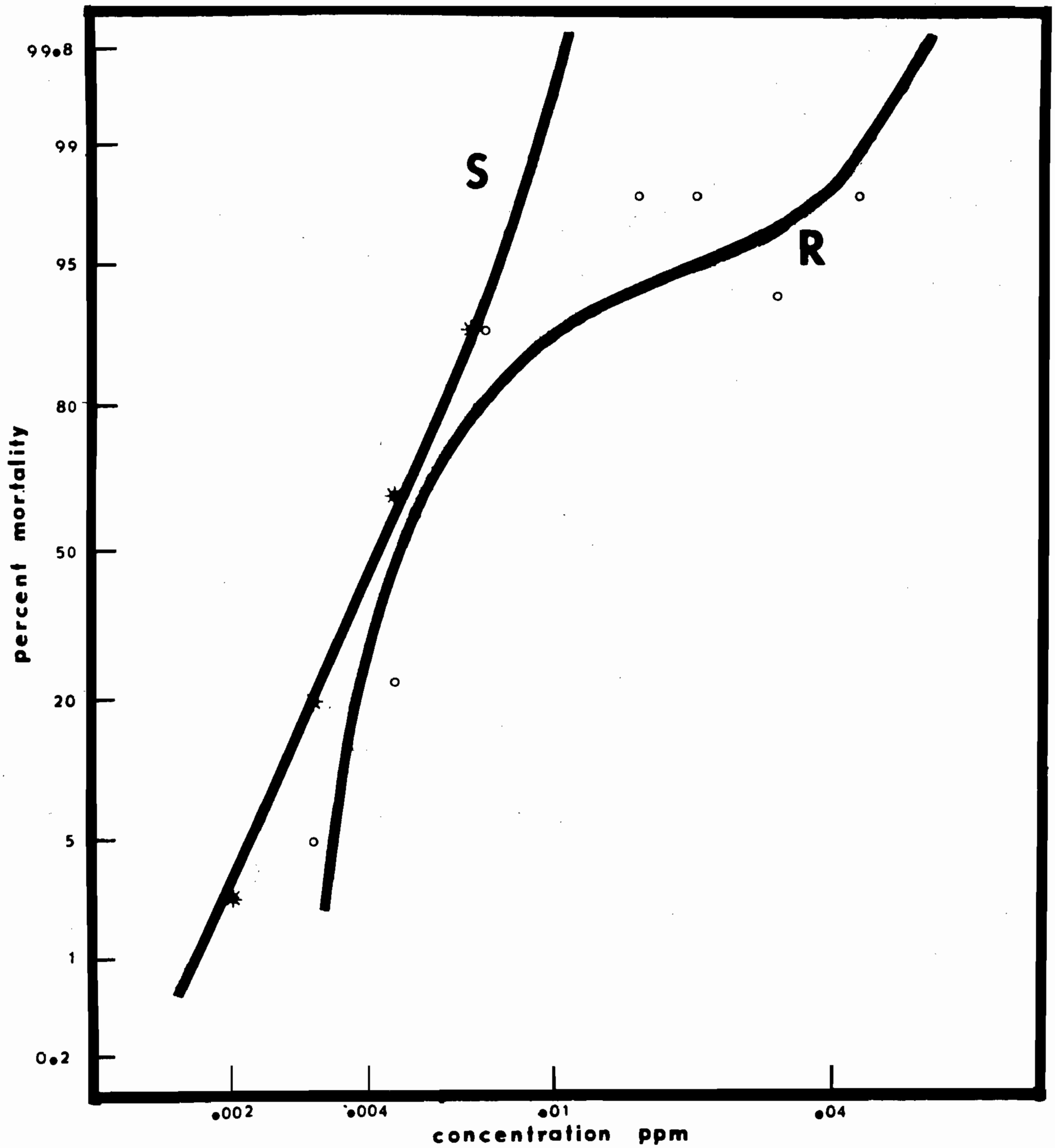

Figure 1.–The biological activity of N-2596 against larvae of susceptible and OP-R-strains of *Culex tarsalis*.

EFFECTS OF DIMILIN™ ON NONTARGET ORGANISMS IN EARLY-SPRING *CULEX TARSALIS* LARVAL HABITATS

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ABSTRACT

A 25% WP formulation of DIMILIN® [1-(4-chlorophenyl)-3-(2, 6 - difluorobenzoyl)-urea] at rates of 0.04 and 0.045 lb AI/acre applied against early season *Culex tarsalis* Coquillett populations in foothill areas was found to be relatively safe to other organisms. Cladoceran populations were greatly stressed by the treatment, but recovered within three weeks. Corixid, notonectid, and mayfly nymphs and aquatic beetle larvae showed some sensitivity, but population declines did not occur. Adult beetles, mosquitofish, and tadpoles tolerated the treatments.

INTRODUCTION.–*Culex tarsalis* Coquillett is one of the most intensively studied mosquitoes in the Central Valley of California, not only because it is a potential vector of viral encephalitis, but also because of its extreme resistance to all commercially available larvicides in some areas.

In the Central Valley of California, *C. tarsalis* females overwinter chiefly in foothill areas and fly back to the Valley in early spring (Bellamy and Reeves 1963, Burdick and Kardos 1963, Bailey et al. 1965, Kliewer et al. 1969); therefore, early spring populations in foothill areas are nuclei from which later season populations in the Valley originate. The effect of a new mosquito larvicide on nontarget organisms in early spring *C. tarsalis* habitats in foothill areas is reported here.

Study Area -- The study was conducted in the Yokohl Valley, about 20 miles east of Visalia, California. Tests were performed at a vernal pond in the Valley and also an isolated lateral pool situated beside Yokohl Creek (an intermittent stream which runs through the Valley).

Vernal Ponds -- This is the most important mosquito breeding habitat associated with the foothill areas. The pond used was about 40 x 50 feet, oval in shape, with a depth of 12 to 15 inches. Water is supplied by subsurface seepage and remains most of the winter months to the early part of May. The water was darkly colored by decaying organic debris accumulated on the bottom of the pond. No vegetation was found in the pond proper.

Isolated Lateral Pools -- The pool used is one of many situated along the creek banks, it is narrow and elongate, and has a surface area of about 0.088 acre (255 x 15 feet). It contained moderately clean water throughout the flow season, although the pool favored the accumulation of organic debris. Vegetation was scant, except in spring; duckweed and algae were abundant.

MATERIALS AND METHODS.–A 25% WP formulation of DIMILIN® [1-(4-chlorophenyl)-3-(2, 6-difluorobenzoyl)-urea] was applied by hand can sprayer (Schaefer et al. 1975).

The biological activity of the compound was determined by monitoring pre- and post-treatment populations in the laboratory, and supplementary samples from the treated pool and pond were also taken daily for 3 weeks (Miura and Takahashi, 1973, 1975).

A total of 36 taxa including plankton, insects, snails, fish, toads, and algae were observed in the study area (Table 1).

RESULTS AND DISCUSSION.–Application of 0.045 lb AI/acre on a 10-day schedule eliminated early generations of *C. tarsalis* larval populations from these foothill breeding sites (Schaefer et al. 1975). Table 2 summarizes the side effects of the 0.04 lb/acre application on populations of planktonic organisms. It is apparent that the compound will reduce the cladoceran population, at least temporarily, but the population will recover within a short period of time. A characteristic reaction of the treated population in early spring was not only a reduction in numbers, but also the production of ephippia; numerous ephippia (carapace cuticle containing diapaused eggs which are resistant to adverse environmental conditions) were observed in the treated water. Copepod and mayfly populations might be affected, but population levels were too low to determine this. Seed shrimp tolerated the treatment. The 0.045 lb/acre applications did reduce the cladoceran population as expected (Table 3). Some nymphal stages of corixid and notonectid bugs also were affected. Table 4 summarizes the effects of the 0.04 lb application on aquatic insects, mosquitofish, and tadpoles. The compound is probably safe to these organisms. No abnormal population changes after treatment were revealed by trapping, although a few dead nymphal bugs and beetle larvae were occasionally observed in the treated water; affected insects often started to ecdysis but failed to exuviate from the old skin and died. Adult beetles, mosquitofish, and tadpoles demonstrated tolerance.

The toxicity of the compound under natural conditions probably does not persist long. For example, bioassay of water from the treated pond showed that the mortality of laboratory-reared cladocerans was about 57% at 72 hr after treatment, and no mortality occurred at 96 hr. Table 5 shows the biological activity under laboratory conditions. As expected, mayfly nymphs were susceptible; mosquitofish fry showed surprising tolerance, even 100 ppm for 96 hr exposure did not kill them. Western Spadefoot Toad tadpoles also were tolerant.

Table 6 shows the comparison of relative toxicities of DIMILIN® and fenthion on nontarget organisms. Fenthion is chosen here because it is the most commonly used mosquito larvicide in California (Murray 1974). A "Tolerance Index" is used to express relative toxicity because it explains effects of toxicants on nontargets better than "Median Lethal Concentration" (Miura and Takahashi 1974). It is apparent that both DIMILIN and fenthion used for *C.*

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Table 1.—A list of organisms found in the test area, Yokohl Valley, California, 1974.

Organism	Stage	Organism	Stage
Rotifers		Dytiscid beetles	
Asplanchna spp.	Juvenile & Adults	*Hydroporus* spp.	Adults
Water fleas		*Deronectes* spp.	Adults
Moina spp.	Juvenile & Adults	*Oreodytes* spp.	Adults
Simocephalus spp.	Juvenile & Adults	*Laccophilus decipiens*	Adults & Larvae
Alona spp.	Juvenile & Adults	*Agabus* spp.	Adults
Copepods		*Ilybius* spp.	Adults
Cyclops spp.	Juvenile & Adults	*Acilius* spp.	Larvae
Seed shrimp		Whirligig beetles	
Cypridopsis spp.	Juvenile & Adults	*Gyrinus punctellus*	Adults
Cypricercus spp.	Juvenile & Adults	Hydrophilid beetles	
Mayflies		*Hydrophilus triangularis*	Larvae
Callibaetis spp.	Nymphs	*Tropisternus lateralis*	Larvae & Adults
Siphlonurus spectabilis	Nymphs	Chironomid midges	
Dragonflies		*Chironomus* spp.	Larvae
Pseudoleon superbus	Nymphs	*Corynoneura* spp.	Larvae
Plathemis spp.	Nymphs	*Procladius* spp.	Larvae
Damselflies		Snails	
Coenagrion spp.	Nymphs	*Physa* spp.	Juvenile & Adults
Water boatmen		Frogs and Toads	
Corisella decolor	Nymphs & Adults	*Hyla regilla*	Tadpoles
Hesperocorixa laevigata	Nymphs & Adults	*Scaphiopus hammondi*	Tadpoles
Back swimmers		Duckweeds	
Notonecta unifasciata	Nymphs & Adults	*Lemna minor*	
Buenoa spp.		*Lemna gibba*	
Water striders		Stone Warts	
Gerris incurvatus	Adults	*Chara* spp.	

Table 2.—Effects of DIMILIN on nontarget organisms: Applied at 0.04 lb/acre against *C. tarsalis*, April 22, 1974.

	April						May			
Organism	22	23	24	25	26	29	1	3	6	8
	No. of organisms in the pre-treatment water (2,250 ml) and held in the laboratory									
Water fleas	379		338	328	343	108	105	97		
Ephippia	0		10	14	14	14	20	26		
Copepods	21		12	10	12	5	5	5		
Seed shrimp	22		23	19	19	18	17	16		
Mayfly N[a]	6		4	4	4	4	4	2		
Beetle L[b]	6		6	6	6	5	5	3		
	No. of organisms in water (2,250 ml) collected immediately after treatment and held in the laboratory									
Water fleas	104		12		0	0	0	0		
Ephippia	0		25		28	30	25	28		
Copepods	7		7		2	2	2	2		
Seed shrimp	45		41		38	32	32	35		
Mayfly N[a]	3		3		0	0	0	0		
	No. of organisms in water (2,250 ml) collected daily from field									
Water fleas[c]		5	1	0		0	0	0	0	0
Ephippia		34	149	28		130	120	87	83	54
Copepods		5	5	2		2	2	2	1	1
Seed shrimp		44	84	35		51	71	109	87	52
Mayfly N[a]		0	0	0		0	1	1	9	2
Beetle L[b]		2	4	2		4	2	7	5	6
Chironomid L[b]		1	3	1		2	0	2	3	8

[a]N = Nymphs

[b]L = Larvae

[c]Sixteen water fleas were collected from the treated pond on May 12, 1974 (20 days after treatment).

Table 3.—Effects of DIMILIN on nontarget organisms: applied at 0.045 lb/acre against *C. tarsalis*, April 15, 1974[a]

Organism	April 15	17	18	19	21	24
	No. of organisms in the pre-treatment water (2,700 ml)					
Water fleas	6	3	3	3	17	
Copepods	10	10	8	8	8	
Seed shrimp	87	63	56	54	50	
Corixid N[b]	12	6	4	4	2	
Notonectid N[b]	17	9	9	7	4	
	No. of organisms in the post-treatment water (2,700 ml)					
Water fleas	6	0[c]	0	0	0	0
Copepods	15	15	13	12	10	10
Seed shrimp	145	120	78	79	83	80
Corixid N[b]	12	10[c]	7	7	7	7
Notonectid N[b]	18	15[c]	14	14	14	14

[a]Daily post-treatment collections were not taken
[b]N = Nymphs
[c]Dead organisms were noted

tarsalis control do suppress cladoceran populations; even so, DIMILIN is much safer than fenthion, i.e. TI_{50} of DIMILIN against water fleas was 2.36 and against side swimmers was $>$ 1000, while that of fenthion was 1.4 and 0.84 respectively, which suggests that repeated applications of fenthion on the same habitat might exterminate cladoceran and side swimmer populations.

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Table 4.—Effects of DIMILIN on nontarget organisms: Applied at 0.04 lb/acre against *C. tarsalis*, April 22, 1974 (F. T. No. 74-2).[a]

Organism	April 17	18	19	22	23	24	26	29	30	May 2	3	6	8
Corixid N[b] A[c]	7	12	14	15	33	2	46	19	12	11	2	4	1
Notonectid N[b] A[c]	1	3	5	0	3	0	3	3	1	0	0	0	0
Odonata N[b]	0	0	1	0	3	0	1	0	0	1	0	0	0
L. decipiens A[c]	12	7	8	2	7	1	7	9	6	17	19	12	3
Agabus spp. A[c]	4	17	4	5	4	3	4	3	3	1	7	2	0
Dytiscid A[c]	2	2	1	0	1	1	3	4	4	2	0	3	3
Dytiscid L[d]	0	3	5	0	5	4	5	2	0	0	0	0	0
Hydrophilid A[c]	2	7	2	2	8	0	1	2	0	1	1	1	14
Hydrophilid L[d]	0	1	1	1	0	0	0	1	1	1	0	0	0
G. punctellus A[c]	0	0	0	0	3	0	1	1	1	1	0	2	0
Tadpoles	85	13	15	223	92	12	168	398	140	427	527	258	25
G. affinis A[c] I[e]	21	16	7	27	9	11	5	6	25	25	12	10	17

[a]No. of organisms collected by 3 minnow traps
[b]N = Nymphs
[c]A = Adults
[d]L = Larvae
[e]I = Immatures

Table 5.—Biological activity of DIMILIN against organisms associated with mosquito breeding habitat in foothill areas.

Organism	Stage	No. Tests	No. Reps	No. Organism Container	Exposed (hr)	% mortality @ ppm	
Side swimmers	Mixed	2	3	5 , 15	48	> 50	0.8
Mayflies	Nymphs	2	3	30	168	50	.002
						90	.003
Mosquitofish	Fry	1	1	10	96	0	100
Western Spadefoot Toad	Tadpoles	2	1	20, 25	192	0	5

Table 6.—Comparison of relative toxicity of DIMILIN and fenthion to nontargets as used in *C. tarsalis* control.

	Dimilin[a]		Fenthion[a]	
Organism	TI_{50}[b]	TI_{90}[c]	TI_{50}	TI_{90}
Water fleas	2.36	5	1.40	1.04
Copepods	> 1000	> 1000	166	132
Seed shrimp	> 1000	> 1000	60	47
Side swimmers	> 1000	> 570	.84	.74
Mayflies	2.86	2.14	- -	- -
Mosquitofish	> 1000	> 700	> 40	> 20

[a]Fenthion: OP R-strain *C. tarsalis* $LC_{50} = 2.5 \times 10^{-2}$, $LC_{90} = 5 \times 10^{-2}$

Dimilin: OP R-strain *C. tarsalis* $LC_{50} = 7 \times 10^{-4}$, $LC_{90} = 1.4 \times 10^{-3}$

[b]TI_{50} = tolerance index at $LC_{50} = \dfrac{LC_{50}\ \text{nontarget}}{LC_{50}\ \text{target}}$

[c]TI_{90} = tolerance index at $LC_{90} = \dfrac{LC_{90}\ \text{nontarget}}{LC_{90}\ \text{target}}$

INVESTIGATIONS ON THE MODE OF ACTION OF DIMILIN® (TH60-40) AGAINST MOSQUITOES

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ABSTRACT

Exposure of 4th instar larvae of *Culex pipiens quinquefasciatus* to a series of concentrations of Dimilin indicates that response is dose-dependent in larvae and pupae as well as adults. This is in contrast to the effect of methoprene (Altosid®) which is not manifested on treated larvae but only on the resulting pupae and adults.

The sensitivity of the four larval instars of *C. quinquefasciatus* and *C. tarsalis* was found to be approximately (LC_{50} 1.6-2.7 ppb). However, brief exposure (30 min.) of larvae of different ages revealed four peaks in sensitivity which are congruent with the occurrence of ecdysis. This is in agreement with the suspected inhibitory effect of Dimilin on chitin synthesis. From the practical standpoint this property enhances the utility of the compound against asynchronous populations.

Quantitative and qualitative measurements of sensitivity within the 4th instar and pupal stage indicate that in *C. fatigans* the "sensitive" period begins ∿ 15 hours before pupation and continues for 2 hours after pupation.

Pupation in larvae is delayed by 21.7% if exposure to sublethal concentrations has occurred within 48 hours after eggs hatch.

Culex tarsalis strains (susceptible, OP-multiresistant, and Altosid-selected) were found to be equally sensitive to Dimilin irrespective of the type of insecticide resistance present. A slight tolerance to Dimilin (1.5x-2.3x) was found in *C. quinquefasciatus* resistant to OP's or carbamates.

RESIDUAL TESTS WITH ABATE, CHLORPYRIFOS, DDT, FENTHION, AND MALATHION AGAINST *AEDES SIERRENSIS* (LUDLOW) IN FABRICATED TREEHOLES

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ABSTRACT

The residual activity of several insecticides in fabricated treeholes was tested against the larvae of the western treehole mosquito. At rates of one pound of technical material per 50 treeholes, malathion lasted 1 year, fenthion and Abate® lasted 3¼ years; and chlorpyrifos gave complete control throughout the 4½-year test period. At rates of one pound of technical material per 100 and 200 treeholes, Abate lasted 3 years, while chlorpyrifos gave complete control throughout the 4½-year test period.

INSECT DEVELOPMENTAL INHIBITORS:
MULTIPLE APPLICATIONS OF DIMILIN® AND ALTOSID® TO *GAMBUSIA AFFINIS* (BAIRD AND GIRARD)

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ABSTRACT

Populations of the mosquitofish, *Gambusia affinis* (Baird and Girard) were exposed separately to Altosid®(isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate) and DIMILIN® [4-chlorophenyl)-3-(2, 6-difluorobenzoyl)-urea] in experimental ponds to determine the effects of multiple treatments over a 5-month period. Five applications of Altosid 10% flowable at 0.03 lb AI/acre and dimilin 25% WP at 0.05 lb AI/acre were made at 1-month intervals. There was no visible adverse effect to the treated populations; their fluctuations and numbers were similar to those of the control.

The mosquitofish *Gambusia affinis* (Baird and Girard) has been recognized since the early 1900's as a potential control agent for mosquitoes (Krumholz 1948). Since its introduction to California in 1922, researchers in this state have continued to show that it reduces numbers of mosquito larvae in the field (Washino and Hokama 1967, Bay 1969, Hoy and Reed 1970, Hoy et al. 1971, 1972) and, at present it is still the only effective bio-control agent which is routinely used by mosquito control agencies.

Nevertheless, as Legner and Medved (1972) pointed out, the mosquitofish does have limitations in temporary water situations such as pastures. In these situations even if mosquitofish were present (as in drainage sumps), it is likely that another control measure would be required. Among the newest control measures that could be used are 2 synthetic "Insect Developmental Inhibitors" (Schaefer and Wilder 1972), Altosid® (isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate) and DIMILIN® [1-(4-chlorophenyl)-3-(2, 6-difluorobenzoyl)-urea].

We studied the effect these compounds have on *G. affinis* when the fish are exposed repeatedly to mosquito controlling concentrations. Prior laboratory tests show that the fish survive concentrations greater than 80 ppm of both technical Altosid (Miura and Takahashi 1973) and dimilin (Miura et al. 1975). Also a 40-day simulated field test with Altosid SR-10, a microencapsulated formulation, yielded no apparent toxic effect at 0.1 lb AI/acre (Miura and Takahashi 1974). Mosquitoes, on the other hand, are very susceptible to both compounds (Jakob 1972, 1973; Schaefer and Wilder 1972, 1973; Mulla et al. 1974; Hsieh and Steelman 1974; Schaefer et al. 1974) and are 100 to over a million times more susceptible (depending on species) than *G. affinis*. Field tests with Altosid against *Aedes nigromaculis* (Ludlow) showed effective rates as low as 0.0125 lb AI/acre (Schaefer and Wilder 1973) and effective rates with dimilin against *Culex tarsalis* Coquillett as low as 0.025 lb AI/acre (Schaefer et al. 1974). Rates for this study were effective on field mosquitoes but were below the acute toxicity of *G. affinis*.

MATERIALS AND METHODS.—We used artificial ponds, 9 x 80.45 m with a 1:3 slope along each edge, at the Kern Research Area (Bureau of Sports, Fisheries, and Wildlife, U. S. Department of Interior) in Kern County, California. The water level in each pond is controlled by a plumbing system connected to a 15-acre reservoir maintained by a deep well pump. Water depth was maintained at 27 cm in all ponds. The input plumbing was screened to prevent immigration of predators through the system. Each pond was seeded with about 3,000 fish which were netted with an 8 mesh seine from stock ponds established at the research area a year before.

The fish were trapped biweekly with 42 x 22 cm barrel-shaped wire minnow traps lined interiorly with 18 x 16 mesh aluminum screens. One trap was placed along each of the short (9 m) sides, near the water edge and submerged so that the entrance holes were about 4 cm below the water surface. After a 2-hour trapping period, the traps were emptied into 26 x 42 x 10 cm pans for counting. Those which contained more than an estimated 200 were emptied into a 1,000 ml graduated cylinder containing 500 ml of water and were measured volumetrically to the nearest 5 ml. The contents of the cylinder were then poured into a pan where 5 samples of fish were netted for counts and volume measurements in a 250 ml graduated cylinder. The total amount of fish was calculated from the volume and number of samples.

Spray material was measured on an analytical balance and held in 50 ml glass screw-top flasks for transport to the field. In the field each material was mixed with 5.5 l of water in a 3-gal stainless steel hand sprayer fitted with a 1.5 m boom and a flare tip.

Applications were made once a month (for a total of 5) by walking around the perimeter of each pond while extending the boom towards the middle. The canister was shaken 2 to 3 times during each treatment to prevent settling of the material. Initial pressure was 40 - 50 psi. One pond was treated with Altosid at the rate of 0.03 lb AI/acre with a 10% flowable formulation, and the other was treated with dimilin at 0.05 lb AI/acre with a 25% WP.

In order to determine the effects of these compounds on nontarget invertebrates in the ponds, 6 or more 450 ml dipper samples were taken from the banks at approximately equal intervals and brought into the laboratory for examination under stereomicroscopes.

Air temperature readings were obtained with Science Associates minimum and maximum thermometers read by personnel at the Kern Wildlife Refuge (B.S.F.W., U. S. Department of Interior). Dissolved oxygen and water temperatures were taken on trap days, between 10:00 a.m. and 12:00 noon, with an International Biophysics Corporation no. 500-051 analyzer.

RESULTS AND DISCUSSION.—Extreme air temperatures varied from a minimum of -1°C in April to a maximum of 43°C in July. A sub-freezing temperature was reached only on one day and was preceded and followed by maximum temperatures of 19.5°C and 21°C respectively. During the test months, the maximum air temperature never fell below 16°C. Although the maximum air temperature exceeded 40°C for several days, there was sufficient (10% or more) vegetation to provide cover from direct radiation thus keeping the water temperature at lower levels. Since the temperatures in general were moderate, it is doubtful that they adversely affected the fish populations (Figure 1). Dissolved oxygen readings for all ponds varied between 4 ppm @ 24°C to 7.2 ppm @ 24.5°C on the bottom and between 4.8 ppm @ 24°C and 7.6 ppm @ 27°C, 2 to 7 cm below the water surface. As these oxygen concentrations are well above the minimum criticals of 0.2 to 0.9 ppm (Sjogren 1972), it was apparent that oxygen content in the water was not a limiting factor in this study. Two measurements of water pH with Hydrion H-20 pH papers in all ponds yielded readings of 8.4 to 8.8.

Figure 1 shows the numbers of fish captured in the Altosid and dimilin ponds as compared to those in the control. When results from the entire 5 months of trapping were plotted, it became apparent that the treated populations of *G. affinis* were following the same pattern as that of the control. Therefore, it appears that exposure to either Altosid or dimilin at the rates used did not effect *G. affinis* population growth even when the fish were exposed to multiple applications over a 5-month period.

Organisms caught in the traps besides fish were adult and larval hydrophilid and dyticid beetles, and corixid and notonectid bugs.

The invertebrate sampling did not yield useful quantitative data, probably due to predation by the fish. Copepods were most abundant in the samples followed by Cladocera, ostracods, chironomid larvae, mayfly naiads, beetle larvae, damselfly naiads, traces of ephidrid larvae and corixid nymphs.

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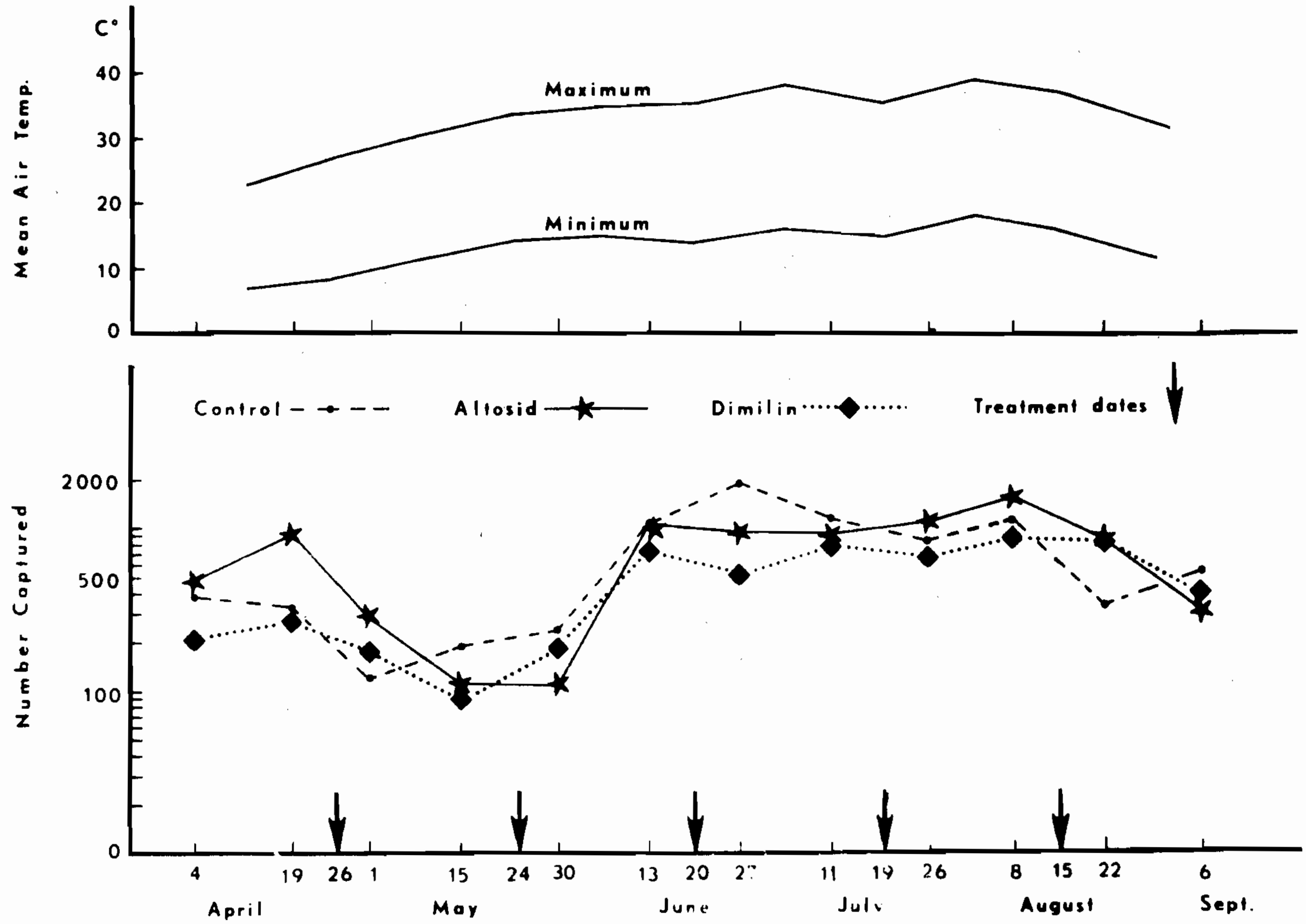

Figure 1.–Top--Average daily minimum and maximum air temperatures between trap periods. Bottom--Semi-monthly trap collections of *Gambusia affinis* from experimental ponds at the Kern Research Area, Kern County, California. The Altosid pond was treated @ 0.03 lbs AI/acre with an SR-10 flowable formulation, the dimilin @ 0.05 lbs AI/acre with a 25% wettable powder.

FACTORS INFLUENCING DIAPAUSE IN *CULEX TARSALIS*

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ABSTRACT

In the natural setting of the Sacramento and San Joaquin Valleys, *Culex tarsalis* enters diapause in fall with the halt of autogenous development, cessation of blood feeding and the building up of fat body. Laboratory attempts to induce similar events have been undertaken. In the laboratory, short days (8L - 16D) evoke the development of fat body. Autogenous mosquitoes show continued ovarian development with retention of eggs. The genetic basis of diapause is under investigation.

EFFECTIVENESS OF THE HEMOGLOBIN CRYSTALLIZATION TECHNIQUE FOR MOSQUITO HOST BLOOD MEAL IDENTIFICATION

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ABSTRACT

Increased reliability of the hemoglobin crystallization technique, used to identify mosquito blood meals, was obtained using a buffered reagent. Reference crystals have been prepared for over 90 mammalian and 10 avian species.

To establish the reliability of this technique, ten mammalian host species (*Sus scrofa, Canis familiaris, Equus caballus, Lepus* sp., *Sylvilagus* sp., *Ovis aries* (Suffolk), *Capra hircus, Mus musculus, Cavia porcellus* and *Homo sapiens*) have been tested against four mosquito genera, (*Aedes, Culex, Anopheles* and *Culiseta*); six and 24 hours after ingestion.

The host species was found to affect the reliability of the of the technique. The success of crystallization was reduced with human blood meals. Twenty-four hours after ingestion, mouse blood meals were no longer identifiable. With all the other host species, however, characteristic crystals formed and were identifiable 24 hours after ingestion. Blood meals from dog, horse, and guinea pig, in many cases, were identifiable 48 hours after ingestion.

Only one mosquito species, *Aedes nigromaculis*, adversely affected the process when tested with cottontail and mouse hosts. Identification of hosts from the common Californian mosquitoes, *Anopheles freeborni* and *Culex tarsalis*, was very reliable.

For the majority of hosts used and mosquitoes tested, there were no problems with this technique and blood meals could be easily and reliably identified up to 24 hours after ingestion.

THE CHANGING ROLE OF THE PROFESSIONAL ENTOMOLOGIST IN MOSQUITO CONTROL AGENCIES

R. Lee Norland[1] and Charles Beesley[2]

Since the first organized mosquito control districts were formed in California, their numbers have increased steadily as agriculture and the human population expanded. Mosquito control has become more complex, requiring better trained personnel. To better meet the need, many districts have selected managers with formal training in entomology. Other districts have responded by employing full-time entomologists. The following table shows the recent increase in entomologically-trained personnel. (These data were kindly supplied by Dr. W. Donald Murray, Secretary-Treasurer, CMCA.)

		No. of Agencies Having:	
Year	Agencies	Manager-Entomologist	Separate Entomologist
1964	56	12	7
1974	62	23	21

Many districts also employ separate technicians with biological or entomological specialties, including fish biologists and research technicians. There are now approximately two dozen managers or entomologists with advanced degrees in entomology and zoology, six of whom have Ph.D. degrees. These resident scientists represent an extraordinary capability for applying analytical techniques to control operations and for evaluating program efficacy. Although the entomologist's role varies substantially from one district to another, it is probable that the professional entomologist will have a major influence on guiding mosquito control in California in the future. The purpose of this paper is to offer a broad yet realistic definition of the entomologist's role in a modern abatement district, under the direction of or in collaboration with the manager.

TRAINING OF PERSONNEL.—The entomologist should have responsibility for training other employees. His training equips him to teach in a classroom setting facts and concepts that field personnel need to cope with field situations they are likely to encounter.

New seasonal employees should be taught mosquito biology, control methodology and safety procedures. The entomologist should utilize training aids, including formal lectures, training manuals, slides, preserved specimens of mosquitoes and their predators, and live specimens. Classroom instruction should be followed by field demonstrations of sampling and control techniques. This phase of training may be delegated to the operator's field supervisor, under direction of the entomologist.

Prior to the mosquito season, refresher seminars should be given permanent field personnel. Recent developments in control techniques and new methods which are to be used should be explained. Mosquito identification should be reviewed.

The entomologist should conduct study sessions to help employees prepare for state certification examinations, using the training manual as a guide.

ROUTINE TECHNICAL DUTIES.—The entomologist should be responsible for assessing progress of technical operations. The manager should be kept aware of the current status of control efforts and of adjustments that are needed. Consistent and comprehensive field sampling should be maintained, combined with a valid laboratory bioassay program adequate to detect control failures quickly, and to allow early corrective action to be applied effectively.

Seasonal surveillance through larval and adult sampling should be included, with sufficient trained technical personnel available to do the necessary analysis of entomological and chemical-resistance samples required. Several of the larger districts now maintain comprehensive technical program analysis to guide field operations.

Winter routine duties include preparation of seasonal reports, supervising equipment calibration, planning strategy for the coming year, and planning and preparing public information programs.

TECHNICAL CONSULTATION.—In addition to routine duties such as advising the manager of day-to-day control problems and their alternatives, it is the entomologist's duty to make certain that the manager is accurately informed of the pest situation and the entomological ramifications of alternative courses of action.

The entomologist may also be called upon by the manager to provide technical information to the Board of Trustees on the prevailing entomological situation through periodic reports. The Board should also be informed of trends and findings in mosquito research, and how these pertain to their respective districts.

The entomologist is often asked for entomological information and advice. Agricultural concerns may want to know the possible aquatic side effects of a pesticide application. Radio, television or newspaper personnel often request information for the public media. It is the entomologist's responsibility to provide factual information.

RESEARCH.—Research traditionally has been considered a primary prerogative of the universities, with relatively little need for ongoing research programs by local agencies. Also, until the California Legislature assigned mosquito research exclusively to the University of California in 1966, the State's Bureau of Vector Control engaged in research on mosquitoes. Many abatement districts formerly functioned only as operational entities and were dependent upon the state agencies for guidance. Recent developments have significantly altered this pattern.

As a consequence of increasing budgetary constraints, growth of university research staffs has been unable to keep pace with need in mosquito control and related fields. The universities have had to redirect research programs to the

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long-term concerns with the state, although some pure academic research still continues. Many districts have therefore initiated field research and technical development seeking solutions to immediate problems, in some cases arising from past inattention due to the lack of technical personnel. The need for immediate research attention is aggravated by increased community awareness by taxpayers who expect corrective action when necessary, for example, when the agency is confronted by insecticide resistance. Thus, the district that has technical expertise to practice control through pragmatic and methodical efforts, not by crisis, will be in a much better position to operate successfully during the years ahead.

The scope of an active research program within an abatement district should be determined by the agency's needs and resources. When university personnel may not be able to become physically involved with the research problem, they are usually more than willing to provide advice and guidance. This resource should be utilized. Also, abatement district entomologists with research backgrounds may be able to apply for grants as co-investigators on research grant proposals with university research workers.

Research findings should be published promptly. Several entomological journals should be utilized to broaden the channels of communication. Publication costs should be borne by the district.

OTHER PROFESSIONAL OBLIGATIONS.–It is the entomologist's duty to keep well informed of advances in his field. The district should subscribe to the major entomological publications, but the entomologist should subscribe to journals he wishes to retain for his personal library. The entomologist should be granted library leave time. Local college and university libraries should be utilized for searching out topics of interest and perusing biological journals. Some districts have an education policy permitting employees to enroll in job-related courses. These districts may be willing to grant educational leave to the entomologist for periodic courses and could provide for fees and incidental expenses.

The entomologist should participate in extracurricular scientific functions, for it is imperative to maintain a constant flow of thought and interchange of ideas. The cumulative results of these efforts will benefit participating districts. He should be an active member of the California Mosquito Control Association and the American Mosquito Control Association. Both organizations sponsor annual meetings where the entomologist has an opportunity to present reports on his studies and to be updated on the work of others. The CMCA Entomology Committee also presents an intrastate program each year, which affords in-depth reviews on major topics of concern. Most districts currently underwrite the cost of travel by the entomologist to these meetings.

Local colleges and universities, public schools, youth groups, and other organizations afford an excellent opportunity for the entomologist to acquaint others in the community with his science and its significance in their daily lives.

Mosquito control is evolving rapidly toward a sophisticated technology requiring a more thorough understanding of the natural and physical sciences. Districts which employ a professional entomologist should consider a person with a postgraduate degree, and should be prepared to provide full support, including an adequate budget and a trained technician.

Key responsibility for insuring that mosquito control districts move forward on a firm scientific basis lies primarily with the entomologist. His guidance is essential if new technology is to progress efficiently from development to application.

AN ADMINISTRATIVE PROGRAM OF MOSQUITO SOURCE REDUCTION

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The time has come for California's mosquito abatement agencies to take a hard look at ourselves and ask some blunt questions. Where are we heading, and how are we adjusting to this era of rapid change? How are we really meeting the challenges of resistance, regulations, economics and environmental influences? What actually is source reduction all about? What are it's limits? And does source reduction offer a more responsible alternative for tomorrow?

The term "source reduction" for many years has been used loosely, vaguely, and conveniently to refer in general to a physical portion of our total programs. In this way it has been possible to rationalize that we are all similarly engaged in a physical effort to reduce mosquito sources. Because of the absence of an accepted definition of the term "source reduction" we have been provided with a basis for producing more lip service than results in relating to this endeavor. As a matter of fact, this vagueness has allowed for occasional ridicule in such expressions as "the ball point pen is the most used tool in source reduction".

For whatever may be the combination of reasons, there has been an inclination toward foot dragging in terms of programming for and accomplishing source reduction. Perhaps the procedure for setting up an administrative source reduction program is not understood any better than is our need for agreement on an applicable definition of source reduction.

Consequently, before any details are set forth, the following general definition of "source reduction" is herewith submitted for hopeful acceptance and application: – "Source reduction is actually mosquito prevention *per se*". Specifically, source reduction is a combination of mental and physical activity that might be best described as a preventive process which includes public relations, technical assistance and physical correction. It is not solely a physical function as we have erroneously given reference for many years.

If this definition is acceptable, it follows that a much needed change in our work reference from mosquito control to mosquito prevention is indicated, to open the door to a whole new concept for programming to meet our requirements for tomorrow.

However, if we are still uncertain about tomorrow and retain doubts as to the need for a change in program direction, let us add up our yesterday for California's mosquito abatement agencies. We are nearing the end of the third decade of an era essentially dedicated to chemical control and repetitive spray programs wherein we have worn out numerous pesticides; developed extreme resistance; encountered increasing regulations; and now find our costs of control have skyrocketed beyond what the public should any longer be expected to assume. Realistically, we must face the fact that we have actually continued to spray and re-spray many of the same mosquito sources week after week, month after month and, regrettably but true, year after year. The best way to summarize our yesterday is to admit it sounds like it should be yesterday, since it brought us only to the mosquito source, rather than to the cause and responsible solution of the mosquito source. Further, as we analyze our mosquito sources, we must conclude that the vast majority of mosquito breeding areas are of man-made origin rather than from natural causes. This fact should result in the conclusion that the "owner" of the mosquito source, and not the public agency, is the legally responsible party and that our programs should be adjusted accordingly.

If we can agree that a change from our past is indicated and an alternative approach is needed, then I suggest that we have reached the starting point of what I refer to as administrative source reduction programs. Since source reduction is mosquito prevention *per se*, it should therefore be recognized that administrative involvement is the key to the future transition. It is essential that the administrator be the underlying influence in this approach, since the first consideration in mosquito prevention is reallocation of responsibility for mosquito breeding, consistent with our State Health & Safety Code. In addition, it should be apparent that since an administrative source reduction program is a direct relationship between the agency and the responsible party, it must begin with (1) purposeful planning; then (2) provision for effective communication (both written and verbal); (3) inclusion of technical assistance; and finally, (4) physical advice and/or equipment assistance to provide for correction of mosquito breeding sources.

1. Planning - This phase consists of three main considerations:
   a. Policy - By the Governing Board, which provides the necessary basis for a revised program, outlines emphasis and reasons for change.
   b. Publicity - Through the news media (prior to and during transition) where revised policy is made known to the general public and the reasons therefore.
   c. Timing - By the Administrator to provide for effective execution of the preventive program. It is recommended that a late fall or winter start be utilized to relate to the mosquito season of the oncoming year, since this provides the all-important period for understanding and acceptance while mosquitoes are not an urgent matter.
2. Communications -
   a. Written - By the Administrator (includes informational letters; letters requesting appointments; follow through letters; and citations, if needed).
   b. Verbal - At the administrative level, usually by Assistant Manager for initial contacts, coordinated as needed with appropriately timed meetings with source reduction representatives. This is the negotiation period.
3. Technical Assistance - By Source Reduction Specialists where surveys and know-how provide logical and economical answers to elimination or reduction of mosquito breeding sources, and advice on water management.
4. Physical Correction - Depending on which agency, the availability of equipment and/or working relationships with private contractors.

There are three fundamental principles which determine the probabilities for success in accomplishing the desired results in an administrative source reduction program.

1. There can be no exceptions made in this program, and all mosquito producers – public or private, large, medium or small – must be approached in a similar manner. The only recommendation is that the largest problems be given high priority consideration, along with any public mosquito breeding sources.

2. The entire program must be designed to expect results and all communications (written and verbal) should be approached accordingly. Results defined, means successful transfer of responsibility for mosquito breeding to the responsible party with an agreed upon conclusion of necessary steps to be taken.

3. Since successful public relations is initially more important than technical assistance in producing agreement, we must recognize that sensitivity and finesse in our communications are essential for accomplishing our desired results. We must keep in mind that people do not think or react alike and adjustments in time and approach are necessary during the negotiation process. It should therefore be apparent that the initial verbal contact should be made at the administrative level to provide a follow through of program intent.

Failure to properly utilize these principles usually leads to unnecessary confrontation and could eventually result in the initiation of needless legal abatement actions. It is therefore urged that sufficient planning and timing be utilized to provide a positive type program which approaches the responsible party in a cooperative manner and offers the necessary assistance to enable a mutually agreeable solution to the problem.

For the benefit of any skeptic who perhaps wonder if this approach will phase out the need for pesticides, the answer is NO! It is inconceivable that the day will ever come when this will be possible; however, this administrative source reduction program places pesticides where they belong, as an assist to a program rather than being a program. As to the second question of "how can you determine compliance and conclude that a reasonable degree of source reduction has been accomplished by the responsible party?", the answer is that each agency should have in its policy that ALL mosquito sources must be reduced to the practical minimum. As such, the agency must reserve the right to determine what is a "practical minimum".

This preventive process program places the public agency in the proper perspective in relation to the mosquito producer. Rather than continuing to look sideways at careless or extreme water usage, this source reduction emphasis coincidently results in major gains in water conservation along with maximum land usage. It enables the only evident way to avoid many of our regulations through increased biological control and the opportunity to use less toxic pesticides, as well as providing probably the only sound way to cope with resistance. Perhaps of greatest concern today, this administrative source reduction approach dramatically reduces the cost of operation in terms of pesticides and the even higher cost of pesticide application.

This administrative approach is essentially a program of education in how not to produce mosquitoes. Rather than trying to carry on a general program of public education, the administrative source reduction approach is instead aimed directly at the known mosquito problems and the people who cause these problems. Since our records provide us with the knowledge of where our mosquitoes come from, it appears logical and inevitable that we reassign responsibility to where it belongs and practice mosquito prevention in the public interest.

An administrative source reduction program also requires an evaluation of personnel needs for the future. While in past years we have leaned toward the scientists in fulfilling our requirements where technical proficiency has been of prime concern, we now can look in another direction for able assistance. Based on the recognition that to overcome water problems, we must relate to "people problems", and should therefore look for competence in public relations as the foremost factor in choosing key administrative personnel. It should be apparent that in order to prevent mosquitoes we must have successful communications, both written and verbal, as we assume a direct relationship between the agency and the mosquito producer. Particularly is this true in the verbal contacts which must be made in the majority of cases to clarify and justify the need for acceptance of responsibility by the owner of the mosquito source. To this end the agency representative must be capable as an educator, as well as a salesman, but also he must be an advisor with a common tongue.

The case for mosquito prevention programs in California is herewith submitted. At best, we are currently "spinning our wheels" and not keeping pace either with the desired level of mosquito control, or following the trend of government for today. We simply cannot continue to absorb the cost and responsibility for sloppy water usage which produces mosquitoes, when we have another alternative. This alternative should be loud and clear: we must stop "baby sitting" the mosquito producer and truly reassign responsibility to where it belongs. Yes, this can be done in a program which defines "source reduction" as mosquito prevention *per se* and proceeds accordingly.

VERBAL COMMUNICATION TECHNIQUES IN ACCOMPLISHING SOURCE REDUCTION

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While this paper relates to verbal techniques in accomplishing source reduction, I believe it should be clearly indicated that our program approaches source reduction at the administrative level as a means of preventing mosquitoes district-wide. Ours is a program which is based on effective communications in all forms with the mosquito producer.

Four years ago we attended meetings on resistance in the southern part of the San Joaquin Valley, and we had one of two choices to make; wait for another miracle-insecticide, or come up with a different method to combat mosquitoes. We met with our Board of Trustees and came up with a new revised policy of operations, using the greatest weapon posible – the word "resistance". We used the news media to communicate with the public, and informational letters to mosquito producers, using mosquito resistance to the maximum to justify the changes in our program.

To date we have changed our routine spray program of the past into a program of mosquito prevention, which I can honestly say is receiving respect from the farmers, rather than resentment. At first, people were curious as to how our revised policy of operation would affect them, because many felt we were discontinuing our spray program entirely.

This leads us into verbal communication with the public to attain the desired results. Proper conditioning of the mosquito producer is most important. This may be accomplished through the news media by explaining the agency's policy, and by effectively-written communications from the administrator. The letters must positively indicate that corrective steps are expected within a designated period of time, and strongly suggest they contact our office to arrange an appointment.

Appointment Time! This is where the fun begins. This is when, in the first few minutes, your program is either received or temporarily rejected. I say temporarily rejected, because in our program we accept no other alternative than to obtain results.

There are several important qualities needed for successful verbal communications.

A. Directness – I believe your approach should be right to the point, and it should be stated where the responsibility for correction lies. The farmer has received from one to three letters from the mosquito district, and he knows this meeting will probably cost him money. From the very beginning he will be on the defensive, and is not in the mood for small talk. It is most difficult to prepare in advance, what you are going to say, because you have no way of knowing what his reaction is going to be. A method of approach I have used quite successfully is that we have a mosquito problem on your property and need your help. First of all, he begins to think, "How can he help me solve a mosquito problem I don't have!" because in most cases he doesn't believe he is raising mosquitoes or even has water standing on his property. He must understand the importance of correcting all mosquito problems to a practical minimum, and that everyone is being contacted and expected to make the necessary corrections. You must thoroughly know his particular mosquito problems, methods of correction, approximate costs, and local equipment and operators that are available.

B. Sincerity – You must be sincere and honest in your approach, and above all, remember you are a type of salesman, and the product you must first sell is yourself. If you cannot convince the farmer, hopefully without making legal threats, that the correction on his land is necessary, then you haven't really sold the program – or yourself. He must be convinced that regardless of size, every mosquito producer in the district is going to be contacted, and that necessary corrections must be made, with no exceptions allowed.

There must be mutual respect between you and the farmer, and sometimes this may be difficult. How do you change the minds of some people who have never respected the mosquito control program or the personnel working for the district? Let's not fool ourselves . . . . . we have created this feeling of disrespect by spraying the same breeding areas year after year, and getting our jeeps stuck in the same bog holes time and time again. It takes time, effort and a lot of hard work to regain this respect, and you cannot do it by sitting behind a desk or merely sending letters requesting correction of their mosquito problems. You must work with them in the field, get to know them, and also allow them to get to know you. After a period of time they will realize you are trying to work with them and they will begin to trust you and your recommendations. At this time the best publicity you and your program can receive is by word of mouth from neighbor to neighbor. Today people in general are tired of governmental agencies telling them what they can or cannot do. When they realize we are trying to work with them and not against them and when you make every effort possible to work together, this is when you will start regaining their respect and cooperation.

C. Knowledge – First of all you should have knowledge of agency policy for operations. In October 1971 and effective in 1972 our board of trustees revised our policy of operations. Because of resistance we can no longer do unlimited spraying of mosquito sources, but will do a limited spray program on those properties where mosquito breeding sources are corrected and reduced to the practical minimum. This includes maintenance of ponds, sumps, ditches and drains.

Secondly, a knowledge of practical farming is essential. You should understand farming problems such as the different crops -- clover, alfalfa, row crops, etc. One of the most important is irrigation procedures on the different crops.

A third item of importance is practical knowledge of source reduction. You must understand people and be able to communicate with them. One of the most important points to stress is that the majority of mosquito problems can be corrected by a practical solution rather than a complicated, expensive plan.

D. Common Tongue and Common Sense – When talking to farmers, we should refrain from using large words or phrases which only we in mosquito control understand.

I think common sense is the most important. If more people will use common sense and think before speaking, our big problems may be reduced to smaller problems.

These key words which I have stressed today – directness, sincerity, knowledge, common tongue and common sense are what I have used for the past three years in our successful source reduction program. It has worked for me and it can work for anyone who thinks in terms of mosquito prevention.

A GRAPHIC METHOD OF DETERMINING ACREAGE

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A technique by which coordinates on a graph are used to determine acreage has been developed because a method of quickly estimating the number of surface acres in a body of water was needed by workers who make on-the-spot acreage estimates.

The technique involves pacing along two sides of an area and comparing the number of paces to corresponding numbers on a graph -- something on the order of a curving multiplication table. Although relatively accurate, it is not intended to replace the more exact methods of acreage determinations – such as engineering techniques or aerial photography – because the graph-method has at least the following limitations:

1. The reliability of the method will vary with the skill of the pacer and, since the subject is area, any errors in pacing would be squared.
2. The precision of the pacer in turning a 90° angle is very important.
3. The graph will not work on very long and narrow shapes, nor with parallelograms whose acute angles are less than 80°.
4. Irregular shaped areas in rough terrain might be very difficult to classify.

DIRECTIONS FOR USE OF THE GRAPH-METHOD.– A full, geometrical pace (measured from the heel of one foot to the heel of the same foot when it next touches the ground) is five feet long. The pacer should strive to maintain this measure. Ninety-degree turns are desirable when pacing the second side of an area because errors become significant whenever deviations greater than 10° from a right-angle occur. Correct initial classification of the shape which is to be measured is important.

Square or rectangular areas:

1. Begin pacing at any corner and pace off a full side. Use the number of paces as one of the coordinates.
2. Pace off any adjacent side and use the number of paces as the second coordinate.
3. Look at the graph to see where those two coordinates intersect at (or near) a curved line. Since the curves represent acres, the point of intersection represents the approximate number of acres in that particular plot.

Circular areas:

1. Pace off an area as long as the diameter of the circle.
2. Multiply the number of paces by .9 and use the product for both coordinates.

Oval areas:

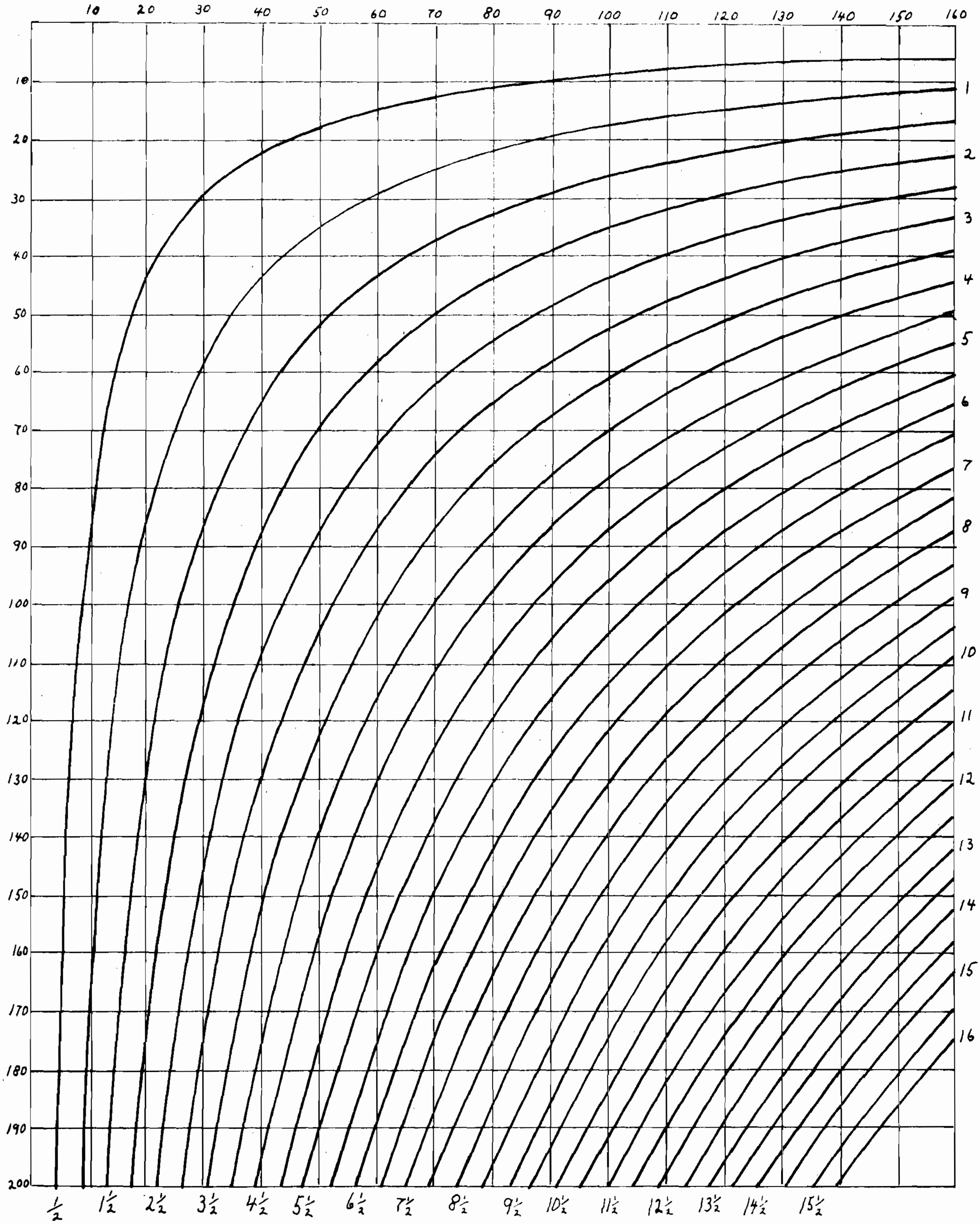
1. Pace off a distance equal to the long axis of the oval and multiply the number of paces by .9 to obtain one coordinate.
2. Make a 90° turn and pace off a distance equal to the short axis of the oval. Multiply the number of paces by .9 to obtain the second coordinate.

Right-Triangle areas:

1. Pace off the shortest side of the triangle and use the number of paces as one coordinate.
2. Pace off the next-to-the-shortest side of the triangle and use one-half of the number of paces as the second coordinate.

Acute, Equilateral, or Obtuse triangular areas:

1. Pace off what appears to be the longest side of the triangle. Use one-half of the number of paces as the first coordinate.
2. Make a 90° turn and pace until you are abreast of the apex of the triangle. Use the number of paces as the second coordinate.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GENETICS OF NEW MUTANTS IN *CULEX TARSALIS*

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ABSTRACT

Viable and easily detected gene mutations are basic to facilitating studies directed towards genetic approaches of mosquito control. Ten such mutants -- spontaneous or induced by EMS -- have recently been isolated from various geographic strains of *Culex tarsalis*, and established as stocks. Of these, four are recessive autosomal eye mutants – two affecting eye color and two the structure of the compound eye. Two other mutants affect the structure of the female palp and one the structure of the male palp; linkage has not yet been determined but they are recessive. Three mutants affect the color of the ventral abdominal scales in both sexes and are autosomal. Of these one is recessive and the other two show incomplete dominance as their mode of inheritance. Some of these mutants will be combined in a marker stock for genetic studies in this species.

AEDES SIERRENSIS (LUDLOW) AS A VECTOR OF FILARIAL WORMS

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ABSTRACT

Certain biological attributes of *Aedes sierrensis* as well as laboratory data suggest that this species has a high vector potential for transmission of the dog heartworm *Dirofilaria immitis.* Experimental work also was conducted on the salt marsh mosquito *A. dorsalis.* Development of the nematode in these two species is reported for the first time. The following California mosquito species also are known to support development of the dog heartworm and also are possible vectors: *Aedes cinereus, A. fitchii, A. taeniorhynchus, A. vexans, Anopheles freeborni, A. punctipennis, Culex pipiens pipiens, C. pipiens quinquefasciatus, C. tarsalis,* and *C. territans.*

The salient features which support the contention that the treehole mosquito is a strong candidate as a vector of dog heartworm are as follows:

1. Feeds readily on dogs in nature.
2. Vector of another filiarial worm in California: *Setaria yehi* in deer.
3. Widespread in California (recorded from 52 counties), breeds in close proximity to man and dogs in many urban and suburban areas.
4. Adults are long-lived.
5. Adult populations appear to remain localized if host populations are available thus increasing the chance of multiple feedings.
6. Laboratory studies indicate that adults support the development of ingested filiarial worms to the infective stage.

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MOSQUITO PROBLEMS ASSOCIATED WITH MAN-MADE IMPOUNDMENTS IN WESTERN AND MIDWESTERN UNITED STATES

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ABSTRACT

The development of man-made impoundments in the western and midwestern United States has caused many serious mosquito problems. Failure to incorporate such preventive measures as shoreline conditioning, marginal drainage, vegetation control, and water level management into the design, construction, and operation of impounded water projects results in the production of mosquito and other vector populations that increase the risk of vector-borne diseases. Methods that will minimize mosquito production in impoundment projects are reviewed. The value of retrospective studies for correcting existing mosquito problems and evaluating recommendations for long-term mosquito prevention on water resources projects is discussed.

INTRODUCTION

In the western and midwestern United States various types of man-made impoundments serve a wide range of needs in the conservation, management, and utilization of water resources. These impoundments, both publicly and privately developed, range from large lakes or reservoirs with surface areas of thousands of acres to small farm impoundments of less than one acre. In most cases, the large impoundments serve multiple purposes, which may include those associated with navigation, flood control, hydroelectric power generation, irrigation, municipal and industrial water supply, and fish and wildlife. Examples of the small impoundments, usually developed to serve a single purpose, include livestock watering ponds, log storage ponds, waste stabilization lagoons, fish and waterfowl refuges, recreational reservoirs, and floodwater detention reservoirs such as those developed by the Soil Conservation Service. The combined total area of these small impoundments in the western and midwestern States is substantial because so many have been built.

Studies conducted over the past 4 decades have shown that man-made impoundments frequently become prolific sources of mosquitoes.

NATURE AND SCOPE OF MOSQUITO PROBLEMS ASSOCIATED WITH MAN-MADE IMPOUNDMENTS

Methods for controlling the mosquito problems associated with impounded water projects in the Tennessee Valley have been evaluated and reported for many years (Bishop and Gartrell 1944; Christopher and Bowden 1957; Gartrell 1951; Gartrell et al. 1972). The most important public health problem concerned the control of malarial mosquitoes where the basic principles for source reduction of mosquito-producing habitats associated with impounded water were clearly demonstrated during the 1930's and early 1940's by the Tennessee Valley Authority (Hess and Hall 1943, 1945; U. S. Public Health Service and Tennessee Valley Authority 1947; Wiebe and Hess 1944). In the western and midwestern regions of the country, mosquito problems associated with impounded water projects were not widely recognized and were not intensively studied as were earlier malaria outbreaks in the vicinity of man-made impoundments in the southeastern States. Nevertheless, mosquito problems associated with impoundments in the Midwest have been documented (Edman 1964; Harmston 1964), and there has been concern in certain western and midwestern States relative to the apparent relationship between sporadic outbreaks of encephalitis among humans and horses and the production of vector mosquitoes in habitats associated with water resource projects (Hansen 1952; Hess 1958). These have been particularly associated with the development of irrigation agriculture (Harmston et al. 1956; Hess et al. 1970). Researchers in public health agencies and elsewhere have conducted studies to gain a better understanding of mosquito problems in the diverse ecologic habitats throughout the western and midwestern States. Effective mosquito control measures need to be implemented because of the increasing number of problems that are directly associated with water resource development and use.

The results of extensive studies on mosquito control at large impoundments in the southeastern United States have already been cited. Likewise, articles have been published on the results of studies dealing with mosquito control problems associated with various small impoundments such as log storage ponds (Harmston et al. 1960; Ogden et al. 1960), floodwater retarding reservoirs (Carreker 1965), and waste stabilization lagoons (Beadle and Harmston 1958; Myklebust and Harmston 1962; Rapp and Harmston 1964; Rozeboom and Hess 1944).

The aforementioned studies on mosquito problems associated with impounded water have provided basic information on the types of mosquito-producing habitats frequently produced by such projects. These habitats include shallow areas, usually in the upper reaches and tributary embayments of the impoundment, which contain emergent vegetation of flotage and are subject to prolonged or intermittent flooding during the mosquito breeding season. Undrained depressions, borrow pits, and marshy habitats within the summer fluctuation zone; seepage habitats below dams; and ponded water behind dikes and levees are also typical areas in which water may stand long enough to produce mosquitoes. The extent and productivity of such habitats, and the kinds of mosquitoes produced therein, depend upon the interaction of various diverse factors. These include location and topography of the impoundment basin, the type and amount of vegetation subject to shallow inundation, and the frequency and duration of flooding (which may be affected by climatic conditions), and the multipurpose functions of the impoundment.

Despite the apparent complexity of factors which influence mosquito production associated with artificial impoundments, it has been demonstrated that conditions favoring mosquito production can be minimized or eliminated by incorporating source reduction measures into the planning, construction, and operation of impounded water projects. Various types of measures that have been shown to be effective when used singly or in combination include shoreline modification through deepening and/or filling (Hess and Hall 1943; Rozeboom and Hess 1944; Rees and Winget 1969), marginal drainage to assure that potential ponding areas subject to flooding at high pool elevations will be dewatered at lower pool elevations (Gartrell and Kiker 1948), removal of vegetation and flotage from protected embayments (Rees and Winget 1969; Gartrell and Kiker 1948), provision of drains to eliminate or minimize seepage habitats below dams; installation of dewatering facilities or the use of other measures to minimize mosquito breeding in ponded areas behind dikes and levees (Gartrell and Kiker 1948), and use of water level management to control or interrupt mosquito production (Hess and Kiker 1943).

THE PROGRAM OF THE PUBLIC HEALTH SERVICE RELATED TO MOSQUITO PROBLEMS ASSOCIATED WITH WATER RESOURCE DEVELOPMENTS

In recognition of the apparent relationship between water resource development and production of so-called "encephalitis mosquitoes", the Public Health Service (PHS) in 1952 established a Water Projects Section in its Vector Control and Investigations Branch to guide the PHS program for the prevention and control of mosquito problems of public health importance associated with water resource developments (Hansen 1952). Headquarters of this program was in Salt Lake City, and field offices were established in several of the western and midwestern States. The major objectives of the new program were to investigate the nature and scope of mosquito problems associated with water resource projects; to develop effective control measures compatible with the various phases of water use; to assist the various construction agencies in "building" preventive measures into new projects; and to promote active interest in minimizing mosquito production in existing water resource projects. These objectives have been pursued with varying levels of budgetary support, staff, and facilities within the Center for Disease Control (CDC). Various reorganizations and staff relocations have occurred. The present center for this activity is the Water Resources Branch in CDC's Vector-Borne Diseases Division at Fort Collins, Colorado. For the most part, these activities have dealt with irrigation-related mosquito problems, including those associated with water storage and distribution facilities, management of water on irrigated lands, and drainage. To a lesser degree, the activities have dealt with mosquito problems associated with water impounded for purposes other than irrigation, including those of flood control, recreation, and fish and wildlife enhancement. In addition to being closely associated with various Federal agencies involved with water resource development and use, the activities have been coordinated and conducted in cooperation with counterpart groups in State and local health departments. Cooperative pre- and post-impoundment studies conducted from 1960 to 1966 on the Crooked River Project in Oregon demonstrated that certain soil and water management measures beneficial to agriculture are likewise effective in eliminating mosquito-producing areas (Water Resources Council 1968).

CDC evaluation reports have been transmitted to water resource development agencies; in these reports the various measures for minimizing production of mosquitoes that should be considered in planning, constructing, and operating water resource projects are outlined. Since the 1950's, these activities have involved office reviews of plans of proposed projects and, in a relatively few cases, a pre-construction survey of the project area. The data obtained from pre-construction site surveys are extremely valuable in assessing the potential impact of project development on mosquito problems and in determining pre-impoundage corrective measures, but such surveys have been severely limited by restrictive budgets.

Retrospective Studies.–Only a few post-construction, or retrospective, field studies have been made of projects that were reviewed and evaluated prior to construction to determine if recommended preventive measures were incorporated into project construction and operation. The effectiveness of such measures, or the need for any additional corrective work, was also evaluated. Such retrospective studies are an important aspect of the present program. Since specific preventive measures are not readily available for all potential problem areas that may result from project construction, these studies should provide better insight into the types of problems that have developed. They also are expected to provide the opportunity to improve recommendations for long-term control.

Retrospective field surveys were made in 1974 of the following U. S. Army Corps of Engineers' projects: the John Day Project, Oregon-Washington; the Lower Granite Project, Washington-Idaho; and the Lewis and Clark Lake, Nebraska-South Dakota. The following Bureau of Reclamation projects were also surveyed: the Seedskadee Project, Wyoming; and the Baker Project, Upper Division, Oregon. These studies showed that the development of all of these projects created important and extensive mosquito habitats. The studies also showed that the construction agencies did not follow the vector control measures recommended in the pre-construction evaluation report. Representatives of these agencies have expressed a willingness to implement control measures.

During 1975, work is planned in cooperation with the Bureau of Reclamation and the Corps of Engineers at two water resources project locations. At each site the vector-producing areas will be delineated, and corrective measures will be initiated. Fish and game interests will be considered in any habitat modification proposals so as to minimize mosquito production while maintaining wildlife habitat.

SUMMARY

In summary, most mosquito problem areas associated with the wide variety of impounded water projects have resulted from conditions that could be minimized or eliminated by adequately preparing the impoundment basin during construction and implementing source reduction measures following impoundage. In most cases, personnel in agencies that construct and operate these projects lack basic knowledge about mosquito biology and the principles of mosquito control. Without such information and knowledge, agencies such as the Bureau of Reclamation, Soil Conservation Service, and the U. S. Army Corps of Engineers find it difficult to incorporate recommended vector control measures into the construction and operation of their projects. Indeed, the complexity of certain conditions associated with various impounded water projects may also confound entomologists and vector control biologists with much experience in vector problems associated with water and related land resource developments. On the brighter side, recent retrospective studies and subsequent contacts with water resources development agencies have resulted in measures being implemented to minimize mosquito production at selected man-made impoundments.

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DUGESIA DOROTECEPHALA FOR CHIRONOMID CONTROL IN SHALLOW WARM WATER HABITATS[1]

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ABSTRACT

The planarian *Dugesia dorotocephala* (Woodworth) is a potentially effective biological control organism which offers possibilities for chironomid midge larval control. In January 1974 we first observed a significant mortality of *Chironomus* spp. larvae in an aquarium stock culture of *D. dorotocephala*. Complete natural destruction of chironomid larvae by *Dugesia* was also periodically observed by one of us (E. F. Legner) in portions of the Coyote Creek and other drainages in the Los Angeles area since 1971. Therefore, carefully controlled laboratory and large scale field experiments are being undertaken to determine predation effectiveness of *D. dorotocephala* when artificially inoculated in naturally breeding populations of chironomid midges. Preliminary results indicate that over 85% control of several chironomid species may be attainable in practical utilization.

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MOSQUITO CONTROL THROUGH VERTICAL DRAINAGE OF PONDED TAILWATER IN FLOOD-IRRIGATED PASTURES

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Around Fresno, California, *Aedes nigromaculis* (Ludlow) is the most abundant and pestiferous mosquito that breeds in irrigated fields. Its chief habitats are the low areas and the ponded tailwater in flood-irrigated pastures. Without much doubt *A. nigromaculis* in recent years has increased in importance around Fresno due to a rather massive movement of families to outlying, small-acreage, residential sites that have been carved out of larger farms by real estate developers. The new owners of the small-acreage sites indulge in a wide variety of construction activities ranging from moderately priced dwellings to elaborate homes with beautiful landscaping, patios, swimming pools, and other outdoor recreational facilities. In some instances all of the land in excess of that required for the house and yard is used for income crops such as fruit and nut trees, or grape vines; but since one of the principal forces motivating the movement to the country is the desire to own a horse, a great number of owners end up with small pastures.

Water rights from the original farms generally go with the newly created homesites, and this so-called "ditch" water, purchased from irrigation districts, quite often is supplemented by irrigation water from privately owned, newly bored or previously existing wells. Thus most new owners have access to an abundance of irrigation water. The combination of small, flood-irrigated pastures, the new owners' lack of experience in irrigation, and the abundance of water, results, almost invariably, in gross overflooding, ponded tailwater, and a high level of mosquito production. But the average small-pasture owner can see no practical solution to the dilemma of mosquito production other than to proceed as though it is a regrettable but integral part of the irrigation process and as such the problem does not devolve on the owner but on the mosquito control districts.

To date both the Fresno and the Consolidated mosquito control districts have in fact provided excellent larval control in the suburban pastures through the laborious process of searching on foot for and hand treating the pockets of larvae that develop during every watering cycle. But the control practice is burdensome and expensive and, in addition, gives promise of becoming more so unless a viable solution evolves for decreasing larval production.

The intractableness of the problem induced us to reexamine vertical drainage as a process that might be developed into a tool that the control districts could use in the small pastures. This report summarizes some of the findings and conclusions made in vertical drainage research in 1974.

MATERIALS AND METHODS.—Our boring rig was described in the 1973 proceedings of the CMCA (Lewis et al. 1973). Our 6-inch-diameter, 8-ft-long auger has since been lengthened to 12 ft, however, and the boring rig has been modified to the extent that boring is a one-man operation.

All holes bored in 1974 were 11 ft deep and were filled with coarse, ¾-inch aggregate as were the holes we bored in previous years. Our hole-filling operation has evolved into a rapid, mechanized, one-man task also with the aggregate conveyed to the holes with a hydraulically powered auger that is attached to the rear of a modified dump truck.

Most fields that produced heavy mosquito populations had areas with continuously standing water that had to be pumped out at least 4 days before we could bore in them. We evacuated those ponded areas with a unit consisting of a self-priming, centrifugal pump powered with a 1-cylinder gasoline engine. The unit was mounted on a 2-wheel trailer.

RESULTS AND DISCUSSION.—The first small field we attempted to drain produced mosquitoes in low spots that were scattered somewhat at random in 6 of the 9 checks. We bored from 2 to 5 holes per low spot for a total of 18 holes, which thereafter caused the water to disappear from the low areas before emergence could occur.

But in all other small pastures we found ourselves dealing primarily with tailwater at the lower ends of the fields. Almost invariably mudholes were involved, and sometimes ditches or remnants of ditches contributed to the ponding process. The mudholes, ditches, and other ponded areas either remained inundated between waterings or would not dry sufficiently to support our equipment. To get the soggy areas dry between waterings we rigged up the pumping unit described above and used it to evacuate the water from the problem areas, a task that had to be completed at least 4 days before we could bore.

Then we bored in 9 more small pastures. Three had ditches as well as muddy areas along their lower ends. In 2 pastures we bored only in the ditches since we judged that they were deep enough to drain off the extra ditch water providing the drainage holes would handle a sufficient volume of water. But in the pastures without ditches we bored only a proportionate share of the holes along the lower ends of the checks while by far the greater number were bored for varying distances up field in order to evenly encompass the total of each ponded area.

During the course of the investigation it became evident that concentrating the holes in collecting ditches along the lower ends of the pastures yielded better results per hole than the even distribution of holes up field in individual problem areas. The explanation was that as water drained downward, the receding water lines, in the sloping, nonditched fields left bare an ever increasing number of holes up field that contributed nothing to ongoing drainage, whereas in ditches that were laid out on the level, every hole drained off its proportionate share of water on a continuing basis.

Up to that point in our investigations we had associated collecting ditches with return systems only, but the results clearly indicated that we should examine further the possibilities of substituting drainage holes in collecting ditches for up field boring. But since we lacked ditching equipment of our own, we were limited in 1974 to making observations on only 2 additional collecting ditches.

In the first collecting ditch, made for us by the owner, we bored 10 holes across the lower end of a small, narrow pasture on which we previously had bored an inadequate number of holes up field. At the lower end of this field, water ponded to a depth of about 18 inches and extended up field for about 75 yards. In the two watering cycles that we followed, no mosquitoes emerged during the first, but some did during the second. We estimate that about 10 additional holes would give adequate drainage at every watering.

In the second collecting ditch – also made for us by the owner – we bored our holes in a wide, rather deep channel across the lower end of a 15-acre, non-suburban pasture that regularly produced a multitude of mosquitoes from a considerable area of ponded water. We were prepared to bore as many as a hundred holes in the ditch but limited the number to 35 when our auger penetrated into a deep, very permeable, gravelly stratum, probably an old stream bed, that we judged would take water at a fast rate. The 35 holes did in fact drain off the surplus water within a matter of hours, a rate that exceeded our expectations, and even our most extravagent hopes by several fold. If vertical drainage would work in all fields as in that one, the process undoubtedly would be adopted by the owners of virtually every problem alfalfa field and pasture in California's Central Valley as a cheap but effective substitute for return systems.

As to the over-all efficacy of our boring process, before the season was over we obtained what we considered to be adequate drainage in 11 of the 12 fields we bored in. The one pasture, as mentioned above, needs approximately 10 more holes.

We believe that fewer holes would have given adequate drainage in most pastures where we bored if collecting ditches had been used. More importantly though, we declined to bore in most fields we examined because the ponded surface areas involved extended so far up field that more holes would have been required than seemed practicable. We believe that if collecting ditches are used, vertical drainage would be a practical operation in most or all of those fields.

By our standards, we have obtained adequate drainage when all water drains off within 3 to 4 days of the cutoff time. But adequate drainage by that definition did not always prevent mosquito production. For two of the owners on some occasions ran water on their fields for a period of 5 full days. Under those conditions, large 4th instar larvae were present when the water was cut off and some emergence did occur even though drainage was completed within the allotted time span.

As to future research plans, we are equipping with appropriate implements a small farm-type tractor with which we can make our own collecting ditches. We have plans also that will further improve the efficiency of our boring rig.

As to the length of time a set of drainage holes may remain functional, in the only field where our observations extend for more than one season, the holes are still functioning after 3 full years of use. If holes in other situations would remain unclogged for a comparable length of time, our opinion is that vertical drainage through bored holes has a very world-wide potential for mosquito control since a large proportion of the *Aedes* mosquitoes produced throughout the world breed in periodically flooded areas in alluvial soils along rivers, estuaries, lakes, bays, seas, or oceans.

ACKNOWLEDGMENTS.—We gratefully acknowledge the help and advice given us by the following: Donald A. Merritt, Manager, Consolidated MAD, and Richard C. Husbands, BVC & SWM, California State Department of Public Health, for Peer reviews of the manuscript; T. Cheney of Valley Foundry and Machine Works, Fresno, California, and George Ogden, Jr., Clovis, California, for advice and assistance in the design and construction of our equipment; the personnel of the Consolidated and the Fresno MAD's whose advice and assistance were essential to us; and to all members of our laboratory staff.

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THE OCCURRENCE OF AN *ENTOMOPOXVIRUS* IN A FIELD POPULATION OF *CHIRONOMUS* SP.[1]

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In January, 1974, an *Entomopoxvirus* was discovered in a colony of chironomid midges established with larvae collected in November, 1973, from the A-3 flood-control channel in Orange County, California. This midge species is abundant in the Coyote Creek area. It is a member of the *Chironomus decorus* complex, similar to the species referred to as *Chironomus* sp. no. 51 by Biever (1965).

Poxviruses were first described from insects by Vago (1963) and have been described from four species of midges, two from Europe and two from the United States (Granados 1973). Information on the epizootiology of *Entomopoxvirus* in natural populations is scant.

This report presents a morphological description of the virus and a general description of the pattern of infection in field populations of *Chironomus* sp.

METHODS AND MATERIALS.—Area of Study: Extensive field surveys were conducted in two Orange County flood-control channels designated as A-3 and A-1, near outflow points into Coyote Creek. The A-3 channel is sandy-bottomed and 35 to 40 feet wide; it contains standing water throughout the summer and fall, though summer run-off periodically produces a measurable flow. Substrate samples were taken from an area between 1 and 2 yards from the south bank. Water depth in this area varied from 2 to 6 inches. The samples were taken approximately a quarter of a mile above the outflow into Coyote Creek.

The A-1 channel is concrete-lined, approximately 12 feet wide, with the bottom covered by a sand substrate varying from 2 to 4 inches. Samples were taken from the swiftest water-flow area of the channel (surface flow rate of 58 to 75 feet per minute), and at a water depth of 2.5 to 3.5 inches over the period sampled. The A-1 samples were taken about 50 yards above the junction with Coyote Creek.

Sampling Technique: Samples were obtained using small coring devices made from cylindrical 40 dram plastic vials. Each device covered 2.75 square inches of the substrate and samples were taken to a depth of 2 inches. Each sample was returned *in toto* to the laboratory, thus preventing the loss of substantial numbers of second- and third-instar larvae occurring when samples are sieved or otherwise volumetrically reduced in the field. Larvae were removed by floatation with concentrated magnesium sulfate. Eight replicate core-samples were routinely taken per site; when populations were low, samples were pooled and up to 40 core-samples taken per site.

Electron Microscopy: Diseased fourth-instar *Chironomus* sp. were fixed by immersion for one hour in cold (5°C) 2.5% glutaraldehyde, buffered with 0.1 M KPO_4. Larvae were postfixed for an hour in 1% OsO_4, dehydrated in acetone series and embedded in Spurr's resin or Epon 812. Thin sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-11 electron microscope.

RESULTS AND DISCUSSION.—Cytological examination of infected larval cells reveals virions similar to those previously described from chironomids as reviewed by Gradados (1973). Each virus particle appears to be composed of three distinct parts. The core consists of a light-staining shell 35 nm to 40 nm thick surrounding a dark-staining internal space. Cuboidal when cut in one plane, the core is biconcave (bar-bell shaped) when cut in another plane. It is surrounded by a dark-staining envelope measuring 25 nm in thickness. Lateral bodies are sandwiched between the envelope and core in the biconcave region of the core. The overall dimensions of the virions are approximately 300 nm by 260 nm by 160 nm.

The mature virions are occluded within proteinaceous inclusion-bodies (IB) that are variable in size. Smears of infected larvae reveal ellipsoidal-shaped bodies up to 8 um in length and 5 um in width. Examination with phase microscopy shows that a variable number of inclusion bodies may develop per cell, yet as many as 10 developing IB have been observed in a single cell.

In accordance with other descriptions of chironomid poxviruses from the United States, inclusion body development appears to be limited to cells cytologically similar to hemocytes. Accumulations of infected hemocytes appear as white spots scattered irregularly throughout the body of the diseased larva. As the infection progresses, the normally deep-red color of the larva gradually pales to a whitish hue. Recognition of infected larvae in early stages of disease is aided by the tendency of infected hemocytes to collect in the dorsal region of the 9th abdominal segment just posterior to the heart.

During August, 1974, an increase in the larval *Chironomus* sp. population in A-3 was followed by a rapid rise in the incidence of *Entomopoxvirus* infection (Figure 2). Disease incidence at the peak of the epizootic was 97.4% of the total larval population. The devastating effect of the epizootic was evidenced by an accumulation of thousands of extended, frankly infected, fourth-instar larvae on the substrate-surface throughout A-3. A rapid collapse of the larval *Chironomus* sp. population to a low level occurred and persisted for two weeks. Increases in the larval population in late September occurred during a period of measureable water flow in the channel (22 feet per minute on 23 September). The incidence of virus infection rose sharply and larval density was subsequently depressed.

The incidence of *Entomopoxvirus* infection in the flowing A-1 channel was at a lower level than seen in A-3 (Figure 3). The larval *Chironomus* sp. populations again fluctuated, but a rapid increase in the incidence of disease was not associated with increases in the host-population

[1]This study was partially supported by the Orange County Mosquito Abatement District.

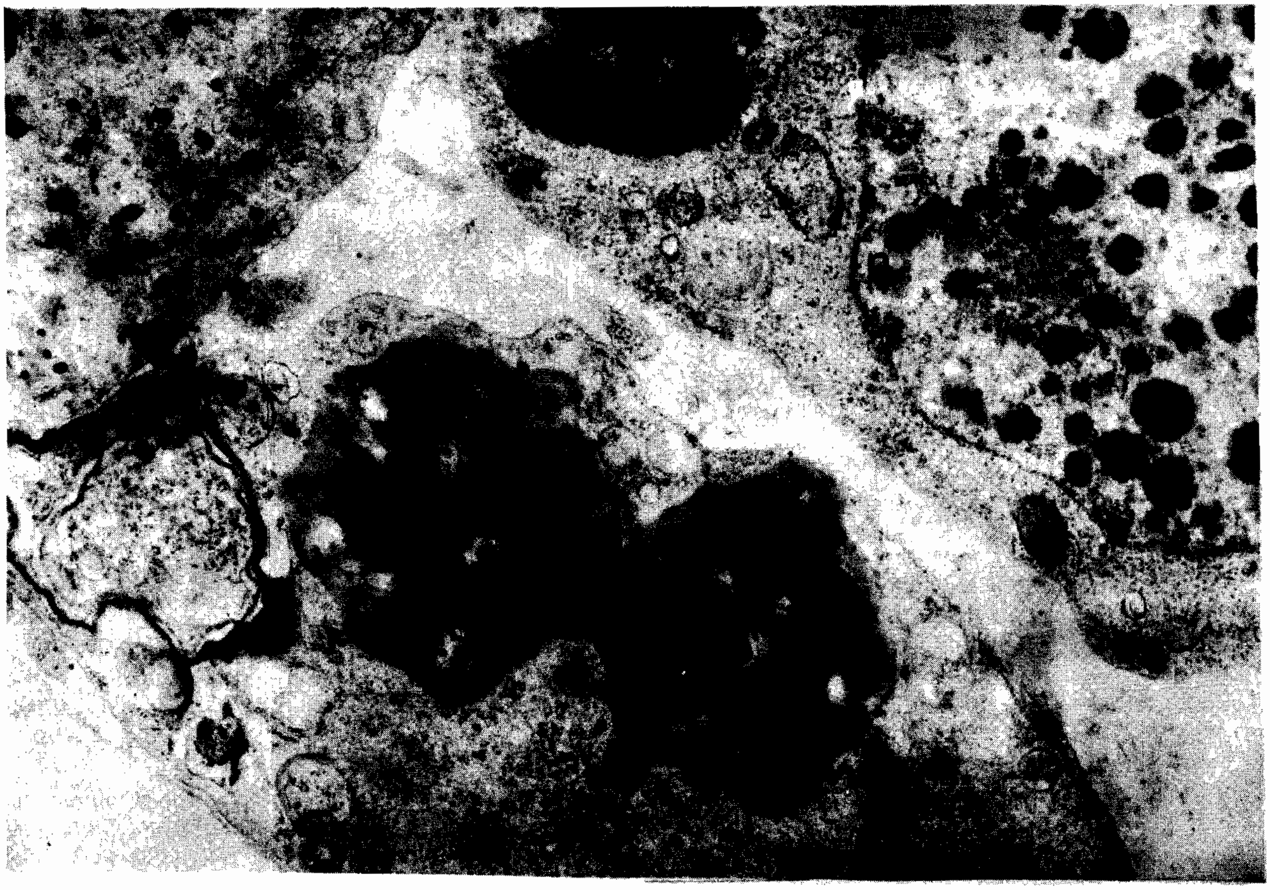

Figure 1.—Virus-infected hemocytes from a fourth-instar *Chironomus* sp. no. 51. n-nucleus, ib-inclusion body, v-virion. X28,500.

density. Peak incidence of disease during September and October occurred during periods of relatively low host density.

The virus infection rates in substrate-samples from a Coyote Creek site below the A-1 and A-3 outflows fluctuated between 0 and 10% during August and September. A different site above the A-1 and A-3 outflows produced no infected larvae during this period. In mid-October the infection rates at both Coyote Creek sites rose rapidly to peak at 36.1% and 36.7% above and below, respectively, on 21 October. Also in October, substrate samples taken from areas of A-1 protected from the main-channel water-flow by sandbars yielded infection rates as high as 52%. Run-off from heavy rains during the last week of October eliminated *Chironomus* sp. populations from all the study areas.

Surveys taken in other flood-control channels in Orange County indicate that *Entomopoxvirus* is not limited to the Coyote Creek area. Collections from the C-2 channel (parallel to the east fence of the U. S. Naval Weapons Station in Seal Beach) revealed infection rates of 6% from samples (averaging 30 larvae per core-sample) on 23 September and 24% from samples (averaging 43 larvae) on 14 October. This midge population was isolated to a small area of thick black mud below a storm-drain outlet. The remainder of the channel substrate was a fine gray clay, devoid of a substantial *Chironomus* sp. population. A site in the D-3 flood-control channel (adjacent to the Santa Ana River near Garfield Avenue) produced an infection rate of 70% from samples averaging 6.7 larvae per core on 14 October. Both channels contained water without a measurable surface current at the time of collection.

Several factors apparently are involved in describing the dynamics of *Entomopoxvirus* infections in a larval *Chironomus* sp. population. Among the more important parameters are the rate of water flow, host density, host age structure, water chemistry, and the nature of virus exposure. An analysis of the role these factors play in the epizootiology of the chironomid *Entomopoxvirus* infection is currently under investigation.

A general understanding of the epizootiology of this virus disease is required before its potential as a biological control agent can be assessed. Nevertheless, it is evident from these preliminary data that under certain ecological conditions the *Entomopoxvirus* does exhibit substantial natural control of *Chironomus* sp. populations.

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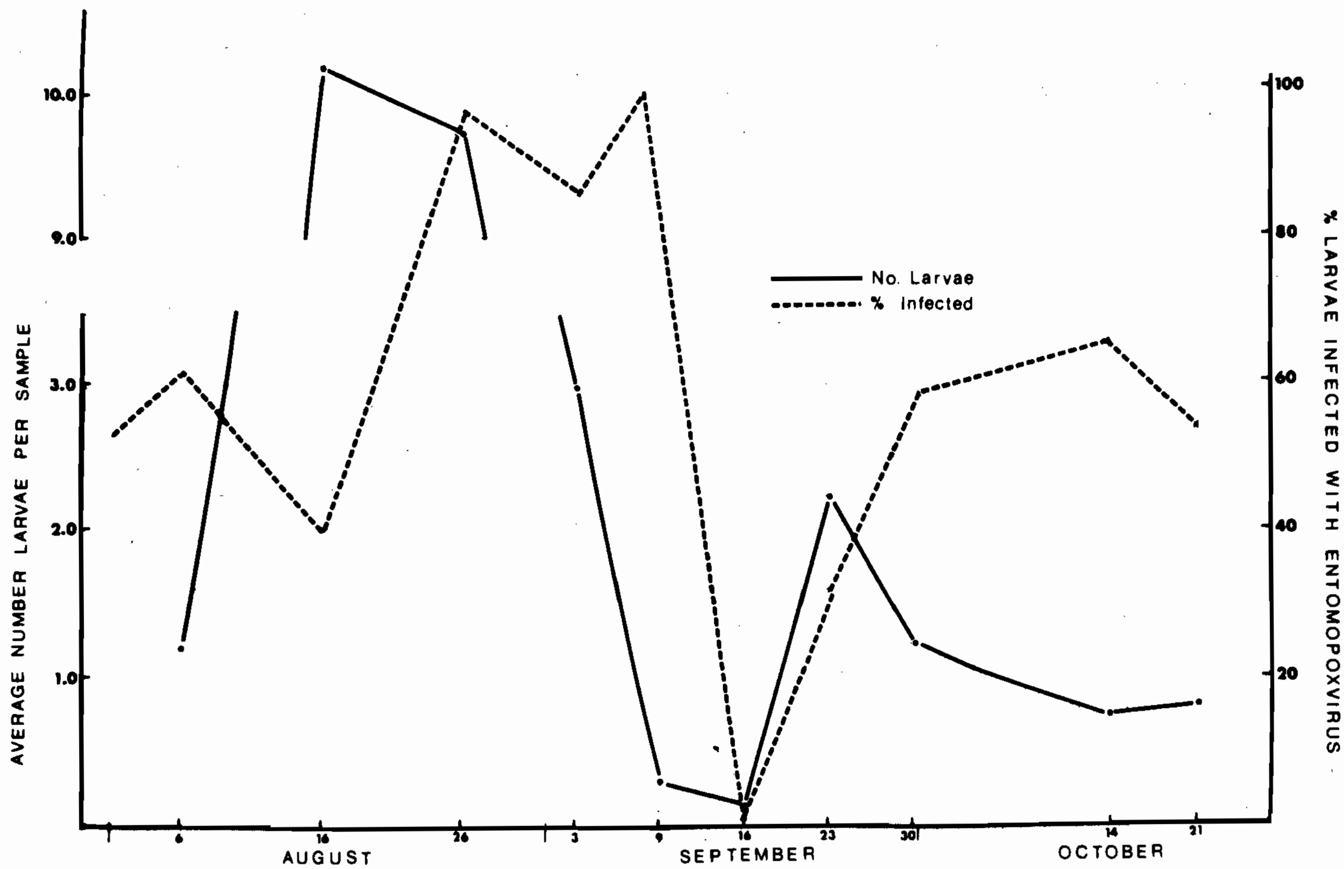

Figure 2.—Results of surveillance of the A-3 flood-control channel for *Entomopoxvirus* infected *Chironomus* sp. larvae. Average is based on a minimum of eight replicate samples each covering 2.75 square inches area of substrate. Average and percent includes all instars collected.

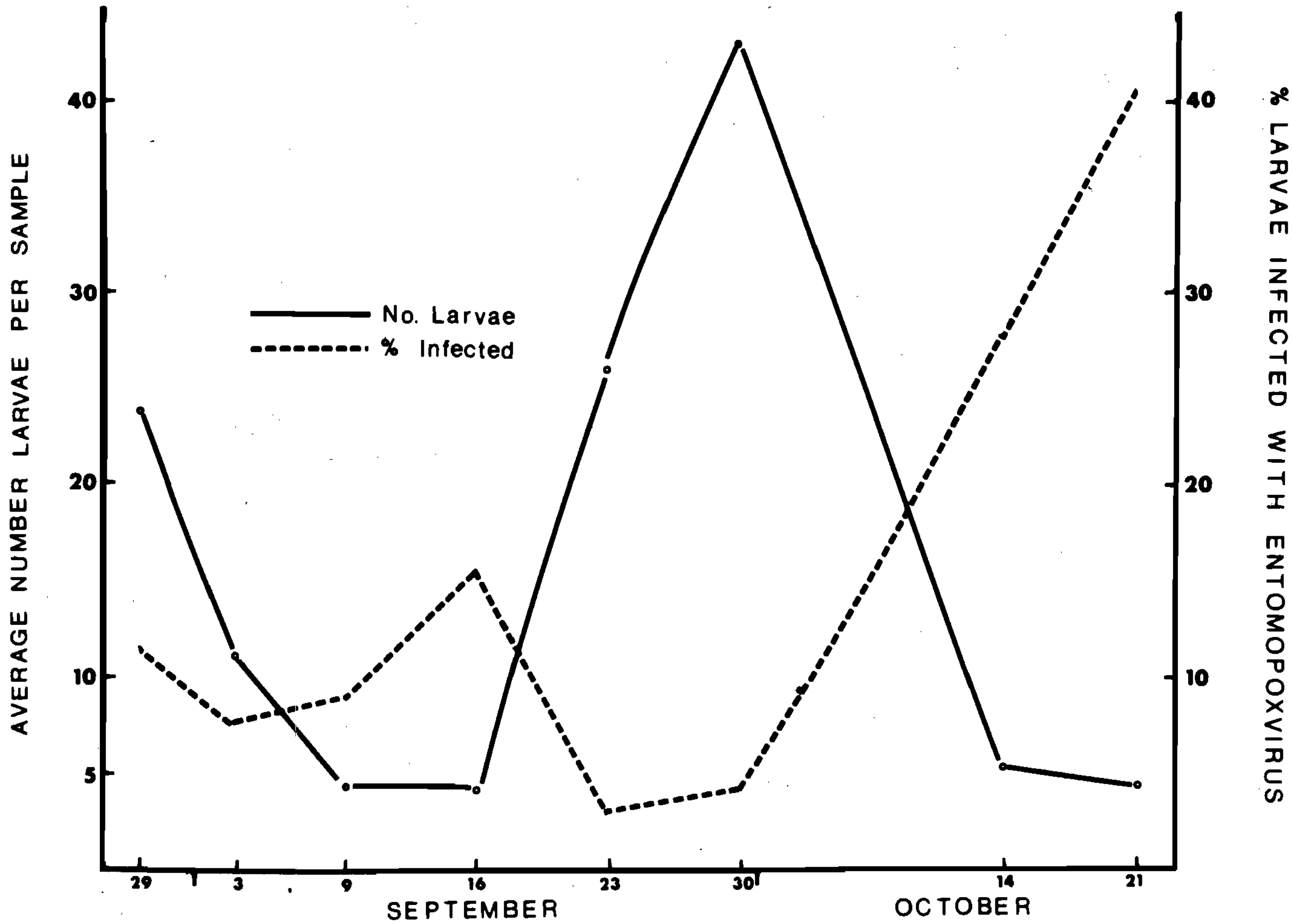

Figure 3.–Results of surveillance of the A-1 flood-control channel for *Entomopoxvirus* infected *Chironomus* sp. larvae. Average is based on a minimum of eight replicate samples each covering 2.75 square inches area of substrate. Average and percent includes all instars collected.

POPULATION DENSITY OF CHIRONOMID MIDGE LARVAE IN RELATION TO POSITION DOWNSTREAM IN PAVED CHANNELS[1]

E. F. Legner[2], R. A. Medved[2] and G. C. McFarland[3]

ABSTRACT

Density of *Chironomus* spp. and combined *Tanytarsus* and *Cricotopus* spp. larvae was monitored monthly in the Coyote Creek, Los Angeles area, for a 4-year period. Regression analyses to determine the effects of position in the drainage system on chironomid density showed a general significant trend for greater abundance upstream, although a few, usually nonsignificant, reversals were detected. This effect was especially pronounced during the summer months when midge densities are greatest. Causes for the effect were not correlated with water quality analyses, but may involve greater nutrient availability and less street pollution upstream that combined may favor adult oviposition and larval development. Other factors such as diseases, invertebrate predators (e. g., planaria), preferred oviposition sites, etc., are under investigation. Reducing this effect, i. e., producing a "down stream" condition upstream, might be significant to midge control in the system.

The proximity of urban development to paved river channels in the Los Angeles basin has focused increased attention on chironomid midge production in such habitats. One channel, Coyote Creek, is unique in that it contains a shallow, constantly flowing stream of highly eutrophied water. Chemical control with known materials and formulations is difficult because of the dilution factor and the neutralizing effect that waters of high organic content have upon many insecticides. Also, there is the danger of wildlife, principally birds and the seacoast fauna, that is posed by concentrations of insecticides sufficient to overcome dilution and organic deactivation. Biological control using fish (Bay & Anderson 1965, Legner & Medved 1973) is difficult because water is not consistently deep enough to sustain effective species such as *Cuprinus carpio* L. and *Tilapia* spp., at the required densities. Finally, whereas chironomids in stable habitats characteristically limit their production through pollution and food destruction, those in Coyote Creek inhabit a substrate that is constantly cleansed and resupplied with new food. The potential for prolonged nuisance from sites such as Coyote Creek is thus substantially greater than from other sources and the control more difficult.

MATERIALS AND METHODS.—The densities of *Chironomus* spp., and *Tanytarsus* and *Cricotopus* spp. larvae combined, were monitored monthly from October, 1970 through 1974. Originally, samples were taken from midstream and near both banks at 12 different comparable sites beginning 1 Km below the beginning of the paved portion of the channel and extending 18 Km downstream. Analyses showed midstream densities, although lower, to be positively correlated with the near bank densities so that only the latter were finally sampled. Sample sites were comparable with one another as to water depth and current velocity. The Southeast and Orange County Mosquito Abatement Districts treated Coyote Creek at irregular intervals with Dursban and malathion in extremely low concentrations that did not favor a higher application rate either upstream or downstream.

A modified Surber sampler was used to collect chironomid larvae (Figure 1). The circular sampler was 15.2 cm diam. and 20.3 cm high, with a 0.5 mm mesh Saran screen extending to 71.1 cm from one end to a screened 150 cc plastic collection vial (Figure 1). The sampler was placed vertically on the substrate, held in place and a metal scraper used to stir the bottom area clean of sand and debris down to the concrete base. Midges and larger debris were collected in the 150 cc vial. Two samples were taken at random per each sample site, and chironomid larvae 5 mm and greater in length were counted in the laboratory with a 6 X magnifier. An effort was made to sample substrates of uniform thickness at all sample sites on any given date.

Data were examined with regression analyses after being visually scanned on graph paper to determine the effects of position in the drainage system on chironomid density.

RESULTS AND DISCUSSION.--Plotted data for the relationship between chironomid density and the distance downstream showed a linear trend. Figures 2-4 show this relationship between chironomid density and the distance downstream after analysis, starting 1 Km below the beginning of the paved portion of the channel and extending to 18 Km.

Figure 1.—Modified Surber sampler used to collect chiromid midge larvae from shallow flowing channels.

[1]Research partially supported by grants and assistance from the Southeast and Orange County Mosquito Abatement Districts.

[2]Division of Biological Control, University of California, Riverside, California 92502.

[3]Southeast Mosquito Abatement District, 9510 South Garfield Avenue, South Gate, California 90280. (Deceased – January 12, 1975.)

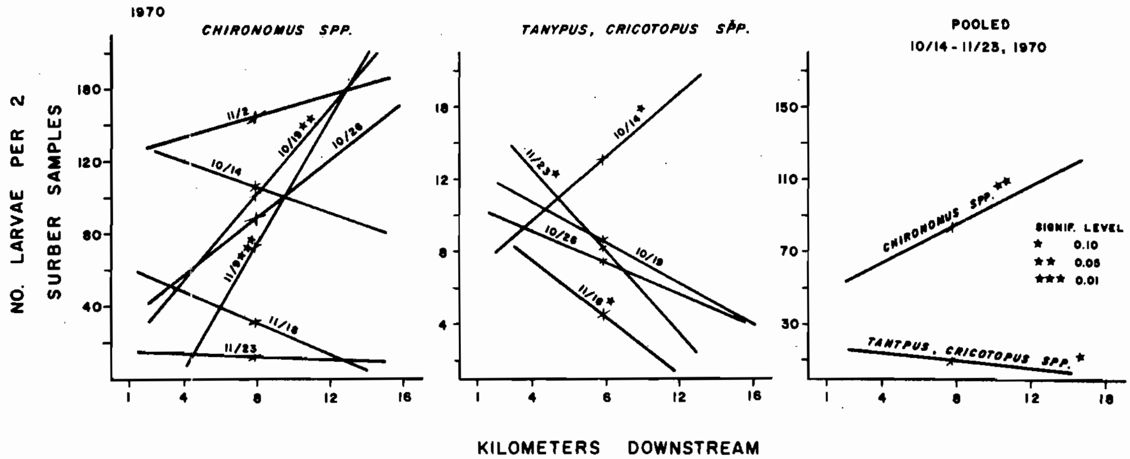

Figure 2.–Relationship between chironomid density and distance downstream in the Coyote Creek. Oct.-Nov., 1970.

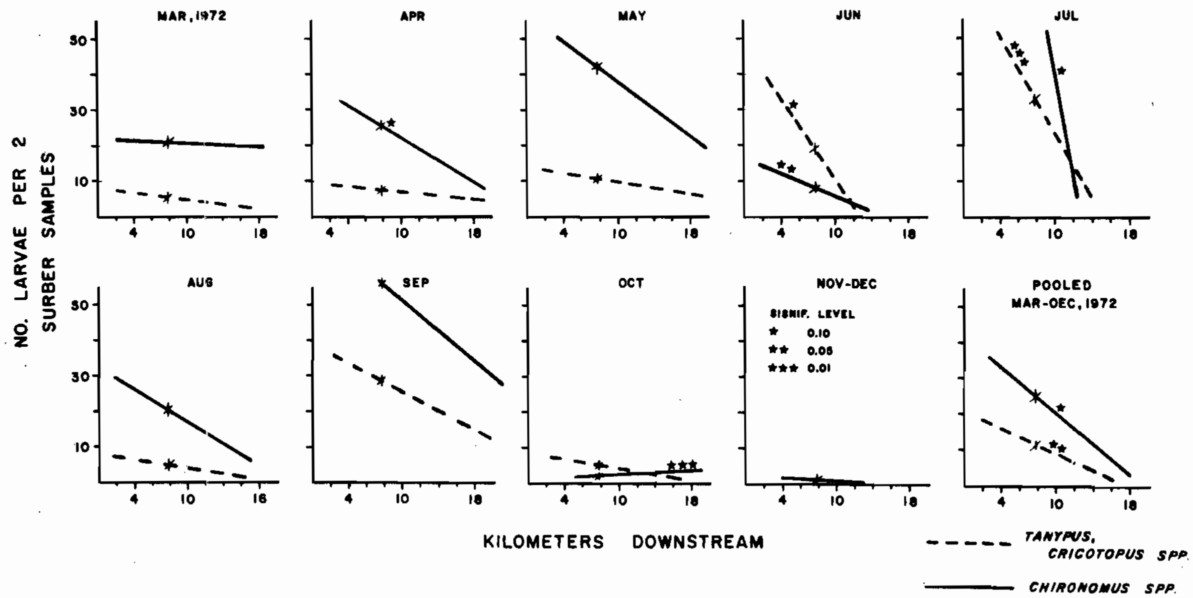

Figure 3.–Relationship between chironomid density and distance downstream in the Coyote Creek. March-December, 1972.

The general significant trend was for a greater abundance of chironomid larvae upstream, although a few, usually non-significant, reversals were detected (Figures 2-4). This effect of position in the creek was especially pronounced during the summer months when midge densities were greatest.

Chironomus spp. in October and November, 1970 when weekly samples were made (Figure 2) show a reversal of the trend which was not repeated in subsequent years when population densities were comparatively lower (Figures 3-4). Similar trends were shown in 1974 samples (data not graphed).

Causes for the trend of greater midge densities upstream may involve greater nutrient availability and less street pollution upstream. However, analyses of water quality at 4 sites along the extent of the 18 Km sample area in March, 1971, (Table 1) did not show any reliable differences that might account for midge density differences. Concentrations of CO_2, HAc, alkalinity and hardness and the pH were not significantly different upstream from those downstream, although there appeared to be some differences in intermediate sample areas (Table 1). Although additional water quality tests at different seasons through 1973 also failed to establish a significant correlation between any water quality measurement and midge density, more detailed measurements may establish a relationship.

Other factors such as flight patterns of adult chironomids, the effects of diseases and invertebrate predators (e.g., planaria, hydra, protozoans) and the relative abundance of filamentous algae as chironomid oviposition stimuli, are currently under investigation. The determination of which factors are capable of producing a "downstream" condition upstream might give a significant clue to midge control in the drainage system.

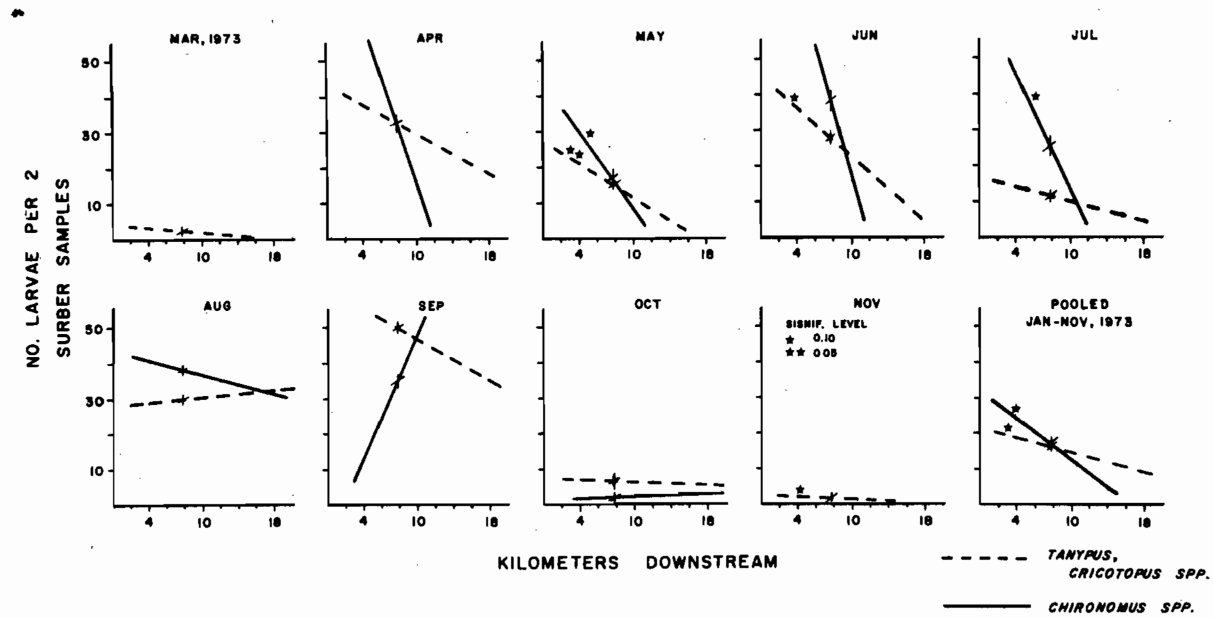

Figure 4.–Relationship between chironomid density and distance downstream in the Coyote Creek. Jan.-Nov., 1973.

Table 1.–Analysis of water quality in the Coyote Creek, Los Angeles basin, at 4 positions downstream during March, 1971.[1]

Position Down-stream (Km)	PPM				
	CO_2	HAc	alkalinity	hardness	pH
1	18.3	17.1	297.0	509.7	8.6
4	0	0	119.0	766.0	9.3
7	10.0	8.6	137.0	521.5	8.7
18	20.0	19.0	284.7	558.7	8.9

[1]3 Sample intervals, replicates per interval; samples made between hr of 1100-1400.

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QUANTITATIVE WATER COLUMN SAMPLER FOR INSECTS IN SHALLOW AQUATIC HABITATS[1]

E. F. Legner[2], R. A. Medved[2] and R. D. Sjogren[3]

ABSTRACT

A water column sampler was developed to quantitatively sample insect predators in shallow aquatic habitats. The sampler eliminates or greatly reduces problems of location and scattering of specimens in the water, sampling time, escape flight of adult specimens, differential phototaxis among organisms, and excessive weight of equipment and samples. Comparisons are made with emersed light traps and 400-ml dipper samples. Time of day activity differences of species groups are recorded.

Increased attention to the value of insect predators of mosquito control in aquatic habitats (Legner et al. 1974) made the development of an accurate sampler imperative for quantitative studies. Problems of location and scattering of specimens in the water, sampling time, escape flight of adult specimens, differential phototaxes, and excessive weight of equipment and samples have been heretofore unresolved. Trials with numerous devices eventually produced an accurate quantitative water column sampler described herein.

METHODS AND MATERIALS

Description and Operation of Sampler.—A graduated hollow plexiglas cylinder, 9.53 cm diam. and 50 cm. long with a ca. 2 m aluminum handle was constructed (Figure 2) to isolate a column of water within. The sampler is operated by plunging the cylinder from a maximum distance of 2 m into the water, fixing its base slightly in the benthic mud. This distance causes minimal disturbance of insects at the sample site. Following fixation the insect fauna contained within the cylinder, including benthos inhabitants, are removed by suction into a perforated polyethelene wash bottle attached to a grease extractor syringe which is manually operated (Figure 1). The wash bottle is removed from the plexiglas cylinder and back-flushed onto a suitable nylon screen which removes the water and retains the insects sampled. Three to five extractions, depending on water depth, remove all the water and contained larval and adult insects in the cylinder, the whole process requiring a maximum of 2 minutes. Photographs of the entire sampling procedure are shown in Figure 1. A prototype of this sampler developed by T. Yamaguchi is shown on page 26 of Usinger (1971).

The nylon screen containing living wet arthropods is then placed into a polyethelene bag and either stored living in an ice chest or killed with the addition of a piece of ethanol-soaked cellucotton.

The weights of equipment and samples are light enough that an operator may easily carry 50 samples at one time.

An aperture of 2 cm cut from the perimeter of the wash bottle base is sufficient for most large insects and small enough to minimize water loss during the transfer from cylinder to nylon screen. The aperture being positioned off center as shown in Figure 1 produces a swirling motion that aids in flushing insects from vegetation that may be located within the cylinder at the time of sampling. One back-flush within the cylinder will dislodge chironomids from the benthic mud.

The depth of the column of water being sampled may also be measured by reading off the graduated plexiglas cylinder, with a suitable adjustment made for benthic penetration.

Sampling Accuracy.—Separate water column samples were taken in square, shallow 4-acre duck club ponds (10-20 cm depth) near Wasco, California on 19 September, 9 and 16 October and 12 November, 1973. There was a total of 16 adjacent ponds in the area totaling 64 acres of water surface. The number of insect species, their density and sample variability was compared in weedy (largely emergent grasses) versus open water habitats in the same ponds, and at different times of the day. Comparisons were also made with the standard 400-ml mosquito dipper and a series of emersed, side-darkened light traps similar to those used by Washino (1969).

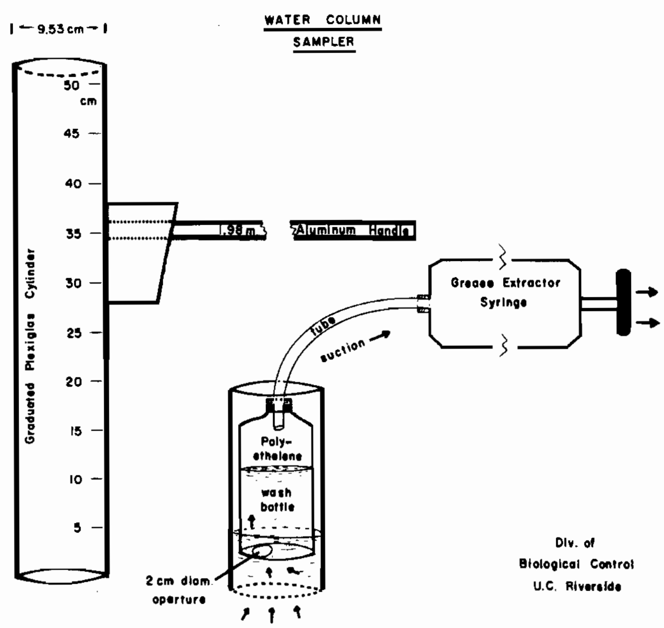

Figure 1.—Water column sampler used for insects in shallow aquatic habitats.

[1]Research performed with the assistance of personnel in the Kern Mosquito Abatement District, Post Office Box 9428, Bakersfield, California 93309.

[2]Division of Biological Control, University of California, Riverside, California 92502.

[3]Metropolitan Mosquito Control District, 797 Raymond Avenue, St. Paul, Minnesota 55114.

Table 1.—Arthropods sampled from 10-20 cm deep duck club habitats near Wasco, California during the period 19 Sept. - - 12 Nov., 1973.

Order and Family	Species[1]
Coleoptera	
Dryopidae	*Helichus productus* LeConte
Dytiscidae	*Agabus lutosus* LeConte
	Agabus regularis LeConte
	Agabus walshinghami (Crotch)
	Copelatus chevrolati renovatus Guignot
	Eretes strictictus (L.)
	Hydroporus sp.
	Hygrotus lutescens LeConte
	Laccophilus maculosus decipiens LeConte
	Laccophilus mexicanus mexicanus Aube
	Rhantus gutticolli (Say)
	Rhantus hoppingi Wallis
	Thermonectus basillaris (Harris)
	Dytiscinae Genus sp.
Haliplidae	*Peltodytes simplex* (LeConte)
Hydrophilidae	*Anacaena signaticollis* Fall
	Berosus infuscatus LeConte
	Berosus punctatissimos LeConte
	Berosus styliferous Horn
	Cymbiodyta imbellis (LeConte)
	Hydrophilus triangularis (Say)
	Helophorus sp.
	Tropisternus ellipticus (LeConte)
	Tropisternus lateralis humeralis Mots
Noteridae	Genus sp.
Diptera	
Culicidae	*Aedes melanimon* Dyar
	Aedes nigromaculis (Ludlow)
	Anopheles sp.
	Culex erythrothorax (Dyar)
	Culex tarsalis Coquillett
	Culiseta incidens (Thomson)
	Culiseta inornata Williston
Tabanidae	*Chrysops vittatus* Wiedemann
Ephemeroptera	
Baetidae	Genus sp.
Hemiptera	
Belostomatidae	
	Abedus indentatus (Hald)
	Belostoma bakeri Mont.
	Belostoma flumineum Say
Corixidae	*Sigara* sp.
Gerridae	*Gerris* sp.
Notonectidae	*Buenoa scimitra* Bare
	Notonecta unifasciata Guerin
Odonata	
Anisoptera (Libellulidae)	
	Aeshnidae sp.
	Libellula sp.
Zygoptera (Coenagrionidae)	
	Enallagma sp.

[1]Species encountered during survey interval.

The bulk of collected specimens were identified to the nearest accurate taxon, and sample specimens were mounted and sent to specialists in the Systematic Entomology Laboratory of the U. S. Department of Agriculture for species identification.

RESULTS AND DISCUSSION

Insect species identified from the duck club near Wasco are listed in Table 1. Further discussion of these species will be in groups that could be practically discerned during the data counting process.

Table 2 shows the average density at different times of day and sample variability of insects secured in one 4-acre pond on September 19. Comparisons are shown with emersed light traps operated during the two hours before midnight. It is immediately apparent that the column sampler secured more species at all sampled intervals of the day than the light traps did after dark (Table 2). However, the light traps attracted two groups of Hemiptera in greater numbers than the column sampler, the Corixidae and Notonectidae. This probably was due to attraction of these species from variable distances and thus a greater sample area. The sample variability as measured by the coefficient of variability indicates that use of the column sampler often reduced variability and in any case estimated most accurately the true population distribution per volume of water. Direct observation of high densities of Notonectidae during daylight hours showed that the placement of the transparent plexiglas cylinder did not scatter specimens which seemed largely unaware of the cylinder being placed around them.

A comparison of the water column sampler with the standard 400-ml mosquito dipper in 4, 4-acre ponds on October 9 is shown in Table 3. Although dip samples were taken rapidly, there were no predators collected with this method. However, mosquito larvae were adequately sampled. Mosquito pupae appeared to be most accurately sampled with the column sampler (Table 3).

The column sampler was further tested in two ponds on October 16 to determine differences between rapid and slow placement of the cylinder both in open water and grass covered habitat (Table 4). The results show that quick placement was more efficient than slow in trapping specimens in both open and grass covered habitats. Also, the grass covered habitat contained the greatest insect biomass. It is interesting to note that variability was relatively constant regardless of habitat or rapidity of insertion (Coeffient of Variability = ca. 200%) (Table 4).

Final comparisons between the column sampler, 400-ml dipper and emersed light traps were made in two ponds on October 16 (Table 5). Results were similar to those secured previously (Tables 2 and 3) even though the average density of insects showed a seasonal decline (Table 5).

The last general sample in the area was made in 4 ponds, 25 samples per pond, on November 12, 1973. Most species showed a further seasonal decline (Table 6), which may have involved both a lack of mosquito prey and a lower water temperature.

Table 2.–Insects collected with water column sampler at different times of the day compared to a 2-hr emersed light trap (22-2400 hrs) collection in a duck club pond near Wasco, California on 19 Sept. 1973.[1]

Order and Family	Collection		Avg. No/cylinder[2] or light trap[3]	
	Stage	Time(hr)	X	C.V. (%)
Coleoptera				
Dytiscidae	larva	1400	0.52	148.12
		1820	0.20	288.65
		2400	0.40	161.35
		0700	0.34	177.47
		light trap	0	--
Haliplidae	larva	1400	0.08	346.00
		1820	0.28	242.21
		2400	0.12	276.33
		0700	0.23	229.74
		light trap	0	--
Hydrophilidae	adult	1400	0	--
		1820	0	--
		2400	0.04	500.00
		0700	0	--
		light trap	0.11	303.00
	larva	1400	0.16	233.81
		1820	0.16	295.31
		2400	0.36	157.94
		0700	0.23	186.48
		light trap	0.78	210.44
Misc. Coleoptera				
	larva	1400	0.04	500.00
		1820	0.08	346.00
		2400	0	--
		0700	0	--
		light trap	0	--
Diptera				
Culex spp.	larva	1400	0.05	447.20
		1820	0	--
		2400	0.04	500.00
		0700	0	--
		light trap	0	--
	pupa	1400	0.05	447.20
		1820	0	--
		2400	0.04	500.00
		0700	0	--
		light trap	0	--
Ephemeroptera				
Baetidae	nymph			
		1400	0.44	197.68
		1820	1.44	109.94
		2400	0.64	99.64
		0700	1.00	111.26
		light trap	0	--

Table 2.– (continued)

Order and Family	Collection		Avg. No/cylinder[2] or light trap[3]	
	Stage	Time(hr)	X	C.V. (%)
Hemiptera				
Corixidae	adult	1400	0	--
		1820	0	--
		2400	0	--
		0700	0.05	426.40
		light trap	1.67	215.90
Cerridae	adult	1400	0	--
		1820	0.05	447.20
		2400	0	--
		0700	0.05	426.40
		light trap	0	--
Notonectidae	adult	1400	0.08	346.00
		1820	0.08	346.00
		2400	0.12	366.33
		0700	0.04	500.00
		light trap	2.33	253.91
	nymph			
		1400	0.60	166.67
		1820	1.00	111.80
		2400	0.68	138.99
		0700	0.95	76.02
		light trap	14.33	222.65
Odonata				
Anisoptera	nymph			
		1400	1.40	145.80
		1820	0.72	147.42
		2400	0.32	195.97
		0700	0.18	219.28
		light trap	0.22	200.41
Zygoptera	nymph			
		1400	1.96	72.87
		1820	1.68	127.11
		2400	2.44	68.05
		0700	2.45	108.16
		light trap	1.00	158.11

[1]Sampler placed rapidly.

[2]25 water column samples.

[3]9 light trap samples.

Table 3.—Insects collected with water column sampler compared to standard 400 ml dipper in 4 separate duck club ponds near Wasco, California on 9 October 1973.[1]

Order and Family	Collection Stage	Pond	No. Per[2] Cylinder x	Cylinder C.V.(%)	400-ml dip x	400-ml dip C.V.(%)
Coleoptera						
Dytiscidae	adult	A	0	--	0	--
		B	0.04	500.00	0	--
		C	0.04	500.00	0	--
		D	0.04	500.00	0	--
	larva	A	0.16	233.81	0	--
		B	0.08	346.00	0	--
		C	0.40	176.78	0	--
		D	0	--	0	--
Haliplidae	adult	A	0	--	0	--
		B	0	--	0	--
		C	0	--	0	--
		D	0.12	366.33	0	--
	larva	A	0.08	346.00	0	--
		B	0.20	288.65	0	--
		C	0.40	204.10	0	--
		D	0.16	233.81	0	--
		A	0			
Hydrophili-dae	adult	A	0.16	233.81	0	--
		B	0	--	0	--
		C	0.04	500.00	0	--
		D	0.04	500.00	0	--
	larva	A	0.16	233.81	0	--
		B	0.20	288.65	0	--
		C	0.52	112.67	0	--
		D	0.08	346.00	0	--
Diptera						
Culicidae						
Anopheles	larva	A	0.20	322.70	0.24	181.58
		B	0	--	0.20	250.00
		C	0.20	250.00	0.16	233.81
		D	0	--	0.28	219.18
	pupa	A	0.36	177.14	0	--
		B	0	--	0.04	500.00
		C	0.12	276.33	0	--
		D	0.12	276.33	0	--
Culex	larva	A	0.12	276.33	0.12	276.33
		B	0	--	0	--
		C	0.20	250.00	0	--
		D	0	--	0.08	346.00
	pupa	A	0.24	248.83	0	--
		B	0.12	366.33	0	--
		C	0.08	500.00	0.08	346.00
		D	0	--	0	--
Tabanidae	larvae	A	0.24	217.83	0	--
		B	0.28	193.43	0	--
		C	0.20	288.65	0	--
		D	0.08	346.00	0	--

Table 3.—(continued)

Order and Family	Collection Stage	Pond	No. Per[2] Cylinder x	Cylinder C.V.(%)	400-ml dip x	400-ml dip C.V.(%)
Ephemeroptera						
Baetidae	larvae	A	2.36	50.25	0	--
		B	1.60	76.54	0	--
		C	4.20	44.54	0	--
		D	0.76	115.70	0	--
Hemiptera						
Belastomatidae	nymph	A	0.04	500.00	0	--
		B	0	--	0	--
		C	0	--	0	--
		D	0	--	0	--
Corixidae	adult	A	0.04	500.00	0	--
		B	0	--	0	--
		C	0.08	509.35	0	--
		D	0	--	0	--
	nymph	A	0	--	0	--
		B	0	--	0	--
		C	0.04	500.00	0	--
		D	0	--	0	--
Gerridae	adult	A	0.04	500.00	0	--
		B	0	--	0	--
		C	0	--	0	--
		D	0	--	0	--
	nymph	A	0.08	346.00	0	--
		B	0.04	500.00	0	--
		C	0	--	0	--
		D	0	--	0	--
Notonecti-dae	adult	A	0	--	0	--
		B	0	--	0	--
		C	0.32	148.75	0	--
		D	0	--	0	--
	nymph	A	0.60	127.28	0	--
		B	0.24	217.83	0	--
		C	0.64	148.77	0	--
		D	0.32	295.34	0	--
Odonata						
Anisoptera	nymph	A	2.64	65.50	0	--
		B	1.20	104.86	0	--
		C	1.08	79.82	0	--
		D	1.12	121.77	0	--
Zygoptera	nymph	A	7.48	45.84	0	--
		B	3.88	92.72	0	--
		C	4.28	59.76	0	--
		D	6.48	55.30	0	--

[1]Sampler placed rapidly.

[2]25 water column or 400-ml dip samples.

Table 4.–Comparisons of fauna collected with slow and rapid placement of water column sampler in weedy vs open water areas in two duck club ponds near Wasco, California on 16 October 1973.

Habitat Character	Rate of Sampler Placement	Pond	% of 23 spp.[1]	C.V.(%)
open water	slow	A	0.27	188.57
		B	0.31	240.64
		avg.	0.29	214.61
open water	rapid	A	0.31	188.21
		B	0.45	209.33
		avg.	0.38	198.77
weed cover	slow	A	0.36	225.92
		B	0.39	195.86
		avg.	0.37	210.89
weed cover	rapid	A	0.43	248.57
		B	0.44	150.25
		avg.	0.44	199.41
		Summary[2]		
open water	- -	- -	0.33	206.69
weed cover	- -	- -	0.40*	205.15
- -	slow	- -	0.33	212.75
- -	rapid	- -	0.41*	199.09

*Significantly greater than previous figure at 95% level.
[1]12 water column samples.
[2]25 water column samples.

ACKNOWLEDGMENTS

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Table 5.–Insects collected with water column sampler compared to standard 400-ml dipper and 2-hr emersed light trap (22-2400 hrs) in two duck club ponds near Wasco, California on 16 October 1973.[1]

		Avg. No. Per[2]					
Order and Family	Stage Collected	Cylinder	C.V. (%)	400-ml dip	C.V. (%)	Light Trap	C.V. (%)
Coleoptera							
Dytiscidae	adult	0	- -	0	- -	0.56	130.77
	larva	0.08	391.00	0	- -	0	- -
Haliplidae	larva	0.12	279.17	0	- -	0	- -
Hydrophilidae	adult	0	- -	0	- -	0.22	198.42
Diptera							
Culicidae							
Anopheles	larva	0.06	436.10	0.11	423.73	0	- -
	pupa	0.02	693.75	0.03	573.33	0	- -
Culex	larva	0.02	722.40	0.07	418.71	0	- -
	pupa	0	- -	0.02	693.14	0	- -
Tabanidae	larva	0.02	693.75	0	- -	0	- -
Ephemeroptera							
Baetidae	nymph	1.54	93.83	0	- -	0.44	228.11
Hemiptera							
Corixidae	adult	0	- -	0	- -	2.22	219.04
	nymph	0	- -	0	- -	0.67	167.72
Gerridae	adult	0.04	499.00	0	- -	0	- -
Notonectidae	adult	0.02	693.75	0	- -	1.67	150.01
	nymph	0.33	210.76	0	- -	8.67	148.21
Odonata							
Anisoptera	nymph	2.02	93.27	0	- -	0.33	212.15
Zygoptera	nymph	3.55	73.57	0	- -	0.22	198.42

[1]Sampler placed in water rapidly.
[2]Cylinder = 50 replicates; dipper = 100 replicates; light trap = 9 replicates.

Figure 2.—Use of water column sampler: (A, B) placement of plexiglas cylinder into sample site; (C) extration of insect fauna with suction; (D) back-flushing sample through nylon screen to remove water.

Table 6.—Insects collected with water column sampler on November 12, 1973 in 4 duck club ponds near Wasco, California.[1]

Order and Family	Collection Stage	Avg. No/Cylinder[2] x	C. V. (%)
Coleoptera			
Dytiscidae	adult	0.06	602.83
	larva	0.01	1000.00
Hydrophilidae	adult	0.02	707.81
	larva	0.11	357.91
Diptera			
Culicidae			
Anopheles	pupa	0.01	1000.00
Culex	pupa	0.01	1000.00
Tabanidae	larva	0.04	489.25
Ephemeroptera			
Baetidae	nymph	1.87	118.70
Hemiptera			
Corixidae	nymph	0.01	1000.00
Gerridae	adult	0.02	714.29
Notonectidae	adult	0.01	1000.00
	nymph	0.10	365.00
Odonata			
Anisoptera	nymph	1.88	105.40
Zygoptera	nymph	5.50	72.03

[1]Sampler placed rapidly.

[2]4 ponds, 25 replicates/pond.

CHIRONOMID MIDGE PROBLEM IN WATER SPREADING BASINS AND FLOOD CONTROL CHANNEL IN THE SANTA ANA RIVER, ORANGE COUNTY, CALIFORNIA[1]

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The intermittent impoundment of Santa Ana River water in spreading basins and a flood control channel in Orange County, California, for the replenishment of underground water supplies, provides nearly 500-700 acres of breeding grounds for the nonbiting aquatic chironomid midges. Since these aquatic habitats are situated amidst residential, industrial, and recreational establishments, severe outbreaks of adult midges are a source of nuisance and discomfort for nearby inhabitants (Figure 1). The nuisance and economic aspects of chironomids in urban and recreational situations in California have been reported by several workers (Anderson et al. 1965, Grant 1960, Grodhaus 1963, Mulla et al. 1971, Mulla 1974).

To understand this complex problem, studies on the nuisance aquatic midges in the Santa Ana River spreading basins and flood control channel of the Orange County Water District were initiated in June 1974, and are being continued. The objectives of these studies are to elucidate the nuisance midge fauna, their seasonal variations, succession, and abundance in the District's water management system, and to establish possible correlations between certain environmental factors and the observed changes in chironomid populations. Such basic understanding of the problem is essential to the development of management strategies and practical procedures for control of nuisance aquatic midges.

Intermittent drying and flooding of the basins and the flood control channel provided an opportunity to study the effects of such rapidly changing physical conditions on the production of midge fauna.

There are 20 basins in the system lying in a series. They are arranged in 4 sets, each containing a minimum of 3 basins. Basins in each set can be dried and flooded independently of the other sets. Water can be drawn either from the basin lying immediately upstream, or from the flood control channel running parallel to the basins. Depth in the middle portion of most basins averages between 3-5 ft, and each basin covers approximately 10 acres of land (Figure 2). The flood control channel is about 360 ft wide, and 2-3 ft deep over most of its course (see Figure 1).

Routine qualitative and quantitative benthic samples were taken from 7 basins, at 4 locations in the flood control channel, and at the sides of the main river at 1 site, using a standard 6 x 6 in. scoop sampler. Drift samples from 3 sites in the flood control channel, and at 1 upstream site in the main river were taken with a 10-in. diameter drift net of 250-μ pore size. Benthic samples from the middle portion of the main river at 1 site were collected with a Suber sampler of 12 x 12 in. frame and 500-μ pore net. Adult emergence in 5 basins and at 1 location in the flood control channel were taken using emergence traps (each trap samples 3.15 ft^2 benthic area). Light traps near the basins were set permanently to study prevalence of adult midges around the study area.

Midge larvae belonging to the genera, *Procladius* spp., *Pentaneura* sp., *Tanypus* sp., *Psectrotanypus* sp., *Tanytarsus* spp., *Chironomus* spp., *Dicrotendipes* sp., *Polypedilum* sp., *Paralauterborniella* sp., *Tribelos* sp., and *Cricotopus* spp. were taken in the area between June-December 1974.

The qualitative and quantitative composition of midge fauna in different sets of basins differed spatially as well as temporally. In addition to the seasonal changes, spatial changes in midge faunal composition in different basin sets probably occurred due to the varied cycles and duration of water impoundments in these basins. This altered midge habitats by gradually changing the chemical and physical nature of substrates, and the chemical composition of the impounded water. Preliminary studies on this aspect showed that established basins contained a higher percentage of clay, silt, and nitrates and total organic matter in the soil but a lower percentage of sand than the newly dredged and flooded basins. Chemical composition of water in the established, newly dredged and impounded basins also differed slightly.

Changes in midge populations between the various basins of an established set flooded in March (4 months before these studies began) were essentially the same from June-September. Here *Procladius* spp. were most abundant of all chironomids. *Tanytarsus* spp., *Chironomus* spp., *Cryptochironomus* sp., *Dicrotendipes* sp., *Psectrotanypus* sp., *Polypedilum* sp., *Tribelos* sp., and *Pentaneura* sp. were taken in small numbers only. A distinct increase in *Chironomus* spp. populations was noticed while basins in this set were being dried for dredging in September. Under newly dredged conditions (i. e. changed substrate and water quality) in the same basins during October-December, *Tanytarsus* spp. appeared as the predominant group, outnumbering *Procladisu* spp. A sharp increase in *Tanytarsus* spp. populations was noticed 2-3 weeks after flooding. *Procladius* spp. gradually declined and disappeared. *Chironomus attenuatus* was taken in considerable numbers.

In another set of basins newly dredged and flooded in June, *Tanytarsus* spp. populations was observed 4 weeks after these basins were flooded. *Procladius* spp. were taken in fair numbers during this time. *Chironomus* spp., *Cryptochironomus* sp., *Dicrotendipes* sp., *Polypedilum* sp., *Pentaneura* sp., and *Cricotopus* spp. were also present.

Chironomis spp. were predominant during June-July in a basin containing a small quantity of turbid seepage water; *Procladius* spp. were present in fair numbers, and *Tanytarsus* spp. were taken in small numbers. Midge faunal composition in the same basin changed markedly after this basin was dredged and flooded in August. Under these changed

[1]Research supported by the Orange County Water and Mosquito Abatement Districts.

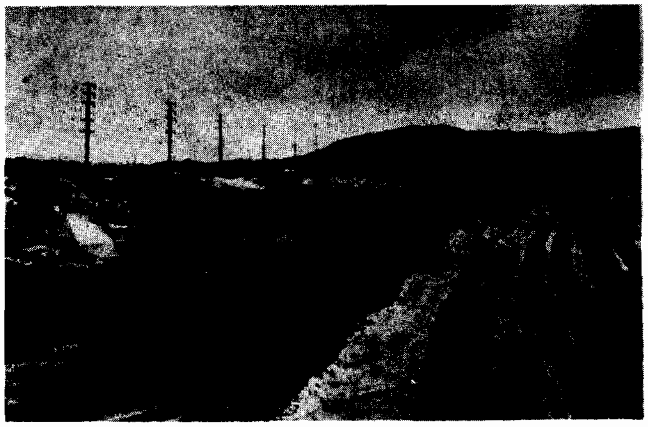

Figure 1.–Flood control channel of the Santa Ana River in Orange County passing through residential-industrial establishments.

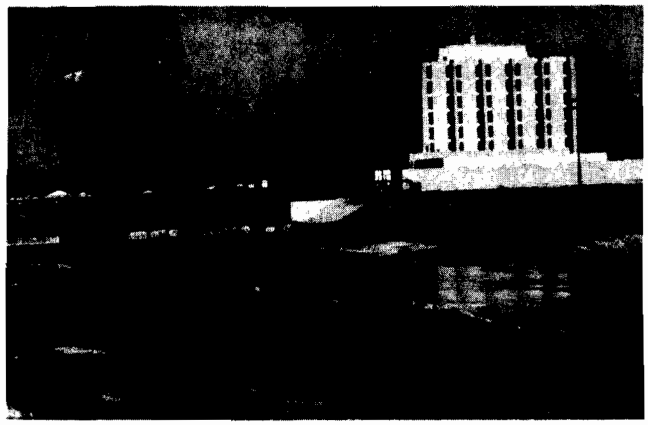

Figure 2.–Spreading basins adjacent to the flood control channel of the Santa Ana River and residential-industrial establishments in Orange County, California.

environmental conditions, *Tanytarsus* spp. and *Dicrotendipes* sp. appeared. Both groups gradually increased and constituted about 42% each of the total chironomids taken during August-December. *Cricotopus* spp. which were absent during the stagnating period in June-July also appeared in fair numbers in later months. *Chironomus* spp. and *Cryptochironomus* sp. were present in small numbers. *Procladius* spp. were rare or absent.

Midge density at various sites in the flood control channel showed production to be as high as in adjacent basins. However, faunal composition, and spatial and temporal population changes in various midge groups in the flood control channel were different from those observed in the basins. At 2 sites in the channel, *Procladius* spp. were predominant during June-September while at the other 2 locations *Tanytarsus* spp. were most abundant during the same period. Overall, in the entire channel *Tanytarsus* spp. seemed most numerous. *Chironomus* spp., *Cryptochironomus* sp., and *Dicrotendipes* sp. were taken consistently in fair numbers. *Cricotopus* spp., *Tanypus* sp., *Pentaneura* sp., and *Paralauterborniella* sp. occurred occasionally. *Tribelos* sp. were rare.

Benthic samples taken from sides of the main river showed that, in general, sandy areas supported more midges than muddy or gravelly substrates. More larvae were present at the sides than in the river middle. *Tanytarsus* spp. were associated with sand, and *Chironomus* spp. were found more in muddy substrates.

Drift data collected at various sites during June-September showed that *Cricotopus* spp. and *Tanytarsus* spp. were most common in the drift at all sites. *Procladius* spp., *Cryptochironomus* sp., and *Dicrotendipes* sp. were taken occasionally in small numbers only. *Chironomus* spp. were rare. Midge pupae were abundant in the drift. Pupae formed 25-50% of the total drift at various sites. The presence of large numbers of pupae in drift indicated that they are constantly carried downstream and emerge in downstream areas.

Data on adult emergence and prevalence collected during August-November indicated that *Tanytarsus* spp. were predominant in the area. *Tanytarsus* spp. females consistently outnumbered males. *Procladius* spp. were present in fair numbers in August and September, and they gradually declined in subsequent months. The females were always fewer than their males. *Chironomus* spp. increased during the fall.

In addition to the above described studies, detailed studies on midge production and their densities in relation to the chemical and physical nature of substrates, and chemical composition of the water in various basins are being carried out.

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BIOLOGICAL ACTIVITY OF INSECTICIDES AGAINST *CULICOIDES VARIIPENNIS* (COQUILLETT) (DIPTERA: CERATOPOGONIDAE

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Culicoides variipennis (Coquillett) is a black biting gnat of considerable economic importance in many areas of the United States. This species is the primary vector of blue tongue virus of sheep and cattle, and has recently been implicated in the transmission of other arboviruses (Nelson and Scrivani 1972). *C. variipennis* can also be pestiferous to humans when adult gnat populations are dense. In Lake County, California, the biting activity of populations from Borax Lake causes extreme irritation to nearby residents. New Jersey light trap collections one mile from the lake have exceeded 50,000 adults per week in summer (unpublished data).

A chemical control program was initiated by the Lake County Mosquito Abatement District to cope with this problem. Several aerial applications of dieldrin were made in 1969. Organophosphorus (OP) larvicides, principally fenthion, and diesel oil have since been applied during spring and summer along the lake shore for control of larvae and pupae. An abundance of aquatic vegetation and highly alkaline water (pH 10) in the lake are thought to have had a deleterious effect on the activity of these insecticides. Fenthion and naled have been used as adulticides; however, the control achieved through fogging has been of doubtful value. Thus, in 1974 it was deemed necessary to make a thorough evaluation of the biting-gnat control program.

Laboratory studies were initiated in July. Our preliminary objective was to characterize the spectrum of insecticide susceptibility of *C. variipennis* larvae and adults to find suitable insecticides for use in the field. Results of this study are presented here.

METHODS AND MATERIALS.—Collections of larvae and pupae were taken from Borax Lake from July through September. Larvae were transferred to enameled pans containing filtered water from Clear Lake and held at 75°F for 24 hours before bioassay. Pupae were placed on moist cotton and isolated in 1-gallon ice cream containers fitted with cloth screen lids. Granular sucrose was placed in the containers as food for adults. Females from 2-4 days old were used in insecticide susceptibility tests.

All insecticides used were of technical grade. Solutions were prepared in acetone on a w/v basis and diluted to give desired serial concentrations. Twenty 4th instars were transferred to glass jars containing 100 ml of filtered water. From ½ to 1 ml of the appropriate insecticide solution was added to each jar. Larval mortality was assessed 24 hours later. Nonmotile larvae were counted as dead. Bioassay procedures for adults have been described by Georghiou et al. (1972) and Apperson and Georghiou (1973).

Each insecticide was tested at 4 or more concentrations. Tests were replicated at least 4 times on different days. The results were plotted on log-probit paper and the log dosage-mortality (ldp) lines fitted by eye.

RESULTS AND DISCUSSION.—Table 1 contains the ld-p data for larvae. The LC_{95} values for methoxychlor, dieldrin, aldrin and chlordane exceed 10 ppm. No ld-p data are available for other populations of *C. variipennis* larvae; however Smith et al. (1959) found *Culicoides furens* (Poey) larvae from Florida to have developed greater than 100-fold resistance to dieldrin. They achieved an average of 50% mortality in resistant *C. furens* larvae at 10 ppm. At the same dosage, we could kill an average of only 46% of the *C. variipennis* larvae tested. This suggests that the larvae have developed resistance to dieldrin. The low activity of other organochlorine insecticides may stem from cross-resistance, since Smith and co-workers found dieldrin-resistant *C. furens* larvae to be highly cross-resistant to heptachlor, lindane and chlordane.

Among the OP compounds tested against *C. variipennis* larvae, malathion (LC_{95} 1.70 ppm) was the least effective while chlorpyrifos-methyl (LC_{95} 0.078 ppm) and ABATE® (LC_{95} 0.072 ppm) were the most biologically active. No definite interpretation of the data can be made in terms of resistance since the population was not tested prior to the application of chemicals to Borax Lake. The LC_{95} values are generally comparable to those achieved against field populations of OP-resistant mosquitoes, as reported by Gillies et al. (1974) for *Culex tarsalis* Coquillett and *Aedes nigromaculis* (Ludlow). ABATE and chlorpyrifos-methyl may be effective compounds for small-scale treatments of *Culicoides* breeding habitats.

Table 1.—Effects of insecticides on *C. variipennis* larvae from Borax Lake.

Insecticide	LC_{50} (ppm)	LC_{95} (ppm)
Methoxychlor	1.0	35.0
Dieldrin	$>$10.0	-
Aldrin	$>$10.0	-
Chlordane	$>$10.0	-
Malathion	0.64	1.70
Fenitrothion	0.20	0.51
Methyl parathion	0.14	0.28
Parathion	0.096	0.23
Fenthion	0.058	0.19
Chlorpyrifos	0.042	0.10
Chlorpyrifos-methyl	0.026	0.078
ABATE®*	0.025	0.072

*0,0,0',0'-Tetramethyl 0,0'-thiodi-p-phenylene phosphorothioate

Results of adult bioassays are shown in Table 2. Propoxur (LC_{95} 3.9 $\mu g/cm^2$) and dichlorvos (LC_{95} 9.0 $\mu g/cm^2$) were the most active while ABATE, dimethoate, and dieldrin exhibited much lower toxicity ($LC_{95} > 160\ \mu g/cm^2$). Chlorpyrifos-methyl and methyl parathion were found to be more toxic than their ethyl analogs. There is no other information on the susceptibility of *C. variipennis* adults to in-

Table 2.—Effects of insecticides on *C. variipennis* adults from Borax Lake.

Insecticide	LC_{50} (µg/cm^2)	LC_{95} (µg/cm^2)
Propoxur	2.1	3.9
Dichlorvos	2.3	9.0
Chlorpyrifos-methyl	4.2	13.4
Chlorpyrifos	6.6	14.5
Methyl parathion	7.2	17.6
Naled	7.9	23.6
Parathion	9.5	26.0
Fenthion	16.0	30.0
Fenitrothion	21.8	32.3
Malathion	16.7	33.9
ABATE®	>160.0	-
Dimethoate	>160.0	-
Dieldrin	>160.0	-

secticides. However, Georghiou et al. (1972) tested a variety of insecticides for effectiveness against *Leptoconops kerteszi* (Boey) from southern California. Chlorpyrifos-methyl (LC_{95} 0.96 µg/cm^2) and methyl parathion (LC_{95} 0.094 µg/cm^2) were the most active compounds tested. These insecticides were more active than their ethyl analogs. Contrary to the results of our study, propoxur (LC_{95} 51.0 µg/cm^2) was found to exhibit low toxicity, whereas dieldrin (LC_{95} 0.27 µg/cm^2) was very active against *L. kerteszi* adults.

Chlorpyrifos-methyl and chlorpyrifos were highly active against *C. variipennis* and *L. kerteszi* adults which suggests that these compounds may be used as general adulticides for ceratopogonid biting-midges.

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MAINTENANCE AND TRANSPORTATION OF BLACK FLY (DIPTERA: SIMULIIDAE) LARVAE IN NONAGITATED WATER

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It is commonly known that the larvae of all black fly species are found in nature only in flowing water. Flowing, aerated or agitated water also is required for long term maintenance or rearing of black flies in the laboratory (Fredeen 1959, Hocking and Pickering 1954, Muirhead-Thomson 1966, Odintsov 1960, Tarshis 1968). Taxonomic research, necessitating the association of immature stages with adults, and biological research on black flies, often requires that living larvae be transported from their natural habitats and introduced into laboratory rearing devices.

Many researchers have had success transporting live larvae of simuliid species from the field to the laboratory with little mortality when such larvae were in transit in containers of water for only a few hours after removal from creeks, streams or rivers. But when black fly larvae were left in nonagitated water for several hours, mortality rates soared. For example, Tarshis (1966, 1968) working in Michigan and Maryland, reported that simuliid larvae would not remain alive longer than 6 and 8 hours in nonagitated water. Because of this transportation survival problem, Odintsov (1960), in Russia, developed a portable device for aerating containers in the field and during transporation. In the U.S. Tarshis (1966) and Tarshis and Adkins (1971) also described portable aerating equipment used in transporting black fly larvae long distances.

Wu (1931), one of the early workers to comment on the survival of black fly larvae in standing water, noted that larvae from cold streams ". . . suffered much more. . ." in transit from the field to the laboratory than did larvae from warmer waters, and she concluded that larvae endured ". . . cold, standing water much better than standing water whose temperature is higher than their natural habitat." Wu (1931) further noted that when larvae were ". . . left in standing water, large numbers die within a short time, as was observed by previous investigators", but in containers of standing water (50 - 57°F ?) she sometimes found that "many" large larvae [of *Simulium vittatum* - ?] lived for 8 - 14 days and a few small larvae lived for 23 days. Fredeen (1959) found that water temperature was critical for the survival and development of black fly larvae of various species, *S. arcticum* larvae only developing to maturity when temperatures remained below 70°F but *S. vittatum* doing so at temperatures up to 92°F. In total, Fredeen reared 8 species at temperatures of 58 - 92°F.

In standing water in petri dishes, Davies and Syme (1958) kept maturing larvae of *Prosimulium fuscum* and *P. mixtum* alive in 1/16 inch of cold water (35-45°F) for 6 weeks. After being starved for several days about half of such larvae filled their digestive tracts in about 24 hours at 40 - 50°F; a few even filled their guts in 24 hours at 35°F. At 40 - 50°F it took most larvae a week to half-empty their guts and over 2 weeks to completely empty them. When 62 larvae of *P. fuscum* and *P. mixtum* were held at 40 - 50°F in ¼ inch of water in a petri dish, the only experiment for which associated density and mortality data were provided, the associated mortality was 19% after 8 days, 61% after 16 days and 85.5% after 23 days (Davies and Syme 1958). For short-term transit, Wood and Davies (1966) successfully transported larvae and pupae of black flies in a thin film of water in petri dishes stacked in a container kept chilled to 35 - 55°F. Similarly, Bailey et al. (1974) noted that the addition of ice to buckets and plastic bags ". . . kept the temperature below 10°C and allowed samples to be held in collection vessels for several hours and greatly enhanced larval survival." Temperature reduction of transport water also has been used to successfully transport various fish species (Cuerrier 1952, Horton 1956, Norris et al. 1960), and Horton (1956) found that post-planting mortality of trout was significantly lower when they were in cool water instead of warm water.

While investigating the potential autogeny of *S. arcticum* and other species of black flies from southern and northern Alberta, Canada, it was necessary to maintain collected larvae alive in transit for several days prior to their being transferred to laboratory rearing containers. We wanted to avoid using air pumps and batteries in the field since snowmobiles and All Terrain Vehicles sometimes were used for transportation. Since several workers had successfully maintained larvae of some species in 1/16 - 1/4 inches of non-aerated, standing water in petri dishes held at 35 - 45°F, we decided to evaluate this technique for long-term transportation. Our methodology consisted of putting collected black fly larvae into styrofoam boxes containing from 1/8 - 1½ inch of natural stream or river water. Larvae attached to rocks were gently rubbed off by hand into water in the styrofoam boxes or into a bucket of deeper water later poured into styrofoam boxes. Other boxes received blades of grass and other vegetation bearing larvae. The styrofoam boxes used were the type available at most commercial stores for a few dollars. These slightly tapered boxes had an inside bottom dimension of 7½ x 14 inches; they were 9 inches deep and had sides either 5/8 or 1 inch thick. During field trips we periodically added enough ice or snow to the styrofoam boxes to keep the water temperature at 50°F or lower. In the laboratory the boxes were put in constant temperature rooms. Dead larvae were counted and removed with a pipette or forceps at 24 or 48 hour intervals.

In the first attempt to transport larvae in nonagitated water late-instar *S. vittatum* larvae were removed from the 35°F water of Flat Creek, near Athabasca, Alberta, on 31 October 1973. Larvae were put into boxes containing about ¾ inch of creek water; one box received larvae on vegetation and the other received only larvae rubbed off rocks. Each box contained about 4,000 larvae. En route from the field to the laboratory these boxes were transported in the unheated, but covered, rear compartment of a pick-up truck where the air temperature varied between 10 and 20°F. The styrofoam boxes were exposed to these temperatures for about 6 hours the first day and 8 hours the second day, during which the water in the boxes was agitated by movement of the truck. The styrofoam transport boxes

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Table 1.—Comparative mortality of simuliid larvae in standing water.[a]

Species	Group No.	Coll. Date	No. of larvae/box	Temp. (± 1°F)	Water Depth[b]	Cumulative Percent mortality after: 24	48	72	96	120	144 hrs.
S. vittatum	I	31-X-73	642	66	1/8	-	14.4	16.8	17.9	21.0	27.7[c]
S. arcticum	II	20-XI-73	273	66	1/16	41.4	72.0	92.0	expt. terminated		
S. arcticum	III	20-XI-73	65	50	1/16	0.0	1.5	6.0	expt. terminated		
S. arcticum	IV	24-IV-74	622	50	1/2	0.0	3.0	-	20.0	-	97.0
S. arcticum	V	24-IV-74	405	59	1/2	27.0	60.0	-	100.0	-	-
S. venustum	VI	29-V-74	1390	54	1-1/4	8.0	17.0	24.0	27.0	35.0[d]	-
S. venustum	VII	29-V-74	1725	75[e]	1-1/4	40.0	95.0	-	-	-	-
S. vittatum	VIII	16-VI-74	1938	48	1	0.6	2.4	14.8	20.6	25.6	-
S. vittatum	IX	16-VI-74	1615	59	1	21.6	39.1	57.5	67.2	-	-

[a]All larvae held in tapered stryofoam boxes with an inside bottom diameter of 7½ x 14 inches. Groups II-V included third to sixth instar larvae; all other groups consisted predominantly of fifth and sixth instar larvae.

[b]In inches

[c]192 hours

[d]108 hours

[e]Temperature began at 68°F but increased to a high of 75°F in transit.

were removed from the truck and held in a heated room for about 24 hours between days 1 and 2. Ice periodically was added to the boxes while they were held indoors at about 68°F. Upon arrival at the Lethbridge laboratory, the only mortality after 50 hours had occurred in one box in which about 1,000 larvae froze en route after becoming entangled in a mesh cloth strainer left in the box. Other larvae in the boxes appeared unharmed. At 50 hours post-collection the dead larvae were removed and about 2,000 larvae from each box were transferred to aerated rearing jars. Two teneral adults also were removed at this time. Larvae and pupae remaining in the original boxes with creek water, and creek water and vegetation, were then held at 65°F, and a third box of larvae with ¼ inch of creek water and vegetation was held at 50°F. There were 800 - 1,000 larvae/box. After 48 hours, during which the water warmed to 65°F, there were 6 more newly-emerged adults in the one box and an 8 and 12% mortality of larvae in the 2 boxes. After 5 days the cumulative larval mortality in the 2 original boxes was 15 and 19%. In the box at 50°F there was an estimated 1 - 2% mortality after 48 hours, and a mortality of 7% after 5 days and 30% after 13 days. On several other occasions black fly larvae were similarly transported successfully from field collection sites in styrofoam containers holding ¼ - 1 inch of ice-chilled water.

The results (Table 1) for other collections show that by using the above technique, and at water temperatures of 48 - 54°F, the cumulative mortality of *S. arcticum*, *S. venustum* and *S. vittatum* larvae from different sources was between 0.0 - 8.0% after 24 hours, 1.5 - 17.0% after 2 days, and 6.0 - 24.0% after 3 days. Larvae in warmer water perished much faster, some reaching a 40.0% mortality rate by 12 to 24 hours with many moribund larvae also noticed then. By 48 hours in water at 66 - 68°F larvae of *S. arcticum* and *S. venustum* had experienced mortalities of 72 and 95%. *Simulium vittatum,* which survives well in both cold and warm water, at times experienced only a 20.0 - 25.0% mortality after 5 and 6 days in shallow standing water at even 64 - 66°F.

The aquatic origin for each of the 9 groups of larvae in Table 1 are as follows: I. Flat Creek, Northern Alberta; II-V. Crowsnest River, Southern Alberta; VI-IX. Flat Creek, Northern Alberta. After their arrival at the Lethbridge laboratory, the Group I *S. vittatum* larvae collected on 31 October 1973, were maintained in aerated containers of river water from 2 November 1973, until they were transferred on November 9 to a styrofoam box containing tap water. The *S. arcticum* larvae collected on 24 April 1974 were held in distilled water; all other larvae were held in water from the collection site. The type of water used did not seem to markedly affect larval survival, but after 24 hours there was a somewhat greater mortality of *S. vittatum* larvae in foodless tap water. Larvae surviving after 2 - 4 days in standing water went on to complete their development when transferred to rearing containers supplied with compressed air and food.

Our results indicate that shallow, nonagitated, ice-chilled water can be used to successfully transport and maintain black fly larvae alive for several days with very little mortality. The success of this technique appears related to the following facts: 1) water at 40 to 50°F contains more dissolved oxygen than warmer water; 2) the metabolic rate of larvae is lower in cool water than in warm water; and 3) in shallow water masses of larvae rarely become entanged and die in globs of the sticky, glue-like substance secreted from the salivary glands of the larvae. Most larvae in shallow water attach to the bottom and sides of the container instead of drifting about secreting numerous glue-like threads.

In areas where it is impractical to rely on ice-water it may be possible to achieve results similar to ours by adding certain anaesthetics to containers of shallow water. With respect to fish transportation, Norris et al. (1960), noted that transport problems and mortality could be alleviated by lowering the metabolic level by temperature reduction or narcosis.

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CONTROL OF *CULEX* MOSQUITOES IN WEEDY LAKE HABITATS IN LOS ANGELES WITH *CHLOROHYDRA VIRIDISSIMA*[1]

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ABSTRACT

European green hydra, *Chlorohydra viridissima* (Pallas) were introduced at the rate of 10,000 each in 3 tule habitats of Harbor Lake and 1 ornamental weedy pond in Los Angeles County, California. *Culex peus* Speiser and *C. erythrothorax* Dyar and hydra populations were assessed monthly. Hydra were well adapted to most habitats tested early in the season, but adaptability declined in drainage channels and at Harbor Lake later in the season. *Culex* populations were reduced 93% by June 5, declining to 78% on July 3, 45% on August 7 and 35% on September 10 in Harbor Lake. However, control increased to 57% on October 16. A slow hydra density decline after the initial introductions was correlated with their suboptimum adaptability to the water. A complete control was recorded in hydra-treated weedy edge habitats of an ornamental pond at Diamond Bar, where an average of 1.2 hydra per sample was recovered in October.

The European green hydra, *Chlorohydra viridissima*, is a potentially effective biological control organism for mosquito larvae. Since Hargreaves (1924) first reported hydra predation, subsequent studies on the destructive capacity of *Hydra vulgaris* Pallas (Hamlyn-Harris 1929, Twinn 1931, Stephanides 1960), and *H. americana* Hyman (Qureshi and Bay 1969) showed hydra to be effective agents against mosquito larvae. Yu and Legner (1973) studied *C. viridissima* and presented successful results of sustained mosquito larval control in outdoor experimental *Culex* breeding habitats in Riverside, California, and against naturally breeding populations of *Aedes nigromaculis* (Ludlow) and *Culex tarsalis* Coquillett in Kern County, California (Yu et al. 1974a, 1974b). This report discusses results of *C. viridissima* inoculations in weedy aquatic habitats in Los Angeles, California and the effectiveness of this species against naturally breeding populations of *C. erythrothorax* Dyar and *C. peus* Speiser.

MATERIALS AND METHODS.–Hydra originating from a stock of the European strain of *Chlorohydra viridissima* secured from the University of California, Irvine, were mass-cultured according to the technique described by Lenhoff and Brown (1970). Water adaptability tests were made in several natural mosquito breeding habitats prior to and during field experiments, using criteria and methods reported by Yu and Legner (1973). Random water samples were taken from 8 selected weedy aquatic habitats in Los Angeles County which included waters of a waste water drainage tule pond, portions of the Coyote Creek, an ornamental pond, and waste-water drainage ditches near apartment complexes (Figures 1-3).

Hydra were inoculated at the rate of 10,000 per treatment replicate in the mosquito habitats using the chilled volumetric method of Lenhoff and Loomis (1957). At Harbor Lake, 3 treatments and controls were selected in a tule covered portion of the lake. At Diamond Bar an ornamental pond in the residential area was treated and compared to other similar ponds in the area. Hydra were inoculated homogeneously within areas with a radius of 1.5 meters (ca. 1 hydra/10 cm^2 of water surface).

Hydra were sampled using 2 different methods depending on conditions found in each habitat. Twelve thin white plastic plates, 30.4 x 15.2 cm were used at Harbor Lake as previously described (Yu et al. 1974a, 1974b), with plates ca. 30.5 cm apart. In the ornamental pond vegetation substrate samples were used. Hydra that became attached to the plates or the vegetation were counted immediately in the field and returned to each respective plot.

Mosquito density was estimated by taking four random spots in each treatment of control, and taking eight, 400 ml dips per spot each month.

Differences between treatments and controls were tested using Student's "t" test (Snedecor and Cochran 1967), after basic data had been transformed to $\sqrt{X + 0.5}$.

RESULTS AND DISCUSSION.–Hydra Adaptability and Mosquito Breeding–Laboratory tests of hydra adaptability to water samples from 8 different localities in Los Angeles County showed that 80% or more of the hydra were well adapted to water found in 6 of the habitats (Table 1). The poorest adaptability was in the Coyote Creek drainage where only 55.5% of the hydra tested were rated as well-adapted. In Harbor Lake, 73.3% of the hydra were well-adapted in June (Figure 1), but declined to 36.7% in October (Table 1 and 2). *Culex erythrothorax* and *C. peus* were the predominant species found breeding in Harbor Lake, and *C. pipiens quinquefasciatus* Say in the ornamental pond (Table 1).

Mosquito Control at Harbor Lake--A significant 95.3% mosquito larval reduction appeared at Harbor Lake on June 5 as a result of hydra inoculations (Table 3). Host destruction gradually decreased to 78.1% on July 3 and 45.2% on August 7, respectively, the differences in density between inoculated plots and the control varying in significance between 95-99%. Host destruction declined to 35.5% on September 10 (only 70% significance). However, it increases to 57% on October 16 (95% significance) (Table 3).

Mosquito density in the controls showed two peaks, one in June with an average of 28.3 larvae/dip and the other in October with 12.9 larvae/dip. Mosquito populations in the control averaged 28.3 larvae in June, while areas that had been inoculated with hydra were significantly lower with an average of only 1.3 larvae/dip (Table 3).

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Figure 1.–Tule pond showing one of four plots used for field predation tests of *Chlorohydra viridissima* against *Culex erythrothorax* and *C. peus*. Los Angeles, Calif., 1974.

Figure 2.–Ornamental pond showing an open and weedy habitat where *Chlorohydra viridissima* were released against *Culex pipiens quinquefasciatus*. Diamond Bar, Los Angeles County, California, 1974.

The hydra population measured as the mean number per plastic sampler was 3.0 on July 3 one month after the hydra inoculation (Table 2). However, hydra numbers gradually decreased to 0.7 in August and 0.3 in September (Table 2). The budding index, i.e., a measure of environmental favorability (Yu and Legner 1973), also gradually decreased from 38.8% in July to 12.5 and 0 in August and September, respectively (Table 2). Hydra could not be detected in the October 16 samples.

Control in an Ornamental Pond--Hydra inoculations eliminated *C. p. quinquefasciatus* breeding from the weedy near

Figure 3.–Weed-covered Coyote Creek (A), and concrete-bottom waste-water drainage channel (B) where *Chlorohydra viridissima* were released against *Culex* spp. Los Angeles County, California, 1974.

shore area of the ornamental pond and reduced the density within a stand of cattails in the same pond when compared to other similar ponds in the nearby area (Table 4). Overall host reduction in the pond by the end of October was 93.1% compared to earlier in the year. Although *Gambusia* top minnows were present in the pond, they were unable to destroy mosquitoes in the weedy cover along the shore, which was thoroughly effected by the hydra. Other ponds in the area produced substantial numbers of mosquitoes from both the shore areas and in stands of cattails even in the presence of *Gambusia*. Hydra recovered on October 31 by means of vegetation substrate sampling averaged ca. 1.2/930 sq-cm.

Hydra Adaptability and Host Destruction--It is interesting to note that the capacity for host destruction appeared

Table 1.–Proportion of well-adapted[1] *Chlorohydra viridissima* to natural mosquito breeding habitats in Los Angeles County, California, compared with standard culture water[2], June-October 1974.

Habitat	Locality	Mean No. Hydra Used: Lab Standard	Mean No. Hydra Used: Natural	Mean No. Well-Adap.: Lab Standard	Mean No. Well-Adap.: Natural	% Well[3] adapted	Mosquito Species Breeding
Tule pond tule covered	Harbor Lake, Harbor City Los Angeles	15.3	17.7	15.3	16.3	92.5	*Culex erythrothorax*
Tule pond waste water drainage	Harbor Lake, Harbor City Los Angeles	15.0	15.0	14.7	11.0	73.0	*C. peus* *C. erythrothorax*
Coyote Creek cement bottom	1.5 mi. E. of Santa Fe Springs, L. A. County	15.0	15.0	14.7	8.3	55.5	*Culex* spp.
Coyote Creek weed covered	Candlewood Country Club 1.5 mi. E. Santa Fe Springs L. A. County	15.0	15.0	14.5	13.5	90.0	None[4]
Ornamental pond	Co. Custom House, Diamond Bar, L. A. County	17.5	17.5	16.5	17.0	97.1	*C. p. quinquefasciatus*
Waste-water drain ditch	Diamond Bar, L. A. County	17.5	17.5	16.5	14.5	82.9	None[4]
Waste-water cat-tail weed	Diamond Bar, L. A. Co.	17.5	18.0	16.5	15.5	86.1	None[4]

[1]tentacles well stretched and body normal (Yu and Legner 1973)

[2]EDTA versine and calcium chloride formula (Lenhoff and Brown 1970)

[3]100 - (No. well-adapted in treated / No. well-adapted in control) X 100

[4]*Gambusia* top minnows present

Table 2.–Average number of *Chlorohydra viridissima* per plastic sample following mass release in *Culex peus* and *C. erythrothorax* breeding habitat of Harbor Lake, and average number of hydra well-adapted in comparison with % destruction, Los Angeles, California. July-October, 1974.

Sample Date	Mean No. Hydra/ Sample	Budding Index[1] (%)	Mean No. Hydra[2] well-adapted	Host Destruction (%)
July 3	3.0	38.8	11.0	95.3
August 7	0.7	12.5	9.1	45.2
September 10	0.3	0.0	8.3	35.5
October 16	0.03[3]	0.03[3]	5.5	57.0

[1]No. Hydra with buds/total No. Hydra X 100.

[2]Mean no. Hydra when total of 15.0 used.

[3]Hydra were not detectable, but oil was floated on water surface and many carcasses of aquatic immature stage larvae were found, alkalinity reached 155 ppm - all available evidences showed that habitat water highly polluted.

Table 3.—Average number of *Culex peus* and *C. erythrothorax* larvae per 400 ml dip in tule pond of Harbor Lake, and % reduction in treated with *Chlorohydra viridissima* as compared with control. May-October 1974.

Sample Date	Treated Mean No/400 ml dip			Mean of Control[1]	Average		% Reduction
	Plot 1	Plot 2	Plot 3		Control	Treated	
June 5	2.5	0.1	-	28.3	28.3	1.3	95.3**
July 3	4.9	0.0	7.3	18.4	18.4	4.0	78.1*
August 7	3.7	N/A[2]	7.8	10.5	10.5	5.7	45.2*
September 10	7.9	0.3	10.5	9.6	9.6	6.2	35.5
October 16	5.4	0.4	10.9	12.9	12.9	5.5	57.0*

[1]% Reduction = 100 - (Treatment/Control) X 100

[2]Sampling impossible due to drying.

[3]Significance at 90% (*), 95% (**).

Table 4.—Average number of *Culex pipiens quinquefasciatus* larvae per 400 ml dip in ornamental weedy pond at Diamond Bar, Los Angeles County, and % reduction by *Chlorohydra viridissima* compared with pre-treatment control. August-October, 1974.

Sample date	Average No/400 ml				
	Open Area	Edge-vegetation	Cattails + Filamentous Algae	Subtotal	Average
PRE-TREATMENT					
July 18	0.0	4.7	1.3	6.0	2.0
August 29	0.0	1.5	0.2	1.7	0.6
POST-TREATMENT					
October 31	0.0	0.0	0.1	0.1	0.0
%Reduction	-	100.0	48.0	92.6	93.1

correlated with hydra adaptability in a given habitat. In the tule habitat of Harbor Lake, the initial adaptability expressed by mean number well-adapted of total hydra tested when 15 hydra were used per replicate, was 11 on July 3; whereas host destruction at the same time was 95.3% (Tables 2 and 3). However, subsequent decreases in adaptability were accompanied with similar decreases in host destruction (Table 2). The correlation coefficient between hydra adaptability and hydra establishment in the tule habitat was r = 0.8439 (95% significance) further indicating the reliability of this relationship.

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THE USE OF THE PLANARIAN, *DUGESIA DOROTOPHALA*, FOR MOSQUITO CONTROL IN RICE[1]

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The planarian *Dugesia dorotocephala* (Woodworth) is a potentially useful Turbellarian worm for biological control of fresh water mosquito larvae. Planaria have been known as mosquito predators since Lischetti (1919) and Stage and Yates (1939) reported that *Dugesia tigrina* (Girard, 1850; Syn. *Planaria maculata* Girard, 1851) effectively consumed *Culex* and *Aedes* larvae which were used as food. Subsequent studies on the destructive capacity of *Dugesia dorotocephala* showed that this species was an effective aquatic predator of the egg, larval and pupal stages of *Culex peus* Speiser, *C. pipiens quinquefasciatus* Say and *C. tarsalis* Coquillett (Legner and Medved 1972, 1974; Medved and Legner 1974). Legner and Medved (1974) observed that complete larval destruction of tested hosts was obtainable both in laboratory experiments, and in semi-field earthen aquaria. Thus, larger scale field experiments are being conducted by us, utilizing natural mosquito populations.

Preliminary results which will require additional replication indicate that up to 80% sustained *Culex tarsalis* and *C. peus* larval and pupal control can be attained. Also, planaria can reproduce at suitable inoculation sites doubling their population density about every 30 days.

[1]Research partially supported by grants-in-aid and assistance from the Southeast Mosquito Abatement District, and the Los Angeles County Flood Control District.

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LARVICIDAL EFFECTS ON MOSQUITOES OF SUBSTANCES SECRETED BY THE PLANARIAN, *DUGESIA DOROTOCEPHALA* (WOODWORTH)[1]

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ABSTRACT

The action of sticky mucous secretions produced by the planarian, *Dugesia dorotocephala*, in paralyzing mosquito larvae and pupae is described. Substances toxic to mosquito larvae were suspected.

Sticky mucous secretions from epidermal glands are produced by various species of *Turbellaria* including the planaria (Castle 1928, Hyman and Jones 1959, Pennak 1953, Storer and Usinger 1965), their function being connected with locomotion and entrapping prey. Larvicidal properties of these secretions on mosquitoes have been emphasized just recently (Legner et al. 1974, Legner and Medved 1974, Medved and Legner 1974). Generally the larvicidal action of mucous was observed in the presence of hunting planarians and thought to operate as a kind of smothering agent. However, further laboratory studies showed a persistent larvicidal activity long after planarians were removed from the environment. Also, closer observations on hunting planarians showed that only a small amount of mucous was necessary to contact a prey and paralyze it, smothering not being required. The current experiments were designed to test the activity of free mucous secretions and water, and acetone dissolved mucous derivatives on mixed cultures of *Culex peus* Speiser and *C. pipiens quinquefasciatus* Say.

METHODS AND MATERIALS.—Experimental culture stocks of the planarian *Dugesia dorotocephala* (Woodworth) were originally collected from Lytle Creek, California in September 1974 (Medved and Legner 1974). Three groups of laboratory experiments were conducted, one group employing a large volume of water for observations of predation, another group a smaller volume for testing the larvicidal effects of mucous secretions, and a final group utilizing minute volumes of water in watch glasses for further secretion studies.

In the first group about 100 matured, 14-18 mm long planaria were placed into 31 X 17 X 10 cm polystyrene aquaria the sides of which were darkened with black tape, and the top darkened with a screened cover (Figure 1). Uniform ages of *Culex peus* larvae were obtained by hatching egg rafts in plastic trays. Planaria were distributed evenly before introducing 50, 2nd and 3rd instar *C. peus* larvae. Planaria predation was observed with a visor magnifying binocular (2.75X) (Figure 1), and immobilized or surviving larvae were counted.

In the second group of experiments 90 mm petri dishes were each filled with 25 ml of test solutions derived from planaria mucous. Twenty 3rd instars of mixed colonies of *Culex peus* and *C. pipiens quinquefasciatus* larvae were exposed to water and acetone dissolved mucous solutions. Mucous aggregates were obtained by starving planaria for 24 hrs followed by an offering of mosquito larval food. This caused planaria to secrete large amounts of mucous which could be removed from their body surface by partial masceration followed by dissolving in acetone.

Aqueous solutions were obtained by centrifuging 50 mascerated, food-stimulated planaria in water. Acetone dissolved preparations were made by emersing 440 mg mascerated similarly food-stimulated planaria in 5 ml acetone reagent. After the acetone had evaporated to about 4.5 ml, 1.5 ml of the final concentration were added to 23.5 ml distilled water containing 20 *Culex* larvae.

The third group of experiments was conducted in 63.5 mm diam. watch glasses. Ten starved planaria were introduced in 1 ml dechlorinated tap water followed by food stimulation with 10, 2nd instar *C. peus* larvae for 10 min. *Dugesia* and prey remains were then removed and the water adsorbed onto blotting paper. Acetone (p. 5 ml) was then added to dissolve the residue mucous secretions. When the acetone had half evaporated, 1 ml distilled water was added. Fifteen 2nd instar *Culex* larvae were then introduced and the watch glass covered. Surviving larvae were counted after 24 hrs.

Mosquito larvae that survived a test sequence were counted and percent mortality calculated with the formula: % Mortality =(A-B)/C x 100; where A = number of mosquito larvae alive in the control, B = number alive in the treatment, and C = number of larvae used in the test. Tests were discarded when the control mortality exceeded 5%.

RESULTS AND DISCUSSION.—Planaria Activity With Mosquito Larvae--Mosquito larvae in the experiments were

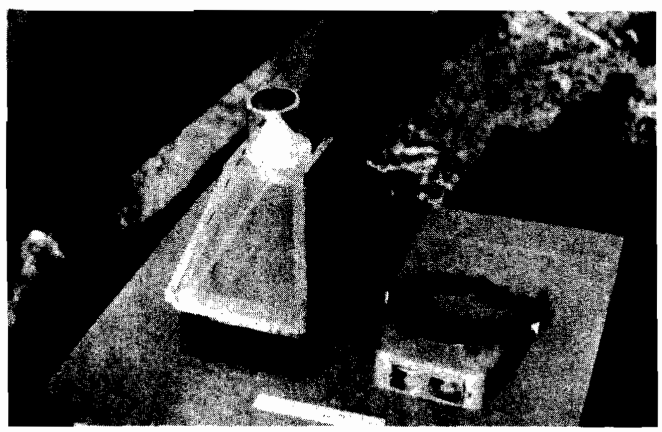

Figure 1.—Plastic tray, 31.5 X 16.5 X 10.0 cm with black tape darkened sides and 2.75 X magnifying binocular, for detailed observations of predatory behavior of *Dugesia dorotocephala.*

[1]Research supported by grants-in-aid from the Southeast Mosquito Abatement District.

Table 1.–Direct and indirect mortality of 50 mixed 3rd instar *Culex peus* and *C. pipiens quinquefasciatus* larvae exposed to 100 *Dugesia dorotocephala* in plastic trays.

Exposure Period (Min.)	MORTALITY[1] Indirect[2] Avg. No.	Indirect[2] %	Direct[3] Avg. No.	Direct[3] %	Total Mortality Avg. No.	Total Mortality %
Planaria Present						
1	6	12	1	2	7	14
10	16	32	6	13	22	45
30	25	49	14	29	39	78
Control (no planaria)						
1	0	--	0	--	0	--
10	0	--	0	--	0	--
30	0	--	0	--	0	--

[1]3 replicates.

[2]A small % recovered.

[3]Larvae engulfed by planaria.

Table 2.–Mortality of 20 mixed 3rd instar *Culex peus* and *C. pipiens quinquefasciatus* larvae exposed for 24 hrs to supernatants of centrifuged mascerated, starved and food-stimulated *Dugesia dorotocephala* in water and acetone.

Treatment	No. Larvae Survived after 24 hrs Repl. 1	Repl. 2	Repl. 3	Avg. No. Alive	% Mortality[1]
Water Derived Supernatant					
Conc. I[2]	20	20	20	20	0
Conc. II[3]	20	19	20	20	1
Conc. III[4]	20	20	20	20	0
Control[5]	20	20	20	20	0
Acetone Dissolved Solution					
Conc. I[6]	18	18	16	17	14
Control I[7]	20	20	20	20	0
Control II[5]	20	20	20	20	0

[1]% mortality = (No. larvae alive in control - No. larvae alive in treatment)/No. larvae used X 100.

[2]No dilution.

[3]Diluted to 0.1 (1:9 supernatant/distilled water).

[4]Diluted to 0.01.

[5]Distilled water 25 ml.

[6]1.5 ml solution + 23.5 ml distilled water.

[7]Distilled water 23.5 ml + 1.5 ml acetone solution.

either killed directly by planaria contact or indirectly by isolated mucous secretions. Direct kill was easily observed, *Dugesia* gliding swiftly to a wiggling larva and grasping it with the strong adhesive-like mucous secretion usually concentrated near the head region. This was followed by enveloping the larva and ingesting its fluid contents by sucking followed by rejection of the remains (Figure 2 and Villee, Walker and Barnes 1973). Indirect kill resulting when mosquito larvae contacted mucous secretions left in the water was more difficult to discern because of the almost complete invisibility of the secretions. A more complete discussion of predatory behavior may be found in Legner and Medved (1974) and Medved and Legner (1974).

Results of group 1 experiments conducted in large plastic trays with 100 planaria showed that mortality of larvae rapidly increased from 12% in 1 min to 49% in 30 min, and that direct kill represented a significantly lower percentage kill than indirect kill (Table 1). The average survival of mosquito larvae after 30 min was only 23%.

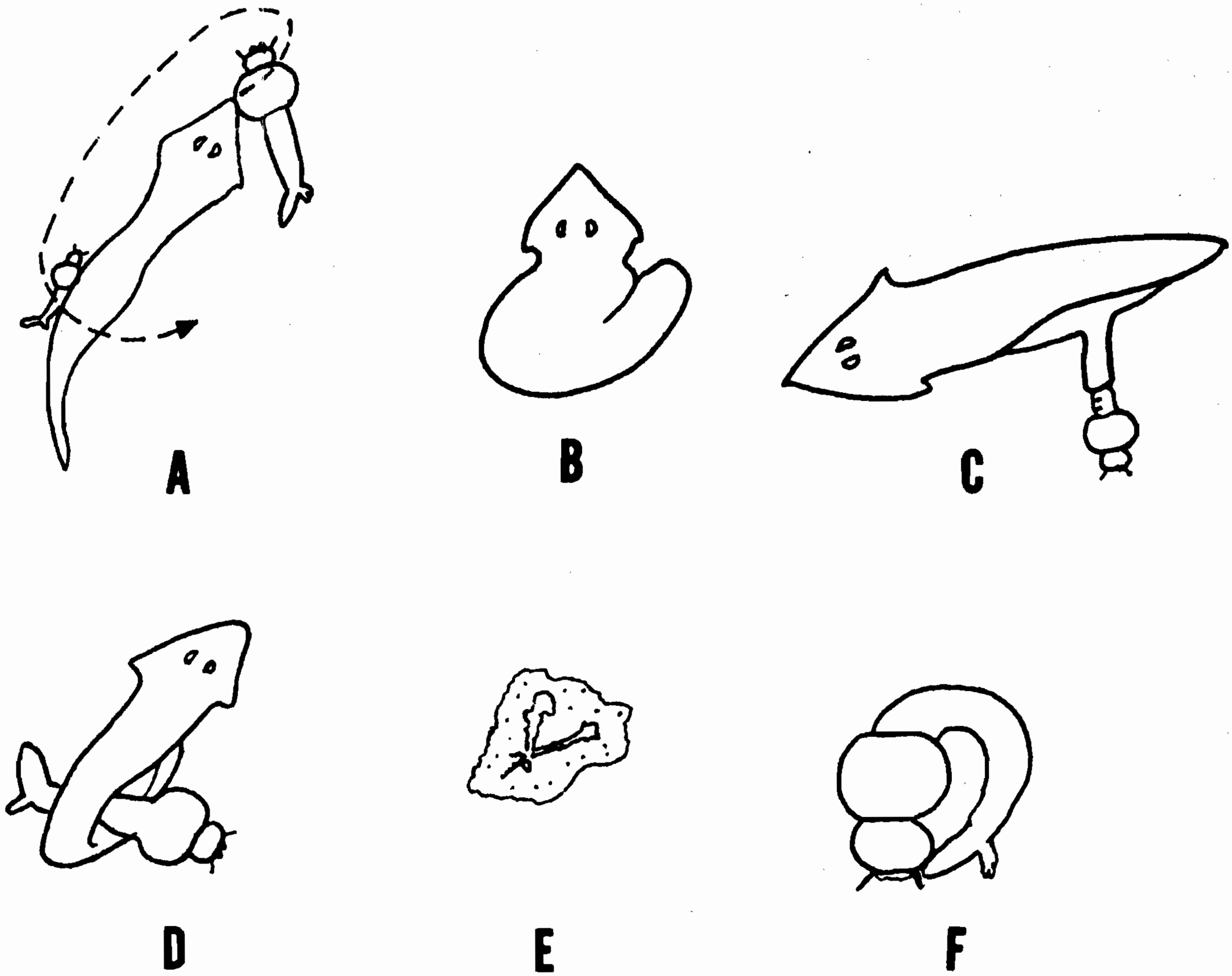

Figure 2.–Diagramatic illustrations showing predatory behavior of *Dugesia dorotocephala:* (A) capture movement for mosquito larvae; (B) enveloping after capture; (C) ingestion of larval fluids; (D) roll-up and sucking position; (E) exuviae residue of 2 *Culex* larvae; (F) symptomatic paralyzed position of *Culex* larva after contact with *Dugesia* mucous.

Observations in group 1 experiments of immobilized larvae without any evidence of bodily contact with planaria suggested the existence of toxic substances. The mucous secretions apparently immobilized larvae by causing a paralysis. In many instances larvae without any sign of mucous contact (i.e., larvae contacted by mucous usually became stuck to the substrate) were immobilized and showed vigorous twisting symptoms of both the abdomen and head. However, these larvae might have contacted minute fragments of mucous that were not discernible visually. Such larvae were unable to surface and eventually drowned. The symptoms of immobilization and paralysis were very similar to that caused by *Chlorohydra viridissima* (Pallas)(Blanquet and Lenhoff 1966, Lenhoff and Loomis 1961), where an eversible thread is released from a nematocyst (Figure 2-F).

Mucous Derived Mortality--A slight but nonsignificant mortality of 2% was detected in group 2 experiments using the supernatant of an aqueous mixture of mucous and water (supernatant further diluted 10 times) (Table 2). However, a significant mortality of 14% was obtained with an acetone derived supernatant (Table 2).

In group 3 experiments a complete kill was obtained with the acetone derivative after 24 hr exposure in 3 replicates, with mortality observed to occur gradually in time. The exact mode of action of the acetone derivative was not determined, but entanglement and suffocation were definitely eliminated as a possible cause. The possible involvement of rhabdites as killing agents was also eliminated in group 3 experiments. Although one might suspect the existence of toxic organic substances in the mucous that become partially dissolved in acetone and only very weakly dissolved in distilled water, further studies are necessary to definitely remove possible direct killing effects of the acetone in these tests. For example, acetone in the controls quickly evaporated and mosquitoes recovered fully after showing initial stress, while the presence of mucous in the treatments might have reduced evaporation rate through adsorption or some other mechanism.

The mucous secretions of *D. dorotocephala* appear to serve two functions which enable this predator to capture its prey. First, the secretions act as a strong adhesive which serve as "pseudo mandibles" enabling the planarian to hold on to its struggling prey. Second, toxic substances in the secretions may slowly (several seconds) paralyze the prey making it further suitable for predation. A characterization of the nature of these substances and their possible synthesis may offer great possibilities for mosquito control in temporary breeding habitats such as rainwater pools and irrigated pastures. The direct inoculation of living planaria cultures may be more suitable for long range control in more permanent water such as rice and duck club ponds.

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TRAINING AND CERTIFICATION

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California is perhaps more fortunate than some other states with respect to mosquito control, in that it has more adequate resources to meet the needs generated by an urgent problem of potential encephalitis and malaria transmission; compounded by a nuisance mosquito problem of gigantic proportions. In the background, there is an extraordinarily serviceable mosquito abatement district act which has been made a part of the Health and Safety Code providing for the formation and operation of local mosquito abatement districts whereby local communities can establish an authoritative organization with taxing power to develop an operational program supported locally, and under the control of a responsible board of citizens selected from within the area of the district. This law grants to the district board of trustees sufficient power including that of entry upon private property to perform a mosquito control operation in a workman-like way.

The role of State agencies including the role of the State Department of Health is clearly indicated as one of providing information, evaluation of problems, surveillance, coordination, and in emergencies, operational assistance to the extent necessary to protect the public health.

The role of the University of California is less specifically defined but within the broad general plan of State funding provided by the legislature, it is clearly indicated that the University is intended to be the primary resource for fundamental research relating to problems of this sort having broad implications upon the better life in California.

Since the State resources must be spread rather thinly over the extensive areas within organized mosquito control, the policy was established long ago of attempting to encourage local mosquito control agencies to be as nearly self-sufficient as possible, with State services provided more on qualitative than on a quantitative basis. Accordingly, local agencies have from the start been encouraged to provide training for their own people in routine daily operational tasks leaving the principal standard setting and training in new procedures to the State agencies. In practice, this has often resulted in the University performing new research, the State Health Department providing interpretation.

Prior to 1972, in some areas of California, resistance to chemical insecticides by mosquitoes had developed to such a high level that practically no synthetic chemical that was available could be used successfully at legal dosage rates. The CMCA and the member agencies recognized the urgency of the problem and proposed that a training program be developed by the State Health Department to elucidate other approaches to mosquito control which could be followed effectively and safely as a substitute for chemical control. Not long thereafter the Congress of the United States passed the legislation which required the EPA to demand that all users of hazardous pesticides be certified by a responsible state government agency as competent to perform the operations required of them. The legislation also required that the EPA set standards and adopt regulations under which the objectives of the laws could be accomplished.

All of this occurred at a time when mosquito control agencies in California were at a crossroads, where the emphasis of mosquito control must be changed from nearly entire dependence upon chemicals to a better balanced program, emphasizing biological control and physical control, with chemical control serving as a supplement.

The CMCA had established a Training and Certification Committee, and upon its subsequent request, the Vector Control Section of the State Health Department reacted by setting up a cooperative plan for conducting the training that would be necessary to prepare workers for meaningful certification. One element lacking was a manual which could be supplied to the field workers to keep and which would provide the study information they would need in connection with their expanding duties and responsibilities and from which the questions for certification could be drawn.

Accordingly, the staff members of the Vector Control Section were invited to contribute subject matter, and the primary technical specialists in vector control were assigned the task of preparing a first rough draft of a manual.

This was hurriedly accomplished as a crash program and circulated in rough form to administrative and technical personnel of all mosquito control agencies for input of comments, criticism, and additional subject matter. The Training and Certification Committee of CMCA had directed that the new manual not duplicate the simplistic instructions already available in a number of other publications, such as how to properly spray a pool, or how to operate a fog machine or a hydraulic sprayer. These were skills already mastered by most of the men to be trained, since many of them have been employed for five to 25 years each and spraying has been a daily occupation for many of them. Instead, the Committee urged that the new manual be more technical than the instructions normally given field operators at entrance levels, giving at least equal weight to biological and physical control and to the safe and effective use of pesticides in a manner that would not be hazardous to the operators or to the public, or to the environment in which operations are normally performed. In most spray programs the proportioning of spray materials and diluents and the selection of chemicals is usually left to the technical specialists who prepare tables which the operators follow in loading spray tanks. However, it was deemed desirable that general information on the mixing of chemicals be included since it was always possible that an operator would be called upon to load a tank when the tables were not available.

Most of the operators who use pesticides had already been instructed in the symptoms of pesticide poisoning and the emergency measures which should be taken in cases of suspected poisoning and those using hazardous chemicals were already under the supervision of physicians skilled in the recognition and treatment of pesticide poisoning. Nevertheless, it was decided that the manual should contain at least elementary information of this sort and that operators should be examined to be sure that they are aware of the hazards and how the materials can be used safely.

The manual was in preparation before the EPA had adopted standards and regulations, but the Committee felt confident that it could reasonably anticipate what would be in them and that they would be substantially in accord with the procedures already in force in mosquito abatement programs. (It should be noted that mosquito control agencies normally apply only a fraction of the amount of insecticide per unit area that may be used in agriculture - for example, the limit on parathion is only 0.1 lb/acre and some agricultural uses are 80 to 100 times as great). It should also be noted that as early as 1956, CMCA with assistance from Vector Control has developed and published A GUIDE FOR RECOMMENDATIONS FOR THE USE OF INSECTICIDES IN CALIFORNIA MOSQUITO CONTROL, which contained major sections on safe usage of "moderately toxic" and of "highly toxic" chemicals. Although now outdated by changes in available pesticides and changes in laws and regulations, this important publication illustrates the principles of safe and justifiable use of toxic pesticides and points up the pioneer efforts of mosquito control agencies in California to safeguard their operations by procedures designed to avoid damage to man and non-target animals, long before EPA had been founded.

However, the manual was revised with partial support by EPA to introduce additional material and to bring it into conformance with Federal and State laws and regulations and will soon be reprinted by the CMCA press, for use for candidates who may be called for examination in the future. The changes which have been made are mainly technical rather than fundamental, so the previously trained operators should easily accommodate to the new specifications.

Based upon the information contained in the manual, several tests have been developed and given, on three dates as indicated in the following table, for a total of 838 candidates taking the test, of whom 717 are now entitled to proudly display the certificate proclaiming them to be "CERTIFIED TECHNICIANS, MOSQUITO CONTROL".

California Department of Health
Vector Control Section
Certified Technician, Mosquito Control
Certification Test Results

	Candidates				Candidates Repeating			
Test Date	Total	Pass	Fail	%Fail	Total	Pass	Fail	%Fail
4/ 5/74	603	555	48	8	--	--	--	--
10/18/74	107	71	36	34	25	12	13	52
5/23/75	128	91	37	29	7	3	4	57
	838	717	121	14	32	15	17	55

Editors Note: The revised "Training Manual for California Mosquito Control Agencies" can be purchased from the:

California Mosquito Control Association, Inc.
1737 West Houston Avenue
Visalia, California 93277

HOW WE SAVED THE ABOMINABLE SNOWMAN FROM MALARIA

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Everyone knows about the Himalaya Mountains, and most people have heard of the Abominable Snowman, but few know the name or location of Nepal – the little kingdom containing these two marvels. I'd never even heard of the place until the U. S. Public Health Service said that they might want to send me there to help eradicate malaria.

My wife and I wanted to be sent to South America because she was of Latin extraction and we both could speak Spanish. We could have fitted right in – and I told my department-head so, This must have impressed him . . . because he said I'd better learn to speak Nepalese.

Unlike Spanish, Nepali is a sanscritic language spoken by about 5 million people. Our State Department had one book on "How To Speak Nepali", but the tape of how the language sounded wasn't available – it was being used in London.

Learning of my efforts to get the tape, someone in Washington said "if you are so interested in learning a foreign language, why don't you and your wife study Spanish? . . . we have some dandy self-teaching tapes and books for it!" Only slightly frustrated, we enrolled in a course on "How To Speak Spanish" while awaiting our travel orders for Nepal to go through diplomatic channels.

While waiting, we brushed up on our geography and found that, if you go south of Alabama 200 miles, and drill a hole straight through the earth, you will come out, on the other side, in Nepal – where the people are Hindu or Buddhist and speak Nepali, Newari, Thibetan, Limbu, Rai, Tamang, Gurung, Thakali, Bengali, Mithili, Hindi, Bhojpuri, Urdu, etc. . . – but no Spanish.

Finally all was in readiness: we each had our Official State Department passports in our hands, twenty or so immunization shots under our skins, new tooth brushes and clean underwear. Our Boeing 707 thundered its way westward – chasing the sun which hung in the sky for 20 hours before we finally saw it set in Tokyo – where the Japanese imigration officials told us we couldn't get off the plane because we had no visas.

Being fluent in Spanish, I soon got them "squared away" and we were en route to our hotel. By the way, have any of you ever ridden in a Toyota taxicab? With your wife and four children? And six suit cases? During the rush hour? On the wrong side of the road? with a Japanese cab driver who can't speak a word of Spanish?

It was even worse in Calcutta, India two days later. The Indian officials took us into separate rooms to question us before they confiscated our passports because they didn't want us to go roaming around India without the proper visas. They didn't speak Spanish either, but they finally agreed that if we would promise to depart India in 24 hours, they would return our passports and let us board a Royal Nepal Air Lines C-47 for Kathmandu, Nepal. We promised, and were en route to our Calcutta hotel. It was quite good – we were housed and fed in a manner reminiscent of the good old days of British "Empiah" and it was with a pleasant feeling of well-being that we returned to the airport the next day for the last leg of our journey to the kingdom in the clouds – Nepal.

Of course there was one small "hitch" at the airport – the Indian officials couldn't find our passports. I diplomatically "explained" to them that my Government would be very upset if those passports were lost or stolen. Let me see if I can recall my exact words . . . MMMM, yes I can. The passports were soon found, and we boarded the Royal Nepal Air Lines ship. Next stop, Kathmandu!

Picture, if you will, mountains so high and breathtakingly beautiful, that you swear they're not real. Imagine a range of mountains whose snow-covered peaks loom so high above the clouds, and block out so much of the clear blue sky, that 29,000 foot Mt. Everest – only 100 miles away – seems lost and insignificant. Feel the airplane lurch and twist as you thread your way through the intervening Mahabarat Range, "only" 14,000 feet high, and look straight out through the window at the mountainsides which border the mountain pass through which you are flying.

Then, just as you are beginning to take a very personal interest in the physical and mental condition of your pilot, the plane's engines slow to a quieter pitch – and you see thousands of thatched, pagoda-like roofs below you. You land, seemingly without descending, in Kathmandu. Oh yes, the immigration men are there – but this time you have an easier time of it because there is a custom in our Foreign Service that all newcomers are met at the airplane door by someone from the mission who steers them through (or around) the "red-tape" and into their new way of life.

Until about 200 years ago, the Florida-sized area which we call Nepal was made up of hundreds of very tiny kingdoms. Then there arose a conquerer named Prithvi Shah. He came from the Gorka District (from which we get the term "Gurka Soldier" for all the Nepalese mercenary soldiers who served with the English.) He was sort of a "hillbilly in silk pants", but he was a strong king and he extended his domain until he ruled over an area larger than California.

One of his descendants, through palace intrigue, was virtually deposed and was forced to designate a member of the Rana Family as hereditary prime minister. The king was relegated to figure-head status and was literally kept prisoner in the Capitol. This state of affairs continued for a hundred years while the Ranas had absolute mastery over Nepal – including the treasury.

The Ranas closed the kingdom to all outsiders and used a great portion of its wealth for their own enrichment. They lived like the maharajas they were – and they built scores of palaces and dwelt in regal splendor until 1950 when King Trubuwan (grandfather of the present king) escaped from his captors and made his way to India – where he called upon his subjects to arise and throw off their Rana masters.

The revolution was successful and the Ranas were deposed from their positions of power. Not having the kingdom's treasury at their command, they couldn't afford to keep their palaces . . . and thereby hangs a tale: have any of you ever lived in a palace? We did. That was what the U. S. Agency for International Development (USAID) had rented for us. It was rather nice, really . . . oh it was a little BIG: the bedrooms were 40 by 50, the tiled floors echoed eerily, it was hard to heat, the ceilings were 16 feet high and our hallway was 160 feet long and . . . I'll bet you that my wife and I are the only people in this room who customarily rode bicycles through the hall on the way to and from the toilet. Hell, we had to! Imagine yourself with amoebic dysentery . . . and bacillary dysentery . . . and viral dysentery . . . and the "john" 150 feet away down a long hall . . . and you with two priceless gifts – the ability to ride a bicycle . . . and speak Spanish!

That palace (called Satya Bhawan – or "True Palace") must have been really something to see in the old days of "Rana-Rule" – but the owner of it, a Rana General, fell on hard times and had to rent it out to USAID – who split it up into six "tenements" for the use of American families.

One day General Satya called upon the Embassy and said that his daughter was to be wed to a young man of royal blood. The wedding was to be at Satya Bhawan, the reception to be held in the 4 to 5 acre courtyard, the King and Queen were to be present, and all the tenants of the palace were invited. I was glad to go. I hadn't seen many real kings or been invited to very many royal weddings – of Hindu couples, that is.

When the big day arrived, the bride and groom, borne on sedan-chairs and resplendent in clothes of colors to rival a rainbow, came to Satya Bhawan. Their approach was heralded by traditional wedding musicians (who belong to the caste of toenail clippers) and who, in my opinion, should stick to the pedicure business. Subsequently, I saw lots of Hindu weddings, but that tune never changed – it was sort of a "It's The Lovliest Night of The Year" played by really independent-type "musicians" who don't really give a damn what the other fellows in the band are doing. The Hindu wedding march goes sort of "twee-deele-dee-dee" with a cymbal and drum accompaniment.

Their majesties arrived in a victorian coach-and-six, the ceremonies got under way and, soon afterward, the wedding banquet began. There was no beef of course – that would have been sacrilege to Hindus – but the food was truly royal, in quality as well as quantity – and we ate from beautiful chinaware.

As the plates were emptied, they were placed on the floor. From time to time, servants would pick them up, take them away to be washed, and return them to the table. During the course of the meal, I left the table briefly. This soured my enjoyment of the party a little because I saw that the servants were taking the dirty dishes to the sewer lagoon where, squating on their haunches, the dishwashers were industriously washing the china between courses. Worse yet, they were not using the New Ivory Liquid. I felt the old urge to ride my bicycle – but now I understood why the urges came so frequently of late.

Meanwhile, back at Satya Bhawan, life in a palace had lost most of its charms for my wife. That is why, during one of my trips to the jungle on the business of malaria control, she pulled a string or three and got us a real house to live in. And she pulled still other shenani – I mean strings – and got the complete staff of servants who had been working for the Director of USAID; who was returning to Washington at the close of his tour. His servants had the reputation of being the best in the kingdom, and the sweeper-bearer (what we would call a butler) was especially good. His name was Kashi, and he seemed to be able to read my mind – and anticipate most of my wishes. The one time he goofed was this: I had been accustomed to mixing my own highballs – and I like Jack Daniels and water. Now, the Nepalese heard me call Jack Daniels "Tennessee Whiskey" so Kashi told me "I fix Sahib's drink. How you do it?"

I said "well, you put in two fingers of whiskey and then fill the glass up with water."

I was busy talking and did not pay attention to the drink he brought – I took one big long drink from the glass and blurted out "My God, Kashi! How strong is this?"

Kashi said "just what Sahib say – two fingers of Tennessee Whiskey . . ." then, as he held his fingers longitudinally up to the glass, I knew I had a language problem on my hands again.

Another illustration of the language problem happened when my boss' wife, Jenny Steffen, decided to entertain some visiting dignitaries with a candle-light-and-silver, "do-the-whole-bit-type" dinner: she had gone to great lengths to have everything just so. She had even procured a special, very delicious fish, called "Bekti", from India to use as her main dish.

Because the smell of the baking fish had permeated the whole house, Jenny told her cook "don't forget the Airwick!" When the cook proudly served the fish, floating in a strange green sauce, Jenny knew two things: 1 - that her cook had not forgotten the Airwick and 2 - she might just as well have spoken Spanish.

Strange, wierd, things used to happen to me over there when I would go away from the comforts of the capitol to seek the haunts of *Anopheles fluviatilis* and *A. minimus:* when I left California to join the "Feds" in 1966, some friends gave me a new Sampsonite suitcase. They said I might be traveling and would need some good luggage. (They were right about the traveling because I literally wore that suitcase out in four years of Federal service.) However, when the suitcase was still almost new, I packed it and took it along on a journey by helicopter to an area about 18 miles from the small principality of Sikkim. We landed quite near a little village situated at an elevation of 7,000 feet.

Now remember, there were almost no roads in Nepal – there were only very narrow mountain paths. Most Nepalese had never seen any kind of a wheeled vehicle. They did not know the wheel because there was no need for such a device and, strange as it may seem, the first wheel ever seen by the majority of Nepalese was on a helicopter . . . that's how quickly the future comes to some isolated peoples . . . anyway, there we were near that little village, being stared at open-mouthed by hundreds of people. They pressed around us in silent wonderment as our marvelous chariot "whop-whop-whopped" its way down the mountain pass.

Feeling somewhat embarrassed by so much attention, I turned to my Nepalese colleague, Shambu Lal Shrestha, and said, "Shambu-Ji, I think these people expect something more of us – why don't we dance or something?" Shambu, even more embarrassed because he was a city boy from the Capitol, said only "aw Vwhit . . ."

After a few more minutes of stares, I said "Shambu-Ji, I've got an idea – why don't you tell them you are the King?"

"Aw, Vwhit"

"Well then, tell them I'm the King! . . . Hell, man, these people expect something"

"Aw Vwhit"

About that time, along the path, there came the complete staff of the Field Unit of the Malaria Office. They told me they had moved to another locale where there was more malaria. This meant that we had a two-day trek in front of us – plus another two-day trek back to the village to meet the helicopter upon its return.

Next day all of us (and our gang of hired porters) were strung out in a long, serpentine path winding through the mountains. Clouds were drifting along below us, sometimes covering us in a deep fog, sometimes vanishing to leave us in blinding sunlight there in the foothills of the Himalaya Mountains. My Sampsonite suitcase seemed out-of-place atop the back-pack of a Sherpa porter, but the thing that was most inconguous was the music that came from the pack of another porter somewhere up the line. That barefoot mountain man had a powerful shortwave transister radio tuned into God-knows-where. (I couldn't understand the language the announcer spoke) but the song (in English) that came drifting out to us over the mountains in that faraway, cloud-hung place was right out of a Grade-B-movie of the thirties: a ceiling fan slowly turns in a steamy, tropical barroom while a Renegade White-Man, dressed in a rumpled, white-linen suit, dances with old "What's-her-name" to the scratching hiss of a 78 rpm record playing "I Saw You Last Night and Got That Oooold Feeling".

The incongruity of seeing that "airplane-type, guaranteed non-crushable, streamlined, two-suiter Sampsonite suitcase-for-the-busy-executive" strapped to the back of that Himalayan porter, and hearing that kooky song so loud and clear, made it all seem so unreal that I had to laugh. Then I was brought back to reality by my own "old feeling" and had to excuse myself and leave the trail. That "old feeling" – which some called "Kathmandu-Complaint", others called "Delhi-Belly", and some called the "Rapti-Ravages" – kept returning to me 20 or 30 times each day until finally (18 months later) I cured myself with massive doses of tetracyclene.

Another item which was "passing strange", happened just after the end of the monsoon rains in 1967: my Chief, Alan Steffen, told me he wanted me to do a one-week evaluation of the malaria situation in Katunje. So he said to me: "Katunje is somewhere out in area 7 – about 100 air miles west of Kathmandu. Think you can find it?"

"Oh sure, Chief, no sweat. I'll find its location and see when the helicopter can fit me into the schedule."

On the helicopter company's 12-foot topographical map I had no trouble finding Katunje. It lay 90 miles west by north-west and the helicopter pilot said he could take me there on Tuesday morning and return for me in 8 days.

On Tuesday morning, my interpreter and I loaded our bedding, food, water, and a medical kit aboard the helicopter. (I also had a couple of bottles of Jack Daniels in case of cobra-bite). We lifted off and headed out over the mountains toward Katunje. Then, when the pilot thought we had gone about far enough, he touched down near some villagers, and my interpreter asked them "is this Katunje?"

"No, its further on toward the west."

Five miles more – "Is this Katunje?"

"No its over that way to the southwest."

Three miles later – "Is this Katunje?"

"No, its across that valley to the south."

Flying across the valley, we saw a new, freshly-whitewashed building, and landed just up-hill from it. When the villagers came running to see the helicopter, my interpreter asked, "Is this Katunje?"

"Yes."

"Where is the Malaria Office? Is it in that new white building?"

"Yes." We finally found it!

After unloading all our gear on the ground I clapped the pilot on the back and said "think you can find this place next week?"

"No sweat, boy! I've got it marked on the chart now – I'll get back to you."

"Now don't forget me – OK?"

"Don't worry, I'll be here – see you next week." As the helicopter lifted away, we walked down to the new building. It was vacant. We were amazed, so we asked: "Where are all the malaria workers?"

"They are not here now – they only come once a month – this is the Panchayet Building." (Panchayet is sort of a grange-cum-town-hall institution.)

"Then can you tell us where the Malaria Office is?"

"Not here, Sahib."

"This is Katenje, isn't it?"

"Oh yes, but there's no Malaria Office here."

"Then why did you tell us there was?"

"Well, you came riding on a Garuda (which is a mythological, parrot-faced, god-carrying creature) – you came like gods riding your Garuda, and we didn't want to disappoint you . . ."

"Oh, you haven't disappointed us!"

After a few more horrified questions, we learned that there might be a Malaria Office in the Katunje over there – or maybe in that Katenje over that way – or possibly in the Katunje which lies in that direction. (It appeared that there were three Katunje's – each one a three-or-four day trek away – for "Katunje" is to Nepal what "Springville" or "Pumpkin Center" is to America . . . we picked the wrong Katunje.)

Realizing that we could never walk to the "right" Katunje (even if we could find it) do the necessary evaluation, and then walk back to the "wrong" Katunje in time to meet the helicopter – and knowing that the pilot probably couldn't find us if we left instructions with the villagers about where we had gone, we aborted the mission, hired porters and prepared to walk back to the Capitol. Even more tantalizing, the buzz of the helicopter was still echoing from the mountain-sides, but there was no way to call him back. I guess the lonesomest sound in the world is a helicopter leaving you in Katunje.

The two porters we had hired agreed to carry our gear for 5 rupees per man each day, so we began hiking toward the south-east in order to intersect the Raj-Path – which was the recently-constructed, unbelievably twisty, mountain road which had been built to connect India and Nepal. We hoped to catch a ride at least part of the way back to Kathmandu.

Three days later, by walking 16 hours per day, we saw the snake-like curves of the Raj-Path in the distance. Working our way toward it, the interpreter and I had inched our way across an extremely narrow footpath which had been cut into the sides of a rocky cliff. We were "taking a breather" when we heard a series of long-drawn-out screams. One of our porters had brushed against the wall of the cliff and had been hurled into space.

His screams, and those of his partner, brought us running back to the edge of the cliff just in time to see him roll to a stop at the foot of the talus which had accumulated at the bottom of that three-or-four hundred foot cliff. Miraculously, he was not killed, because the slight outward cast of the cliff – together with the loose, sliding, talus, had slowed his downward momentum enough for him to escape serious injury. Oddly enough, even the remaining bottle of Jack Daniels was unbroken.

The porter was horribly skinned-up, though, and (to forestall infection) we poured iodine on his wounds – which must surely have hurt him terribly. After we all had a liberal, therapeutic dose of "Tennessee Whiskey", I offered to pay him off and double his pay (in partial compensation for his injuries) so that he could return to his village, but he told the interpreter "tell the Sahib that I have never in my life seen a road or a truck. I want to finish the journey." He finished his journey with a red hide – but walking proudly.

Three days after leaving Kathmandu in a helicopter, I returned in the first vehicle ever seen by that porter – a Mercedes truck which was driven by a bearded Indian truck driver dressed in pajamas. My report to Alan Steffen must have struck the State Departments' funny-bone, because they sent it back to Washington – where it was picked up by a newspaper which used the heading "The Names The Thing".

To understand the enormity of what we had gone through, one should realize that although Nepal is only Florida-size horizontally, she could be Texas-size if all her mountains were flattened out. Those mountains are so steep that often villages only ten horizontal miles apart are strangers to each other. Strangers with different cultures and languages. In some places it may take twenty days to hike a hundred linear miles. And, because the mountain chains run north and south, people who must travel in an east-west direction often find it more convenient to first go south to the plains of India, make the longitudinal traverse, and then re-enter Nepal to reach their destination. Those mountains are not only breath-takingly beautiful to see – they are also breath-takingly breath-taking to walk.

Then, there was the time that ghosts and leopards "put the fritz" on our experiment huts in Butual . . . but that's another story . . .

Although this talk has been given in a light vein, the malaria which once beset the people of Nepal was no laughing matter: prior to 1958, in that tiny Asian kingdom, there were 40 thousand people who died each year from malaria – and an additional 40 thousand who died from causes attributable to malaria. Those 80 thousand deaths mean that one out of every 112 people died each year because of malaria. It was so bad that the native workers who had to cross the band of jungle separating India from Nepal used to pray to the gods "if you let me live – and don't kill me with malaria while passing through this jungle belt – I will leave an article of my clothing hanging on the bushes to show my appreciation." The Nepalese also had a saying about the little cross-roads of Hetaura, in the Rapti Valley: "if you buy one rupees worth of rice there, you will not live long enough to eat it."

The work performed by all the malaria workers – indigenous, third country, or American, was arduous and long – but it had results: all those articles of clothing left in the jungle have, long ago, rotted away and are not being replaced. Hetaura is no longer an isolated cross-roads, but is a thriving town of perhaps ten thousand people who don't fear malaria as they eat their rice . . . And the last I heard, there were 12 million people in Nepal – only 30 of whom had malaria in 1972. None of them died from that killer of men. Many of the hill people have taken up homesteads in the formerly malarious lowlands and have abandoned their farms on the unprofitable mountains.

Some say the Yeti lives in the higher elevations of those mountains – preying on farmers, yak-herders, and the occasional mountain-climber. "Yeti", translated into English, becomes "Abominable Snowman". "Abominable Snowman", translated by my wife, becomes "Obnoxious Snowman" – but she may be referring to those objects built by her Irish playmates during her girlhood in Boston.

It is possible that, growing bolder or seeking additional prey, the Abominable Snowman may some day venture down from the High Himalayas – down even below the old 7,200 foot elevation which once marked the malaria transmission zone in Nepal. If he ever comes down, he can be sure he is safe from malaria – even in the lowlands of the kingdom.

When my tour-of-duty ended, we boarded a PIA airplane for Dacca. Then, continuing our westerly flight, at last we saw the lights of Home. When we landed at Dulles International Airport we had circled the globe – with a two and one-half year stopover in Nepal and intermediate points. At the airport, a U. S. Immigration Official, examining our passports (bearing the stamps and seals of embassies from far-away places with strange-sounding names) said, "Sir, you've really done some traveling and you've been away one heck of a long time! Welcome back! . . . Well, Welcome home!"

My wife turned to me and, quoting from Omar Khayam, said "And, Lo – the Phantom Caravan has reached the Nothing it set out from – Oh make haste!"

Smiling at the immigration man, and quoting Constance Whitworth, I said, "Yeah, but we did save the Obnoxious Snowman from malaria!"

The State Department marked our passports "Void".

THE ROLE OF PHOTOGRAPHY IN TRAINING PROGRAMS FOR MOSQUITO CONTROL OPERATORS

Elmer J. Kingsford[1] and Carlton Pearson[2]

Demonstration and visual aid materials are excellent tools in educational programs, whether at the pre-school, college, or in-service level.

Program planners use two methods to incorporate demonstration and visual aid (D-VA) materials into their programs. The first is to plan the program around readily available D-VA materials. The second is to plan the program and then prepare D-VA materials to fit the program. Frequently these methods are combined to gain the advantages of each with greater flexibility in program planning.

Circumstances frequently make it impractical to bring live or preserved D-VA materials to the training site or to move the training site to the D-VA materials. Bulky materials, rare or unusual materials, hard to copy written materials and even dangerous or unsafe materials can be brought to the training site through the medium of photography. With modern photographic equipment and a little skill almost anyone can take acceptable photographs. Where technical material and needs require greater skill and knowledge, the services of a professional or free lance photographer can be obtained.

Mosquito operator training frequently has need of D-VA materials. Photography can fill much of this need, as there are areas where a picture is indeed worth a thousand words.

Many Districts have experienced the need for their operators to be able to recognize various species of mosquitoes in the field. Keys with pen and ink illustrations are the norm. However, through the use of Micro and Macro photography detailed photographs (slides) can be obtained showing the Morphology and location of structures used in identification. The operator can then compare them with live or preserved specimens. This type of classroom practice can be used for orienting new operators and refreshing the skills of veteran employees.

Through photography, examples of the habitat types which various mosquitoes prefer can be brought to the training site (classroom). This allows the similarities and differences of the habitats to be compared and discussed by and with the operators.

Government regulations being what they are these days the use of slides and/or movies as D-VA's is decidedly beneficial in meeting the needs for employee safety training requirements. They are also good for bringing demonstrations of spray applications and other techniques into the classroom.

Over the past 25 or 30 years workers in the field of mosquito control have developed, in the operator (as well as themselves), a firm and positive attitude toward chemical use. This is as it should be. There has, at the same time, been little effort to establish as firm and positive an attitude toward the use of source reduction and mosquito prevention, the foundations of good mosquito control.

Many operators, therefore, either don't or can't make reliable field decisions in source reduction and mosquito prevention. Handicapped with this deficiency, and forced by legislated loss and/or restriction of many chemicals, the operators are faced with the need to upgrade their skills. There is a need, for everyone, to better understand the operators role in these areas.

The use of photographic D-VA materials is highly beneficial in developing operator skills in applying source reduction and mosquito prevention techniques. It is especially beneficial in building the operator's appreciation of his own role in source reduction and mosquito prevention, through before and after pictures documenting progress in these areas.

In training programs for mosquito control operators, photography is an effective D-VA tool in providing the constant refreshing and updating needed to maintain a dynamic and inovative, mosquito control program.

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FISH POPULATION STUDIES IN FRESNO COUNTY RICE FIELDS

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The effects of stocking rates of *Gambusia affinis* for mosquito control in rice fields have been studied by various authors (Hoy and Reed 1970; Hoy et al. 1970; Hoy et al. 1972). Studies of the fish population increase in rice fields have been limited (Reed and Bryant 1974).

The object of this study was to arrive at a population increase curve which could be used to monitor fish populations during the mosquito control season.

METHODS AND MATERIALS.--Fish were obtained from a local minnow farm and stocked directly into rice fields at a rate of 1/8 lb/acre in a manner previously described (Reed and Bryant 1974). Thirty-four rice fields were studied. Fields were stocked 11 to 18 days after rice seeding. Stocking was accomplished in two weeks with 18 fields ready by May 7 and 16 rice fields 10 to 14 days later. Three fields were selected during each stocking period as experimental controls.

Study fields ranged in size from 20 to 127 acres and each contained at least 12 paddies. Minnow traps were operated for 24-hour periods in the shallow portions of 10 paddies starting 4 weeks after stocking. Mature (gravid) females that were caught were counted and recorded separately. Males and immature fish were counted and recorded together. All fish were returned to the field. The trapping interval was 2 weeks per field and was accomplished so that the age of the fish would be comparable in all study fields over the 2-week trapping period.

Stocked fields were trapped 6 times starting June 1. Experimental controls were trapped 4 times.

Inspection and sampling for mosquito production and population was done by zone operators. A 10-dip mosquito larvae sample and population estimate was made for each field using the routine operational method employed by Fresno Westside MAD (Reed 1970). Rice fields reaching mosquito population estimates of .1 mosquito larvae per dip were candidates for chemical control. Parathion at 0.1 lb/acre was the chemical of choice.

RESULTS AND DISCUSSION.—The data obtained from individual rice fields (Table 1) were analyzed by means of the linear regression method. Only mature, gravid females are included in this analysis, and in 14 of the 34 rice fields the data do not fit a uniform pattern. There were marked differences in the level of the midpoints and the slopes of the regression lines among the remaining 20 rice fields, so no attempt was made to compute a time-catch relationship.

There are many variable factors in a rice field study of this nature which may contribute to variability of data, and it was not practicable to separately study each factor independently. Some of the important factors are as follows:

1. Soil types and slopes.
2. Field water depths, fluctuations, and drainage.
3. Width of paddies.
4. Size of fields.
5. Submerged and emergent vegetation.
6. Weed control during study period.

Table 1.–Captures of *Gambusia affinis* in 6 two-week periods starting June 1, 1974.

Field No.	2-Week Period Numbers 1	2	3	4	5	6
1	1	9	61	72	98	264
** 2	0	10	2°	2°	18	75
** 3	0	25	22°	26	27	75
4	1	2	28°	27	32	105°
5	3	29	65	62	142	123
6	3	13	67	87	111	110
7	18	19	40	38	86	133
8	2	31	32	26	111	120
** 9	1	7	35	28	337	124°
* 10	7	21	46	20	226	180°
11	5	20	°8	75	96	151
* 12	8	31	°7	32	102	204
* 13	5	23	°18	44	27	53
** 14	2	5	°9	28	203	234
* 15	4	38	°9	119	41	°132
16	2	20	°13	99	121	157
17	0	9	°6	73	94	40
18	1	31	66	106	202	190
19	0	10	32	104	164	133
20	6	13	46	86	112	81°
21	3	20	51	191	100	187°
22	0	11	26	64	67	49
23	0	31	30	80	179	239
** 24	3	23	21°	88	101	247
25	3	5	°59	234	181	338
** 26	4	17	52	175	102	302
** 27	1	28	49	269	181	254
28	1	17	57°	132	142	219
** 29	2	24	60	43	194	374
** 30	3	15	114	59	280	204
31	7	21	63	192	205	279
32	2	16	99	143	243	347
33	2	33°	23	248°	166	251
** 34	4	6	33	101	72	254

*Data not acceptable at the 5% probability level.

** Data not acceptable at the 1% probability level.

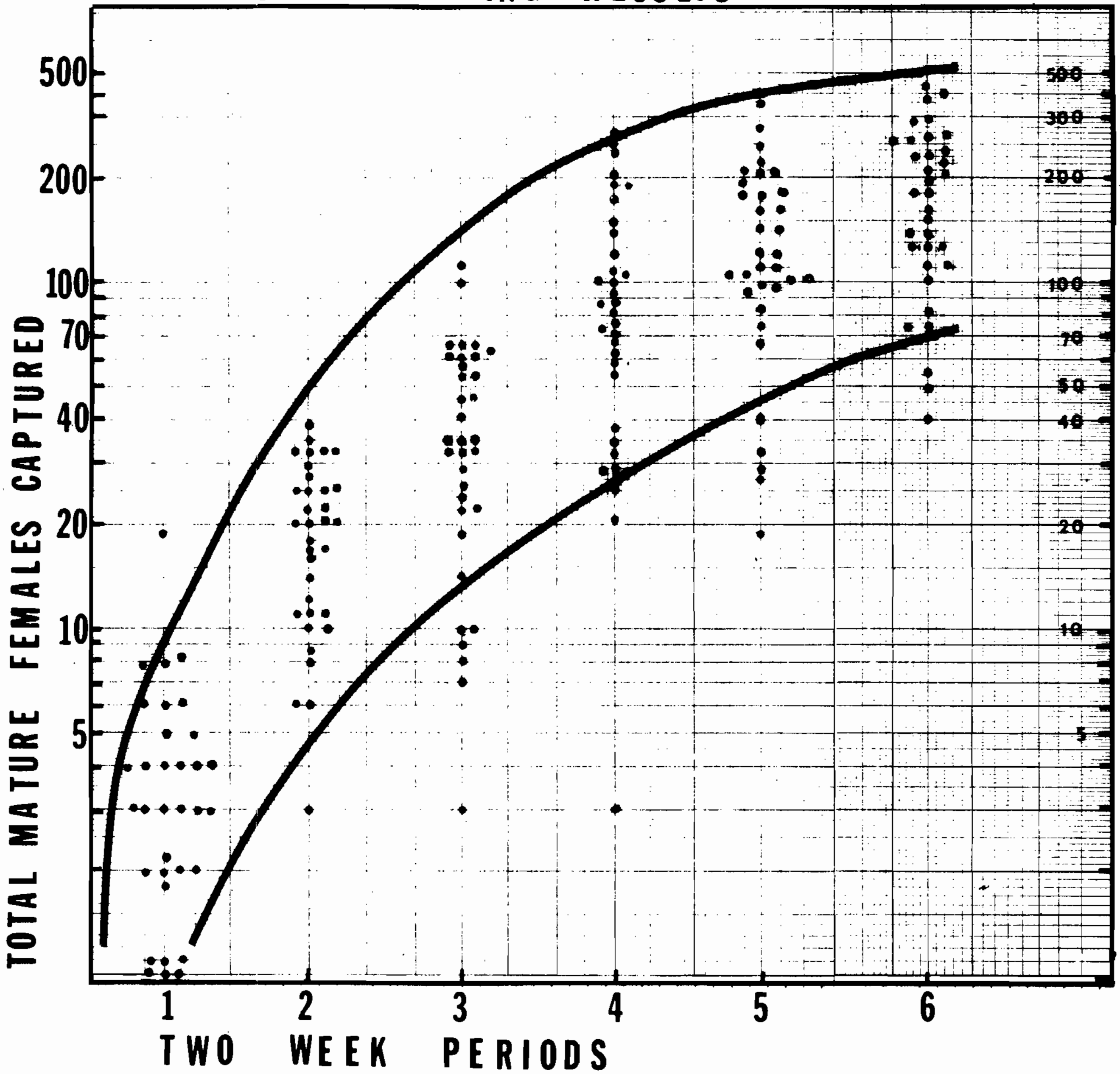
°9 Field sprayed just prior to placement of trap.

9° Field sprayed after trap count was made.

Note 1: There are low counts (under 5 per trap) in at least 4 periods (5 fields), and these are not acceptable at the 1% probability level.

Note 2: Some collections in the 4th or 5th period fell outside the regression line (5 fields): these are not acceptable at the 1% probability level.

FIG. 1 FISH TRAPPING RESULTS



7. Insecticide application during study period.
8. Differences in stocking periods.
9. Number of samples (traps) in fields (usually insufficient).
10. Length of sampling interval.
11. Experimental error.

The use of parathion appeared to be the most important factor affecting fish catches during this study. Treatment of rice fields 48 hours prior to trapping had the most significant effect on the results. (See Table 1, 3rd Period.)

To exhibit the pattern of fish population growth in this study of rice fields, the trapping data were plotted on a 3-cycle log graph (Figure 1) on which the dots show a 3-dimensional effect at each 2-week trapping period. The gross pattern indicates a parabolic curve in the time-catch relationship of stocked fields. The grouping of dots about the midpoint of each trapping period indicates "normal" distribution. The dots located away from the central group indicate variability of the data. The indication of the parabola appears to reflect a small section of the cyclic predator–prey peaks and troughs. In rice fields peaks in fish populations at 1/8 lbs stocking rates may never be reached due to field drainage late in the summer. Measurements of fish populations were only possible during a small portion of the populations increase and well before field population stability was reached. (Figure 1.)

Data summarized in Table 1 were evaluated in relation to the presence of mosquitoes. Figures were ranked in order of the fish caught for each period, and the presence of mosquito larvae was noted. The 12 fields yielding highest fish counts were compared to the 12 showing minimums and to the 10 fields showing medium fish numbers. Mosquito larvae were found in the top 12 fields 11 times, 19 times in the bottom 12 fields and in the 10 mid-range fields 15 times. These data further substantiate the variability of rice fields and appear to indicate that fish stocking rates of 1/8 lb/acre were not sufficient to determine the critical population limits to attain mosquito larvae suppression and/or control.

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LABORATORY COLONIZATION AND SEXING OF *NOTONECTA UNIFASCIATA* GUERIN REARED ON *CULEX PEUS* SPEISER

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INTRODUCTION.–Several species of backswimmers (Notonectidae) have been studied intensively as biological control agents for suppressing mosquito populations (Lee 1967; Ellis and Borden 1970; Garcia, Voigt and Des Rochers 1974; Sjogren and Legner 1974; and Hazelrigg 1974). *Notonecta unifasciata* Guerin, a backswimmer species reported to occur through Western Canada and the United States (particularly in California and Arizona) and in Northern Mexico (Hungerford 1933; and Scudder 1965), appears to be a potentially effective predator of mosquitoes (Hazelrigg 1974; Garcia, Voigt and Des Rochers 1974). Like other backswimmer species reared in the laboratory (Ellis and Borden 1969a; McPherson 1966; Toth and Chew 1972b), *N. unifasciata* can be colonized for study purposes. In addition, immature fifth-instar larvae can be sexed to aid in field release and laboratory studies.

PROCEDURES.–Laboratory Colonization of *N. unifasciata*.–Ellis and Borden reported successful laboratory rearing of *Notonecta undulata* Say for 23 consecutive months (1969a). Their technique, with modifications, was developed for a single colony of *N. unifasciata*. A typical laboratory colony of *N. unifasciata* is shown in Figure 1.

A ten-gallon glass aquarium was filled to a depth of approximately two inches with autoclaved, medium-grade, aquarium gravel. A standard corner aquarium filter-aerator was placed in the aquarium. Two-gallons of equal parts of tap water and double-distilled water were mixed and added, resulting in a pH of 8.15+.2 and total hardness of 35+17 ppm $CaCO_3$; no attempt was made to duplicate water conditions of the collection site (ponds at the Ontario National Golf Course, San Bernardino County, California, containing effluent from an adjacent county sewage treatment plant).

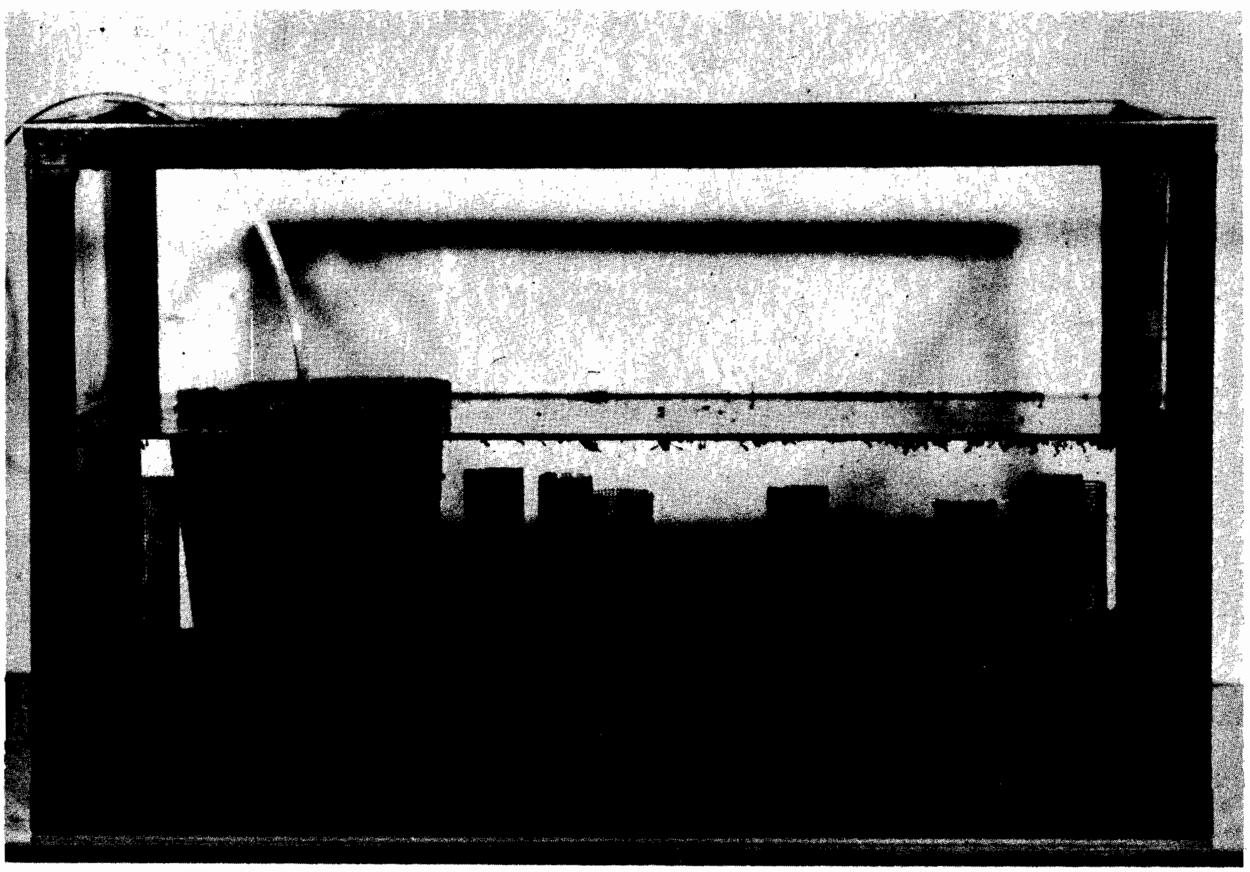

Figure 1.–One of three laboratory colonies of *N. unifasciata*. The cylinders of fiberglass screen placed vertically in the aquarium are resting and oviposition sites provided for the adult backswimmers. The filter-aerator is surrounded by a larger fiberglass screen.

Resting and oviposition sites of 14-mesh fiberglass screen were formed into cylinders measuring approximately four centimeters in diameter and ten centimeters in length and placed vertically in the aquarium (Figure 2). The filter-aerator was also enclosed within a cylinder of 14-mesh fiberglass screen to prevent entry and destruction of hatching backswimmer nymphs and added food (live mosquito larvae) by the filter.

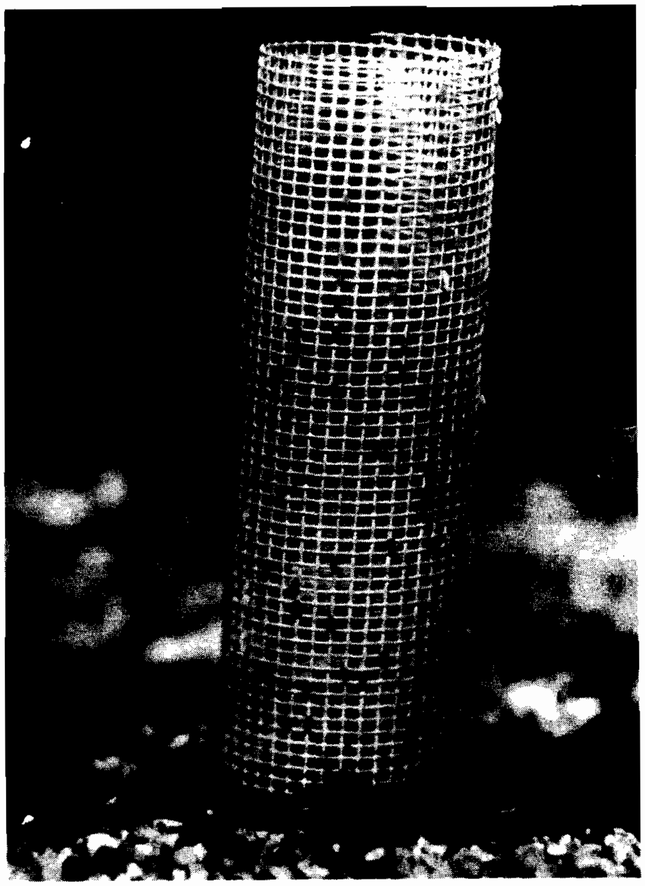

Figure 2.–A resting and oviposition site typical of those placed within the laboratory colonies of *N. unifasciata.* Note the attachment of the eggs to the site.

From the wild or field-collected backswimmers, fifth-instar or preimago nymphs examined and found free of water mite (Hydrachnidae) parasitization (Hungerford 1919; and Bare 1926) were selected for beginning the laboratory colonies. This selection was based on the fact that fifth-instar nymphs molting to adults yield individuals which can be exploited for their mating-age compatibility, longevity, and maximum egg productivity; factors which favor successful colonization. Thirty fifth-instar nymphs were added to the aquarium. These nymphs were not sexed, since observations indicated an approximate 1:1 sex ratio among field-collected adults. The aquarium was covered with a glass plate to prevent the escape of newly-emerged backswimmer adults.

This and similar colonies were maintained at 14 hours of light – 10 hours of darkness cycle and at temperatures varying from 19 to 26°C, depending on the temperature in the laboratory. The water was monitored weekly for pH and total hardness and mainatined at 8.5±.2 and 85±17 ppm $CaCO_3$, respectively, by replacing water lost through evaporation with double-distilled water and by changing the filter-aerators as necessary. The colonies were fed daily on live *Culex peus* mosquito larvae.

Culturing *Culex peus*.–All *C. peus* larvae were laboratory reared from egg rafts collected from an outdoor mass rearing facility (Bay, unpub. data). These were hatched in three-gallon rubberized tubs (Rubbermaid®) each containing approximately 1½ gallons of tap water and one gram of autoclaved, powdered Tetramin®, a commercial tropical fish food. Six egg rafts were placed in each tub and incubated at constant temperatures ranging from 21 to 31°C. Hatched mosquito larvae grew uniformly in size, facilitating the selection and use of particular larval instars as food for the backswimmers. Some larvae were supplied additional amounts of Tetramin to sustain growth. Prior to being offered as food, the larvae were suspended briefly in a one-percent solution of sodium hypochlorite (commercial bleach) and then rinsed in tap water to minimize contamination by a vorticellan destructive to the eggs of *N. unifasciata* (Hazelrigg unpub. observations).

Sexing Immature *N. unifasciata*.–The sexes of adult *N. unifasciata* can be easily distingished by examining the terminal and penultimate and ventral abdominal segments under a dissecting microscope. These segments differ morphologically in each sex as shown in Figure 3. As in the adults, the morphology of the last two ventral abdominal segments of fifth-instar nymphs differs conspicuously in each sex (Figure 4). Examination of the specimens was facilitated by observing them in a narrow container containing shallow water to restrict backswimmer movement.

DISCUSSION.–The colonies were established only to insure a continuous supply of backswimmers for subsequent studies. Nevertheless, these colonies were sustained with minor maintenance for 20 months. Although backswimmers oviposited on the glass sides, particularly in the corners of the aquarium, the cylindrical oviposition sites provided were preferred. These provided an easy means of collecting and transferring the eggs to other containers for rearing and subsequent study.

Sexing immatures of *N. unifasciata* is particularly important in a release program. For example, a desired proportion of the sexes of fifth-instar nymphs (potential reproductive stock) released into mosquito infested habitats as immatures might adjust better than a field release of flight-capable adults and therefore remain in the habitat after emergence. Separating the sexes prior to adult emergence to prevent mating may be desirable when conducting laboratory studies.

The prey used in sustaining these colonies was *C. peus* (easily obtained in this case). Some economy or flexibility in having other suitable prey might be gained by rearing Entomostraca, amphipods or other fresh-water crustacea as used by Ellis and Borden (1969a).

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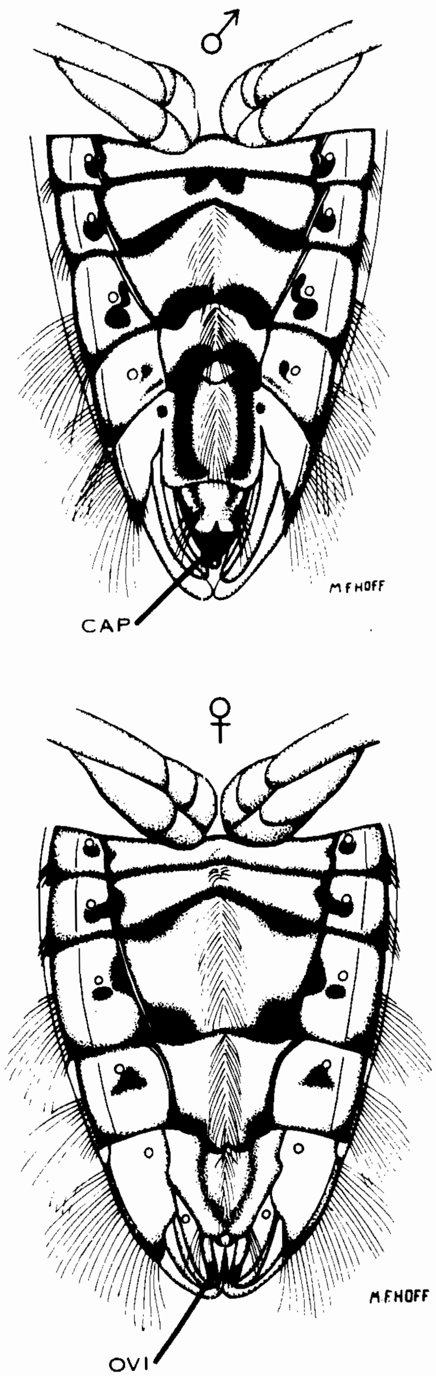

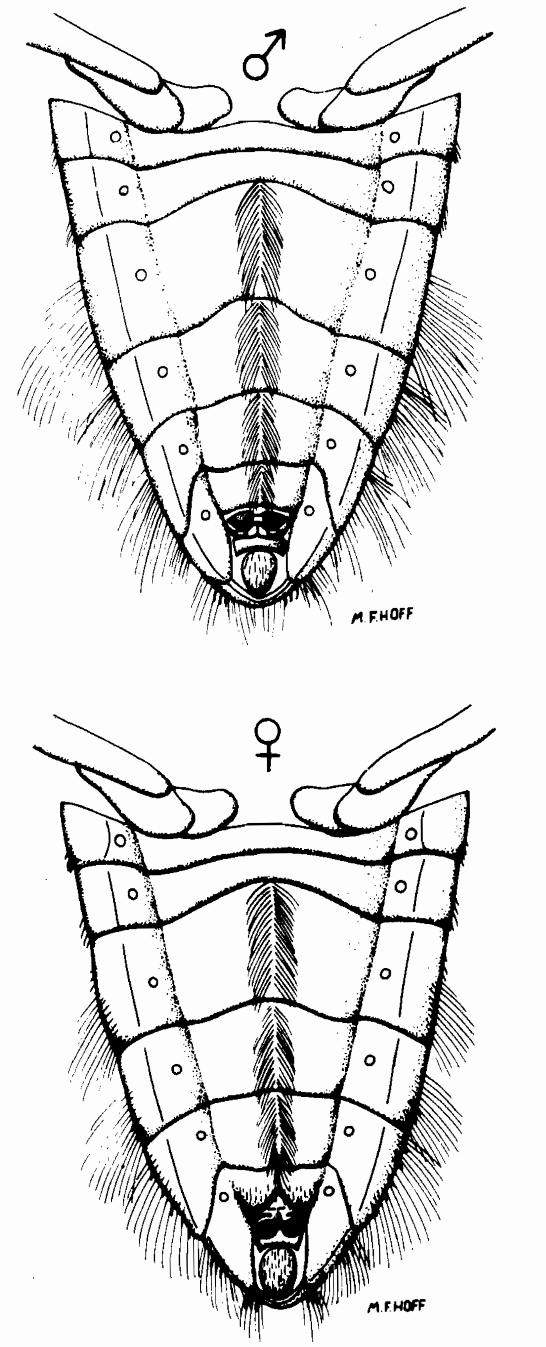

Figure 3.—Ventral view of the abdomen of an adult male and female of *N. unifasciata* (first abdominal segment not shown). The conspicuous appearance of the genitalia below the terminal abdomenal segment is a reliable feature used in distinguishing the adult sexes. CAP - male genital capsule; OVI - female ovipositor.

Figure 4.—Ventral view of the abdomen of a male and female fifth-instar nymph of *N. unifasciata* (first abdomenal segment not shown). The difference in morphology of the terminal segments of the male from those of the female are a conspicuous difference which can be used in sexing the fifth-instar nymphs of *N. unifasciata.*

NIDITINEA FUSCIPUNCTELLA (HAWORTH), A MOTH OF PUBLIC HEALTH IMPORTANCE FROM POULTRY MANURE IN SOUTHERN CALIFORNIA

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The moth, *Niditinea fuscipunctella* (Haworth) (Lepidoptera: Tineidae), enters homes, irritating the occupants by their erratic flight and tendency to contact humans and their food and utensils. This moth may become such a nuisance that governmental agencies were asked to respond either by developing or instituting control measures. During the past few years mosquito abatement districts (vector control districts) have accepted additional responsibilities for abating insects other than mosquitoes. In Riverside County the response was made by the County Health Department, the University of California and the Northwest Mosquito Abatement District.

N. fuscipunctella was most annoying during the avian Newcastle (Hanson 1963) outbreak of 1971-72. Previous minor moth outbreaks have occurred on a four to five year cycle within a fifty mile radius of Riverside. A saprozoic species, *N. fuscipunctella* is recorded from the nests of seventeen bird species and the nest of a *Polistes* wasp (Petersen 1963). In Europe moth flare-ups occur annually during early spring in suspended cage poultry houses. The common name, "poultry house moth", has been approved by the Entomological Society of America.

N. fuscipunctella is a nidicolous species with wide distribution in Europe and Asia. *Niditinea tugurialis* Meyer is the ecological analogue in the Caucases, Turkey, Iran, Central Asia, Afganistan, Pakistan, and India. In the Far East, *Niditinea eurinella* Zagulyayev is the predominate species in China, Korea, Japan and Indochina. According to A. K. Zagulyayev (personal communication), only polar regions are free from *N. fuscipunctella* or related species.

Genitalic examination by Petersen (1957b) of the 28 known palearctic nidicolous Tineidae (Petersen 1957a) showed group tendencies which, when combined with partial biological information, resulted in taxonomic revision and a generic type designation of *N. fuscipunctella*. Later Zagulyayev (1952) synonomized *N. fuscipunctella* as *Tinea eurinella*. More recently, Zagulyayev (1954) expanded on the species genitalic description, supporting Petersen's *Niditinea* generic type description. He subsequently considered, but did not act upon, (personal communication, 1972) placing *N. fuscipunctella* in the subgenus *Acedes*, relative to several grain feeding *Tinea* species. This is of interest since *N. fuscipunctella* is not recorded as reproducing within the whole grain habitat, and such evidence was not obtained during this investigation (Mathlein 1941, McDunnough 1939).

Studies on the biological control of synanthropic Diptera (Povolny 1971) have been conducted in California and worldwide since 1960 (Legner and Poorbaugh 1972), but few reports on pestiferous synanthropic Lepidoptera are available (Legner and Eastwood 1972, McDunnough 1939, Snowball 1941, Thygesen 1971, and Zagulyayev 1968). Because flies and moths are found together, all of this was hoped to lead to simultaneous compatible control of synanthropic Diptera and Lepidoptera.

METHODS AND MATERIALS.—Adult Moth Sampling Method.—Concurrent biological studies on *N. fuscipunctella* indicating photo orientation and larval habitat preferences suggested sampling adult moth night flights, using modified New Jersey light traps. Light traps with 2-watt ultraviolet lamps and a photoelectrically controlled suction fan were placed at the end of central hen coop rows. The traps which were placed 20 feet from structures, suspended 1.5 meters above the ground surface and away from competing light sources, drew adults into 0.3 liter receptacles where dichlorvos (2.2-dichlorovinyl dimethyl phosphate) killed moths within 10 minutes. Collections were counted every 14 days to prevent the trap containers from overflowing. Mean weight of adult moths, based on samples weighed within 24 hours on a top-loading balance (Mettler P-1000), was 0.00254 g.

Larval Moth Sampling and Extraction.—Numbers of moth larvae per liter of manure were deducted from 5 replicated 1-liter habitat samples. Manure was collected in 1-liter plunger cylinders, 10.5 cm long by 10.0 cm in diameter. Moth larvae were extracted with a Berlese apparatus for 48 hours, using a 40-watt incandescent light bulb (Brydon and Fuller 1966). This extraction method is primarily for mobile second through fourth and some fifth instar larvae. Fifth and sixth instars, occupying cocoons, appear in low numbers in Berlese extracted samples. The importance of prevailing larval instars is apparent when interpreting time lag irregularities in concurrent larval and adult moth numbers per sample. One-liter manure cores were extracted at a 45° angle where deposits were less than 45 cm in height. Core samples were collected horizontally where manure deposits were divisible into multiple, 30 cm elevation segments.

When poultry manure accumulates above a 45 cm height, 3 distinct zonal layers occur. These are apparent up to the highest (105 cm) height observed during this study. The manure base pad flares away from the upper core section. The interior basal areas contain the oldest deposits. The flared sides commonly associated with the base are spillages from recently deposited manure not adhering to the section. The manure deposition area was limited by the wire-caged chickens, and was generally observed to be less than the flared basal deposit width. The middle core area ranged in thickness from 15-45 cm. The top is the third apparent zone, characterized by narrowness of width and steeply angled, almost parallel sides.

Two types of poultry housing were chosen for this study. Buildings on Ranch No. 1 are completely enclosed, without windows, and utilized updraft exhaust fans to create forced ventilation. Air movement is adjustable by louvres along the length of the building. Birds are housed in suspended back-to-back cages. Each 1-room environmental structure was 91 x 18 meters with cages suspended 1.5 meters above the ground level. The manure habitat of the moth accumulated for 18 to 22 months, which is the egg-producing expectancy of these chickens. Light within the buidling was regulated to a 13 hour photo-period. Ranch No. 1 was located at the intersection of Limonite and Bain Streets, Riverside County.

Ranches No. 2 and No. 3 were both open-sided, but covered. Manure in Ranch No. 2 was removed annually after accumulating to a 90 cm height. Hens were housed in back-to-back cages suspended 1.2 meters above ground, and were exposed to the environment on all sides. During the spring and summer, manure was exposed to sunlight for more than six hours daily. Rows were oriented east to west.

The housing covered an area 60 x 96 meters. Ranch No. 2 was located at the intersection of Orange and Columbia Streets, Riverside, Riverside County.

Ranch No. 3 was smaller than No. 2, covering an area 80 x 80 meters, with wider aisles and shorter cage rows. The manure habitat was oriented on a north-south axis, limiting direct sunlight exposure to two to three hours daily. Hens were cage-suspended as in Ranch No. 2. Ranch No. 3 was located at Mission Boulevard and Mountain Avenue in Ontario, San Bernardino County.

Ranch No. 4 was located at the intersection of California Highways 55 and 91 in Anaheim, Orange County. A detailed description is found in Legner and Eastwood (1973).

Except for No. 4, poultry ranches were within a 10 km radius of Riverside, California, and were at elevations of 396 to 493 meters. Unless otherwise indicated, all ranches were monitored semi-monthly.

Mean maximum and minimum daily temperatures for Riverside ranged from a high of 33° to a low of 4°C in 1972-1973.

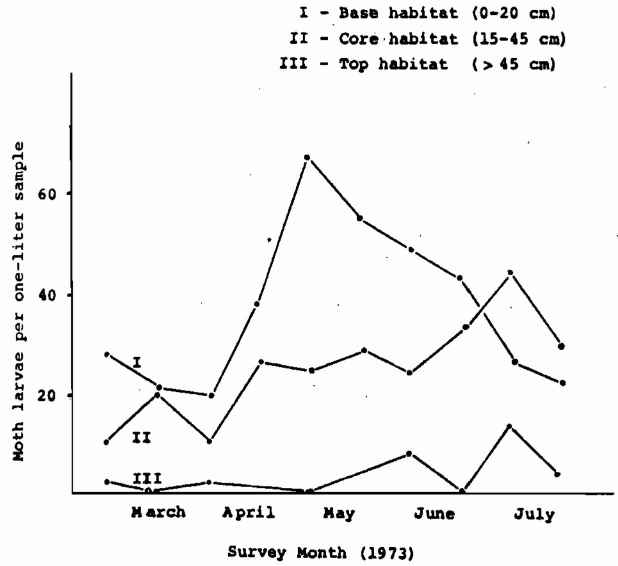
RESULTS AND DISCUSSION.—Larval and Adult Moth Collections In Environmental Buildings.--Larval samples of *N. fuscipunctella* which were collected semi-monthly reached yearly mean peaks in early February and late May. The lowest larval incidence was recorded in December, 1972 and in late July and throughout October, 1973 (Table 1).

Mean numbers of adult moths per 24 hour period were highest in late February and late March, although counts of over 100 adults per 24 hours occurred from February through June. A 14 to 21 day lag was evident between sampled larval increases and the increase in adult moth appearance during the February-June emergence period.

Parasite Collections.--A tineid parasitoid, *Apanteles carpatus* (Say) (Hymenoptera: Braconidae), was first reared from *Tinea pellionella* L. in 1905. Fallis (1942) discussed some environmental factors pertaining to the wasp's life cycle. His studies gave evidence of possible regulative potential on other Tineidae. He noted that *A. carpatus* was reared from *Trichophaga tapetiella* L. and *Trichophaga uterella* (Wilson), both nidicolous tineids. Two adult *A. carpatus also emerged from Pyralis farinalis* L. (Lepidoptera: Pyralidae) obtained from a sample of amassed hen manure. *A. carpatus* was most abundant in fully enclosed, forced-air ventilated hen houses.

Larval and Adult Moth Collections In Open-Sided Buildings.--*N. fuscipunctella* sampled from Ranch No. 2 are recorded in Figure 1 and Table 1. More than 50% of the larvae sampled were located in the base habitat section from March 6 through June 22. Thereafter, larvae appeared in slightly greater numbers in the core habitat section. Base located moth larvae declined in number and percentage between May 2 and June 22. During that period, numbers and percentages of larvae recorded from the core section steadily increased (May - July).

Adult moths peaked in abundance the week preceding the April 3 sample, 27 days after the base habitat larval mean count of 28 larvae per liter sample (Figure 1). Although there were no other adult peak occurrences, total larval counts sharply increased again on May2 and July 6. Fouteen days prior to these dates, an increase in the core section larvae was noted, ranging from 70 to 100% more than in the previous two week period. The data thus indi-



Figue 1.—Numbers of moth larvae per liter of manure, (Ranch No. 2).

Table 1.—Mean numbers of larvae of *N. fuscipunctella* per 1-liter habitat samples extracted at 45° angle to horizontal and collected twice monthly from enclosed building, Ranch No. 1 (5 replicates).

	Larvae/liter		
Survey month	First half monthly sample	Second half monthly sample	Monthly mean
		1972	
November	2.1	3.9	3.0
December	1.6	3.2	2.4
		1973	
January	2.2	5.8	4.0
February	30.6	14	22.3
March	8.0	16	12
April	21	8	14.5
May	11	23.4	17.2
June	6.2	14	10.1
July	0	8.4	4.2
August	8.2	4.1	6.5
September	3.0	1.4	2.2
October	0.59	0.8	0.695

Table 2.–Number of *N. fuscipunctella* trapped in 24 hours by New Jersey light trap at Ranch No. 2.

Sample date (1973)	Weight of moths collected per 24 hours (gms)	Estimate of total moths per 24 hours[a]
March 6	7.27	2860
March 27	14.30	5630
April 3	17.02	6700
April 19	10.53	4137
May 2	10.09	3969
May 21	9.00	3537
June 2	7.89	3100
June 22	4.80	1886
July 6	0.78	308
July 23	0.74	294
August 6	0.31	120

[a]Calculated from weight.

Table 3.–Abundance of three predatory arthropods from poultry Ranch No. 1 (enclosed building), obtained by Berlese extraction, no./per liter.

Sample month	Avg. No. Predators/liter		
	G. nanus	*L. campestris*	*P. sordidus*[a]
		1972	
December	.2	.08	
		1973	
January	.4	.2	
February	.2	.2	
March	.6	.6	
April	.6	.2	
May	.4	.6	
June	.2	.4	
July	0	.6	
August	0	0	
September	.2	0	
October	0	0	

[a]None extracted from this series of samples.

cate that the top habitat section is not suitable for larval development.

Comparable densities of moth larvae in coastal and inland Southern California ranches were found by Legner and Eastwood (1973). In their study the manure habitat attained a height of 34 cm. In this study Ranch No. 2 had accumulated manure to 90 cm in height. Their maximum number of larvae per sample liter was 35 larvae per liter in April while our maximum mean attained was 68 larvae per liter on May 2 (Figure 1). During 1966, larvae on their inland Ranch II decreased on July 12. Our decrease (1972) in moth larvae was similarly noted. Larval increases in the base habitat were preceded 14 to 20 days by larval increases in habitat zones immediately above the base and core sections.

Table 4.–Predators extracted by Berlese funnel after 48 hours ranked to the habitat section of origin from open-sided ranch no. 2.

Survey date (1973)	Larval habitat sampled[1]	Avg. No. Predators/liter			
		L. campestris Adult	*L. campestris* Immatures	*G. nanus*	*P. sordidus*
3/6	a	3	7	0	0
	b	3.2	6	0	0
	c	0	6	0	0
3/27	a	1	4.2	.8	0
	b	0	0	.4	0
	c	0	0	0	0
4/3	a	3	11	3	.8
	b	1.2	14	2.4	0
	c	0	0	1.2	0
4/19	a	4	18	0	2.3
	b	1	3	1.1	1.0
	c	0	0	0	0
5/2	a	0	0	0	0
	b	23	109.5	8	(7 other Staphylinidae)
	c	8	6	0	0
5/21	a	0	0	0	0
	b	18	32	9	0
	c	4	38	0	0
6/5	a	1	0	0	0
	b	13	21	0	0
	c	18	43	0	0
6/22	a	0	0	1.6	1.4
	b	4	37	3	7
	c	13	19	0	0
7/6	a	7	0	1.3	7
	b	7	38	3	3
	c	8 (total, not cat.)		0	0
7/23	a	4	14	0	0
	b	0	0	0	0
	c	0	0	0	0

[1]a = Results for base habitat sample.
b = Results for core habitat sample.
c = Results for top habitat sample.

Table 5.–Abundance of three predators from Ranch No. 5 extracted by Berlese funnel.

Survey month	Avg. No. Predators/liter		
	G. nanus	*L. campestris*	*P. sordidus*
		1972	
December	.05	.4	1.3
		1973	
January	.1	.7	.8
February	.3	.6	1.2
March	.2	.3	2.2
April	.2	.6	3.8
May	.05	.8	2.6
June	.1	.2	1.8
July	0	.1	1.0
August	.1	0	.2
September	0	0	0
October	0	0	0

Predatory Arthropods.--The three most abundant insect predators collected in 1972 and 1973 were *Gnanthoncus nanus* Scriba (Coleoptera: Histeridae), *Lyctocoris campestris* (F.) (Hemiptera: Anthocoridae), and *Philonthus sordidus* Marsham (Coleoptera: Staphylinidae). Individual predators were recorded as numbers per liter of sample. Instars were noted where possible.

Predator abundance from Ranch No. 1 is recorded in Table 3. Ranch No. 1 sustained a population of the parasite, *A. carpatus*. Predators were generally scarce, compared with the biological activity on Ranch No. 2. *G. nanus* was most numerous in January, March, and April, while *L. campestris* was abundant from March through July, when its population declined sharply. *P. sordidus* was not recorded from Ranch No. 1.

In the absence of *A. carpatus*, *L. campestris* was present in the greatest density at Ranch No. 2 (open-sided). Results are recorded for immatures and adults as found in the base, core, and top sections of the sampled habitat (Table 6). *L. campestris* was generally most numerous in the base and core habitats. During May and June, immature *L. campestris* were extracted from the top manure section. This coincides with the appearance of *N. fuscipunctella* in that section, but the cause and effect relationship, if any, is not clear.

Another shift was noticeable in Ranch No. 3 structure in San Bernardino County. This ranch was sampled for 8 months from November through July, 1973. Five 1-liter cylinder samples, randomly collected semi-monthly produced no *N. fuscipunctella* larvae. Samples were withdrawn from a 45° collection angle on a uniform habitat, ranging in height from 25 to 40 cms. CDC (National Center for Disease Control) light traps, situated as previously described for Ranches No. 1 and No. 2, also failed to trap adult moths. Ranch No. 3 was equal in area and hen capacity to Ranch No. 2 of this study.

P. sordidus was consistently the most abundant predator for Ranch No. 3 through the July sampling date. Beginning in August, *P. sordidus* decreased until it approximated the density of *L. campestris* (Table 5).

Ranch No. 4 (open-sided buildings), which was closed January-February, 1973, was referred to as coastal Ranch I by Legner and Eastwood (1973).

SUMMARY AND DISCUSSION.–Before the influx of human population into predominately agricultural portions of Southern California, *N. fuscipunctella* caused but a few isolated complaints. Until poultry operations move to less populous regions, or until they are accepted as a part of rural living and urban agriculture, moth threshold tolerances will dictate control activities. While such thresholds have not yet been established, this study provides descriptions of certain field parameters which tend to influence moth production.

The following are pertinent conclusions derived from the present study:

1. The first 5 larval instars are mobile, seeking out drier habitats prior to forming cocoons. The sixth instar is immobile or is limited in range to its cocoon.
2. *A. carpatus* was recovered only from, or near, closed poultry houses. One ranch which produced numerous adult moths and citizen complaints had fewer *P. sordidus* than either ranches with enclosed buildings, with higher *A. carpatus* counts, or moth free ranches with elevated densities of *P. sordidus*. Effects of *L. campestris* predation in all ranches examined was not determined.
3. During peak months of adult moth occurrence, base sections of manure had the highest density of moth larvae. When manure was greater than 70 cm in height, larvae apparently migrated from the less dense core section into the base section.
4. As moth larvae were rather evenly distributed in the larger manure deposits, the total adult moth population can probably be correlated with the available larval moth habitat. However, on any given ranch, the overall moth density may be kept low by predatory action and other biological and physical factors.

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THE FLY CONTROL PROGRAM OF THE DELTA VECTOR CONTROL DISTRICT

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INTRODUCTION.–The Delta Vector Control District began a full-fledged program of fly control in 1972, upon request of the Board of Supervisors and the Health Officer of Tulare County. Personnel assigned now include an administrator assisted by a biologist, four part-time student inspectors, and up to sixteen federally funded, poverty-level student helpers. . . the District's "bicycle brigade".

The work of the fly control section is divided among rural, agricultural, urban and commercial inspection programs. Larviciding has not been used to effect control, but adulticides have been used upon four occasions, because in each case the fly populations constituted a severe public health nuisance.

THE LEGAL APPROACH.–How does the District engage in an operational vector control program virtually without the use of insecticides? The fly control division uses the much-misinterpreted legal approach. Operationally, a map of each square mile is color-coded; and every source, small or large, urban or rural is given a priority rating for inspection. Forms and inspection procedures are designed to fit the requirements of a hypothetical trial brief. This brief is based upon the Manager's detailed analysis of the statutory steps provided by the State Health and Safety Code under which the vector and mosquito abatement districts function. Ownership of property must be established and photographic evidence taken, and inspection reports and maps of breeding areas, contact and complaint reports, and other pertinent data compiled and periodically reviewed.

With this approach, if at some point the legal remedies of the District were exhausted, or if the legality of the procedures were challenged, the Manager could simply close a particular folder and submit the data to the District's attorney. The fly control division is also fully operational; besides making requests for needed corrections and providing technical and educational assistance, we also utilize source reduction equipment to eliminate breeding areas.

The Delta Vector Control District is more interested in obtaining from the offender the spirit of the law, rather than applying the letter of the law, although we are prepared to do so if necessary. By eliciting from the landholder an appreciation of the spirit of the law, I refer to that higher moral law from which written law is derived: Christ's "Golden Rule" from the "Sermon on the Mount" which states in effect that "one should do unto others as he would have them do unto him" (St. Matthew 7:12). This moral law is translated in the case of vector control into the legal precept that one cannot use his property in such a manner as to deny to others the enjoyment of their property; or to create a hazard to the general health and safety of the community.

The Board of Trustees of the District, sitting as an administrative tribunal, ultimately renders its decision only after an open hearing with the landowner, who even then has additional recourse through the courts. The Board of Trustees constitutes a jury of his peers, whose goal is to obtain his cooperation. The board members are often his friends and are required to be his District neighbors. The District has not yet had any hearings with fly producers, but several mosquito producers have been cited to appear before our Board of Trustees.

How does the public view this program? We have retained the support of our citizenry, who view Tulare County as a unit of localism having unique values and traditions and a quality of environment they wish to preserve and enhance. They also agree with us that cooperative efforts at the local district level is a desirable way of preventing arbitrary actions imposed by a higher and more remote level of government.

The so-called "legal approach" can be positive and dynamic, as well as inhibiting or proscriptive. It provides a procedural outline for the effective management of such districts. The administrative challenge lies in structuring operational programs to fit the provisions of the Code. It need not imply the abandonment of other control methods or summary legal action. It provides the citizen protective legal safeguards against arbitrary action, administered by a special district, specifically created to protect the public while providing equitable treatment to the landholder.

CONCLUSION.–In concluding I would like to make two points: The first concerns the problem of which governmental agency should administer a fly control program; the second concerns the application of the legal sanction.

First, our fly control program is reviewed by a committee, chaired by the County Health Officer. Other agencies include the Agricultural Extension Service and County Planning Departments, the Public Works Department, and the Agricultural Commissioner's Office. The District Manager is in charge of our program, but in many of our inspections we team with personnel from the Environmental Health Section of the Tulare County Health Department. We receive supplementary funding from cities and from the County, and we report to each annually.

The Delta District's program is an integral part of the broader perspectives of County government. Most of the fly and mosquito problems are due to uses and abuses of the environment by man. Fly and mosquito vectors typically develop by reason of environmental misuse. In agriculture, over-irrigation produces an unwanted byproduct, waste water. And if the water stands four days or more, an even less desirable product may arise: mosquitoes. The same formula applies to flies breeding in organic wastes. In fact, the sources in which flies multiply are often as serious a public health menace as the fly vectors that emanate from them. It is therefore logical that our surveillance and control program be closely coordinated with the other agencies of local, county and state government that share responsibility to abate such public health nuisances. For example; representatives of the Water Quality Control Board have rendered outstanding assistance to our program.

The District knows that vector problems cannot be simply "sprayed away". Solving these problems requires planning, education, enforcement, and coordination among the concerned agencies and the landholders. No one special district is equipped to handle all of the complex considerations a comprehensive vector control program entails. A County establishing such a program should not ignore the unique contributions an existing district can make in personnel, entomological expertise, operational and source reduction capabilities, and in executing legal procedures; but neither should a district attempt such a program exclusively and unilaterally.

May I add the somewhat sobering reflection that since the abatement provisions of the Health and Safety Code were first enacted in 1915, they have never been challenged in the courts. It therefore follows that the first few cases processed in the courts will provide the precedents for all subsequent legal actions. Since the actions of one district potentially could adversely affect the legal programs of every other district in the State, perhaps CMCA should consider the formation of a committee on the legal aspects of vector control, to prepare for the test cases which probably are inevitable.

SOME PRECEPTS OF MANAGEMENT IN HUMAN ORGANIZATIONS

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In small organizations, or in subgroups of larger operational units, there may be people who are unable to adjust their activities to their job description, thereby causing unrest in an otherwise smoothly running organization. Animosity, insecurity, and mistrust may result if over-exuberant or misinformed persons wish to assume a responsibility and have to be restrained or requested to observe the limitations of their assigned jobs or their capabilities. Resulting interaction among staff members may hamper normal organizational progress. During the past fifteen years several instances of this sort were observed. It is the intent of this paper to define the applicable precepts and to describe methods which have been employed to prevent disruptions so that the goals of the organization could be attained.

Organizations of a business, professional, or governmental nature always have a primary function, which should have first priority. Secondary effects of an organization, either upon its supporting public or upon other organizations, should take lesser priority ratings. When an organized group fails to achieve its main objective, the group function has not been satisfied. The success of an organization may be limited by various factors. Influencing criteria can be insurmountable physical problems, or personnel motivation problems. Because of these obstacles to success, the practical extent to which a primary function will be satisfied should be defined and periodically evaluated by competent organization specialists.

Any untoward internal friction may inhibit the performance of the primary function of the organization. Ways of preventing friction through effective selection of personnel, clearly stated job descriptions, delineated vertical and lateral chains-of-command, and the wise application of "human relations" have proved to be prime considerations in helping to attain complete satisfaction of the organization function (Gray 1964).

The following outline of situations may apply to everyday personnel and staff experiences:

1. In heterogeneous groups there are apt to be people who will tend to mistake a lack of rigid directions or a display of kindness for weakness in the leader of the group.
2. It follows that those people will not respect a leader because they are able only to respect power.
3. In the absence of a demonstration of power, there is a tendency toward a chaotic power struggle when such persons are present in an organization.
4. Those who respect only power must be shown a powerful image if the organization is to function in an effective manner.
5. To fail to demonstrate strength to persons who respect only power may allow them the opportunity to reduce the organization to a common denominator based on strength, as opposed to common sense and facts.

   Lesser characters, even if active participants in power conflicts, may be forced by the two principal power seeking individuals to assume the role of pawns. These controllable characters provide maneuverability for key personnel who, by the nature of their positions and delegated responsibility, could not publicly associate with a chaos-power concept.
6. To restore order to an organization so disrupted, an excess of power must be employed.
7. The demonstration of power will often create what appears to be greater chaos than in Step No. 3, due to the resistance of ring-leaders who wish to retain newly acquired strength amongst the followers they gained in Step No. 5.
8. This new chaotic disruption must be endured if order is finally to be restored.
9. The leader who cannot, will not, or who is not allowed to endure and quiet this turbulence invites Step No. 3 to recur endlessly, because:
   a. To give in to the people referred to in Step No. 2 will invite complete chaos.
   b. To allow the disruption to continue might destroy any hope for organizational progress or attainment of the primary function.
10. To abate the disorganization which will occur in Step No. 9, the leader must either:
    a. Rid the organization of the disruptor(s).
    b. Deprive the ringleader of his power.
    c. Redesign the organization to obtain homogeneous "esprit de corps" by defining and re-emphasizing or-

ganizational goals, functions of individuals, and the chain of command.

11. The leader must have sufficient power to act. He must:
    a. Have the support of his peers.
    b. Have the support of his supervisors.
    c. Be sure in his position.
    d. Or resign, because:
12. After a reasonable length of time during which Step No. 3 prevails, the leader must be replaced if he cannot attain order in such a fashion that progress and harmony ensue.

In the cases investigated, power struggles developed between two strong personalities. In three of five cases observed, the individuals were placed in a threatened position, either due to their inability to understand leadership's function, or because sufficient information and description of technical concepts were lacking. The latter can occur when unusual or new techniques are used and non-managerial personnel are required to make decisions for which they are not prepared.

In organizations where leadership's role is to offer encouragement and support, personnel who desire strong leadership may be placed in the uncomfortable position of having to make decisions for themselves which they would like others to make for them. When this is the case, greater job and goal definition should be given.

The following steps may prevent or delay disruptive power struggles in small organizations:

1. Develop firm group goals and define the function of the organization.
2. Determine the extent to which you intend to satisfy the function, and impress that point on all concerned.
3. Describe the pertinent jobs within the organization, and the routes of communication. However, overemphasis upon this point may limit the expression of individual ability.
4. Make sure the group leader has the necessary support to accept responsibility for the group. Continual change of leaders detracts from group stability.
5. A leader is necessary for group action. Many groups have leaders in order to concentrate the power and goal concepts of the group in delegated personnel. The opposite is also apparent; a good leader may encourage the support of the workers.
6. In a system where management is either appointed from outside sources or elected from within the group, there should be a degree of separation such that everyone concerned will respect operational decisions which are favorable to the group goals and functions, even though they may not be liked by some individuals concerned. The optimum working situation is one where the success of the individual is a base for the success of the organization in obtaining its goal; but since attainment of the goal or function is the purpose of organized efforts, these should be well defined and periodically evaluated.

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A CHART USED FOR THE TIMING OF CHEMICAL APPLICATIONS ON PASTURE MOSQUITOES

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A successful mosquito larviciding program relies on two factors: Locating the source and timing the application.

The timing of larvicides on mosquito source areas is especially critical in irrigated pastures. Aerial application programs usually treat source areas one or more days after the inspection.

Routine source inspection procedures on irrigated pastures are performed to determine if source areas have been irrigated, and if so, the stage of the larvae.

Table 1.–Pasture mosquitoes.

Days to Pupae	Mean Temp.	Days to Adults	Growth Rate Scale 6-1
	85		1.8
	84		1.63
3	83	4	1.5
	82		1.38
	81		1.28
	80	5	1.2
4	79		1.12
	78		1.06
	77	6	1.
	76		.94
5	75		.9
	74	7	.85
	73		.81
	72		.78
6	71	8	.75
	70		.72
	69		.69
	68	9	.66
7	67		.64
	66		.62
	65	10	.60
	64		.58
8	63		.56
	62	11	.54
	61		.52
	60		.51
9	59	12	.50
	58		.49
	57		.47
	56	13	.46
10	55		.45
	54		.44
	53	14	.43
	52		.42
11	51		.41
	50	15	.40

One of the many operational decisions is the determination of how often and when the source areas in irrigated pastures should be inspected.

The chart was designed as an aid to help with these decisions. Information for the chart came from four sources:

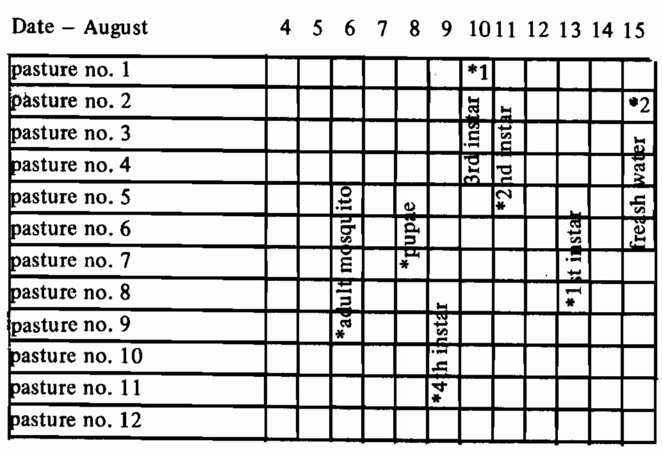
1. Utah Proceedings – the 16th Annual Meeting, from the paper "A Composite Growth Rate Study Between Larvae of *Aedes dorsalis* and *Aedes nigromaculis*" by James J. Peterson.
2. California Proceedings – the 19th Annual Conference of CMCA, from the paper "Review of 1950 Studies of Mosquitoes in Irrigated Pastures" by Thurman, Husbands, Mortenson, Rosay and Arnold.

Table 2.–Example of operational use of chart.

Growth Rate Scale – 1 to 6

1 = 1st instar	2 = 2nd instar
3 = 3rd instar	4 = 4th instar
5 = pupae	6 = adult

Irrigation Date	Mean Temp.	Growth Rate	Mosquito Development	
Aug. 15	66°	Fresh water	Egg	
14	63.5	.62	.62	
13	65.5	.57	1.19	1st instar
12	71.5	.61	1.80	
11	75.5	.76	2.56	2nd instar
10	71.5	.92	3.48	3rd instar
9	68.5	.76	4.24	4th instar
8	68.5	.67	4.91	pupae
7	62	.67	5.58	
6	71.5	.54	6.34	adult mosq.



*Date Source Area Irrigated.

3. From field notes taken by myself in the eastern area of Fresno County.
4. Field observations while with the Klamath Vector Control District in southern Oregon.

The combined information of temperature and larval growth from the four areas was charted and used as a base to proportionately compile the present chart. The larval growth rate indicated on the chart is not 100% accurate and should be used only as a guide.

Water temperatures in the field will vary from the effect of mean temperatures. Deep water stays cooler than shallow water. There are longer periods of darkness in spring and fall; therefore longer periods of cool temperatures in spring and fall compared to mid-summer.

The basis for the chart is the average from the four areas, including data about both *Aedes dorsalis* and *Aedes nigromaculis*. So, the larval growth indicated on the charts is not absolute, but is a useful guide for operations.

The area of the chart that indicates the days to pupae, and days to adult mosquitoes, was used successfully for seven years for coordinating the aerial larviciding program with our routine inspection procedures.

When mean temperatures were 62° and lower, the pastures were inspected weekly; when the mean temperatures were 62° to 73°, the pastures were inspected twice a week; and when the mean temperatures were higher the 73°, the pastures were inspected three times during the week.

This procedure allowed adequate time for both inspection and aerial application before the larvae matured to the fourth instar or pupae.

During the fall of 1973, a time and motion study of this procedure indicated that 80% of the time was used in checking pastures that hadn't been irrigated.

The four major irrigation districts in the county were contacted, the problem of wasted time and motion was explained to them, and their cooperation solicited.

With their aid we were able to acquire daily knowledge as to which mosquito producing pastures had started their irrigation cycle the day before.

The growth rate scale is a modification of the chart that was designed last year for use in conjunction with the date of irrigation, which made it possible to determine each day the growth rate and stage of larvae in each irrigated pasture until emergence.

This system greatly reduced the number of required inspections. It also enables us to choose the larval stage we want to treat and schedule the aircraft accordingly.

Last year this system enabled us to concentrate our aerial application on third instar larvae, providing a 60% reduction in time and motion by eliminating unnecessary inspection of pastures.

The results provided the same standard of mosquito control with less personnel in the field.

COMPARISON OF THREE SAMPLING METHODS FOR MEASUREMENT OF ADULT MOSQUITO POPULATIONS

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ABSTRACT

A study was conducted in Sutter County, California to more precisely characterize the mosquito population being sampled by 1 cubic foot red box, CO_2–baited CDC miniature light trap and standard New Jersey light trap. Six collections were made at bi-weekly intervals from four rural sites from July 10 to September 18, 1974. Males and females of all species were recorded and in addition, the physiological state and age of each female mosquito was determined by the Polovodova method.

The most frequently collected mosquito species in order of abundance were *Anopheles freeborni, Culex tarsalis* and *Aedes melanimon.* Greatest numbers of *C. tarsalis* and *A. melanimon* were collected in CDC light traps while *A. freeborni* was most abundant in New Jersey light trap collections. Highest parity rate was observed in samples of *C. tarsalis* collected by CDC light traps. Lowest rates of parity were observed in mosquitoes collected from red boxes. No females dissected showed evidence of completing more than 4 gonotrophic cyles. Three 4-parous *A. freeborni* and one 4-parous *A. melanimon* were collected by CDC light traps.

ANAUTOGENY AND AUTOGENY IN *LEPTOCONOPS (L.) CARTERI* HOFFMAN

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ABSTRACT

In an effort to establish whether the Valley Black Gnat, *Leptoconops carteri*, survives in nature to take repeated blood meals, as would be contrary to previous opinion, the physiological age of females was assessed by analysis of ovarian activity. Weekly samples of host seeking individuals were taken through the respective 1973 emergence period of two isolated *L. carteri* populations. One population was emergent from the Coast Range, elevation approximately 2,200 feet, eight to ten miles west of Tracy, San Joaquin County; the second population from the floor of the Sacramento Valley just north of Davis, Yolo County.

Upon analysis, females of both populations were observed in which the intima of the ovariole pedicel was dilate or saccate in form. This condition is indicative of prior oocyte maturation and oviposition, and these individuals were judged to be parous. Females lacking this condition which were rated as nulliparous. Parity rates in host seeking gnats from the foothill area ranged from eight to fifty-two percent in weekly samples, and averaged twenty-six percent for the 300 individuals observed. In nulliparous females, terminal follicle maturation was uniformly arrested at stage IIa. This population was therefore judged to be autogenous, and the presence of parous females interpreted as evidence that female *L. carteri* in this area do survive to take a second blood meal.

Parity rates in host seeking *L. carteri* from the Sacramento Valley population averaged nearly 100 percent over a six week period. A few nulliparous females, each of which possessed excessive fat body and follicle maturation in advance of stage IIa, were also collected. These observations were interpreted as evidence that female *L. carteri* of this population undergo autogenous oogenesis of the first gonotrophic cycle. Certain parous individuals also exhibited autogenous oocyte maturation, a condition heretofore underscribed within the Ceratopogonidae.

In 1974, samples of fifty host-seeking female *L. carteri* were taken for analysis of physiological age from the following Sacramento Valley emergence areas: Sacramento Wildlife Refuge, north Colusa, east Woodland, north Davis, and east Dixon. Each sample was observed to be composed of *L. carteri* females from 1973 emergence in north Davis. It appears, therefore, that female gnats from throughout the Sacramento Valley are autogenous for the first and, possibly, a subsequent gonotrophic cycle. Biting gnats from this region are largely parous, but this condition does not necessarily reflect previous blood feeding.

SLOW RELEASE FORMULATIONS OF INSECT GROWTH REGULATORS FOR MOSQUITO CONTROL IN CATCH BASINS

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ABSTRACT

Applications of slow release formulations of insect growth regulators (IGR) were made to 34 catch basins in Fairfield, California and to 48 artificial basins in Davis, California. These formulations, developed at Davis, were in the form of round disks of 6 cm diameter and 1 cm thickness. One disk was applied to each basin. The IGRs used were Stauffer's R20458, Zoecon Altosid and Hercules' 24108. In Fairfield, 16 ppm A.I. of Altosid and R20458 gave 100% control of *Culex pipiens* for 49-64 days and 35-55 days, respectively. At 10 ppm A.I., 24108 gave 81-100% control for 53 days. Substantially better control was obtained with similar and higher rates in the artificial basins. A preliminary cost analysis of expenditures by local mosquito abatement districts for control of catch basin mosquitoes was undertaken.

Such information will be necessary in developing new control measures which are economically feasible for mosquito control in an urban situation. Additional input from local agencies is being requested to this end.

CORRELATES OF MOSQUITO LARVAE DENSITY AND SURVIVORSHIP IN SOME NORTHERN CALIFORNIA RICE FIELDS

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ABSTRACT

Mark-and-recapture techniques applied to *Culex tarsalis* larvae in enclosed sections of six rice fields in East Nicholas (Sutter County) revealed striking inter-field differences in larval survivorship. Rice fields that had high natural populations of mosquitoes displayed high larval survivorship. Over a wider range of fields, mosquito numbers were not consistently related (positively or negatively) to the abundance of any specific predatory species or to the abundance of all potential predators as a group. In one series of survivorship experiments, predators and competitors were excluded from the cages which contained introduced *C. tarsalis* larvae. Although predators reduce the survivorship of mosquitoes in all cages, the variance in larval survivorship between fields is as great in cages without predators as it is in cages with them.

This suggests that nutritional differences exist between fields, or that differences exist in toxin and/or pesticide levels. Laboratory bioassays of water samples from field situations indicated that the latter two factors were not operating. Feeding rate studies performed in the fields indicated that they differed with respect to the density of particles on which mosquitoes fed. Chemical analyses of field water samples indicated that Ca^{++} and conductive levels also varied between fields. Fields with high natural mosquito densities and survivorship had higher Ca^{++}, conductivity levels and faster feeding rates.

SURVEILLANCE AND DETECTION OF *AEDES SIERRENSIS* (LUDLOW)

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ABSTRACT

A trapping system based on CO_2 and rabbits has been shown to be very sensitive for collection of adult *Aedes sierrensis*. Application of this technique to a mosquito abatement program for control was initiated. Preliminary results suggest that the trap will be useful in delineating areas requiring control action as well as monitoring the effectiveness of a control program. Positive correlations were determined between mosquito activity and complaint calls as well as temperature variations and complaint calls. Studies in an uncontrolled oak woodland indicate that adult female populations emerge about 3 weeks before they begin entering the CO_2/rabbit baited traps. The correlation between complaint calls and trapping data and emergence suggest that this is a biological attribute of the species and not a peculiarity of the trapping system.

Studies in different geographic areas revealed large differences in adult populations. It was estimated that population levels in residential areas of Marin are 5 to 10 times lower than in the oak woodland areas of Marin which in turn are 5 to 10 times lower than in a mixed conifer forest of the Sierras.

Patterns of movement were investigated by mark/release and recapture methods. An extremely high recapture rate of 40% of marked mosquitoes was recorded. In addition this mosquito was recorded flying for distances of over one mile. The preliminary data thus far support a theory that in forested areas of the Sierras where wild host density is low during the summer, the mosquito moves from scattered breeding sources until it locates centers of host activity and then builds to extremely high numbers.

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CONFLICT OF INTEREST

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Financial disclosure and conflict of interest are all part of what was called Proposition 9, which was passed by the voters by approximately 70% in the June, 1974, primary election. This act covered many areas – campaign disclosure, limitations on expenditures in campaigns, lobbying regulations, auditing provisions, changes in the state ballot, and conflict of interest and financial disclosure provisions.

A person must now register as a lobbyist if a substantial or regular portion of his time is spent influencing legislative or administrative action. Under the former law, lobbying was generally construed to be dealing with the legislature, but Proposition 9 brought into the law the concept of administrative action, which includes contacts with officials of state agencies. If a person deals with any state level official for 40 or more hours in any two consecutive month periods, or receives $1,000 or more to support such activities, he must register as a lobbyist. The law exempts state employees from the requirement of registering but does not exempt county, city or other local officials or employees. This law, however, does not apply to elected officials.

To register as a lobbyist, one should write to the Secretary of State, from whom he can obtain a 1-page form on which he will list his name, his address, his employer, and for which agencies he intends to lobby. Lobbying without registering can be a misdemeanor, which can be cause for removal from office. There is a $25.00 fee for registering as a lobbyist. A lobbyist incurs certain prohibitions – he cannot spend more than $10.00 per month on any public official; he cannot contribute to any political campaign; he must disclose what money he spends as a lobbyist.

Conflict of interest includes the general requirement that no public official at any level of state or local government may participate in or in any way use his official position to influence a governmental decision in which he has a financial interest, unless such participation is legally required. A financial interest includes items such as a direct investment in a business entity worth more than $1,000, or in real property worth $1,000 or more, or receipt of $250 per month income. The result of a violation of these provisions could be a citizen or a district attorney injunctive relief prohibiting that decision from going forward, or in certain cases the decision could be voided.

Another part of Proposition 9 is financial disclosure for certain public officials. Mosquito abatement district trustees do not come under this section. Those officials who do are elected state officials, members of Boards of Supervisors, the chief administrative officers of counties and cities, mayors, city managers, and members of city councils.

Every government agency, including mosquito abatement districts, will have to develop a "Conflict of Interest Code". This Code does not have to be devised until April, 1976. If a district presently has such a code, it will be applicable at that time. The Code must be reviewed by a "Code Reviewing Body". If a district includes territory only within one county, the body that reviews the Conflict of Interest Code will be the County Board of Supervisors. However, if the district crosses county lines the Reviewing Body is not the Board of Supervisors but the Fair Political Practices Commission set up in Sacramento to oversee all of Proposition 9.

REVIEW IN DEPTH OF THE STATE LEGISLATION DURING THE PAST YEAR

L. R. Brumbaugh

San Joaquin Mosquito Abatement District
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The recent passing of Gardner McFarland was a great loss to all of us. The loss is even greater in that he acquired personal friendships with many of us in private life. He will be well remembered for his many contributions to the Association over the years.

During 1974, Chairman McFarland of the CMCA Legislature Committee, and members of this committee, tried to review over 5,000 bills introduced during the legislative session. Quite obviously, the purpose of this review was to determine which bills would be detrimental or beneficial to mosquito control operations. Of the 5,000 **plus** bills introduced, only 1,545 were passed and became law. Of these 1,545, only a few were concerned with mosquito abatement district operations. Undoubtedly these few may cause some inconvenience – increased costs in mosquito control, or problems in the future. These are as follows:

There were about ten law changes in the County and State Retirement Systems. Most of these changes were additional benefits for employees, which will have some effect on District premium costs.

AB 2528 – State Holidays: March 7, Arbor Day, has been declared as a State Holiday. This might present some problems in the future for mosquito abatement districts.

AB 2546 – Pesticide Chemical Residues: This law prohibits the harvesting of any produce that carries spray residues in excess of permissible tolerance as established by the Director of Food and Agriculture. We do not believe this will present a problem; however it would depend upon how this law is administered.

AB 2792 – Special District Property Taxation: This act requires the Board of Trustees or Directors of any special districts to furnish the Board of Supervisors before the first day of August certain information regarding District finances before a tax rate is set.

AB 2022 – Employment Practices: It would now be unlawful for a District to disclose information as to race, religion, creed, color, natural origin, or sex, unless permission is granted by the employee. Perhaps we should review our application blanks for revision, if necessary, to comply with this new law.

AB 2541 – Agricultural Pest Control License: This law includes a variety of requirements for an agricultural pest control business license. Some of the different requirements are: application for license, demonstration of ability to conduct a pest control operation, and knowledge of nature and effects of the materials used in pest control.

AB 2543 – Agricultural Pest Control Operator's License: Prohibits issuance or renewal of pest control operator's license unless covered by Workmens Compensation Insurance, or self-insured. This means that before a license is issued the applicant would have to prove to the Director of Food and Agriculture that he has insurance coverage.

AB 3197 – Local Health Department: Permits County Board of Supervisors and Local Health District Board to transfer, upon the concurrence of the Director of the State Department of Public Health, to a County Agency, the function of providing environmental health and sanitation services. When such a transfer of function is made, the County Agency must employ a Director of Environmental Health, who is a registered sanitarian. Also, to employ an adequate number of registered sanitarians to carry out such services.

AB 3713 – Helicopter Landing Site Permits: Permits the Department of Transporation to issue permits for thirty days for temporary helicopter landing areas. May delegate this authority to a political subdivision, and such agency may charge a fee for issuance of such permits. Those agencies who have helicopters should investigate this law further.

SB 1340 – Financial Disclosure: Relates to conflict of interest and financial disclosure by certain public officials. So far, board members of mosquito abatement districts are exempt.

SB 1508 – Bidding Requirements: Exempts local agencies from competitive bidding requirements on purchases or contracts for the purchase of gasoline, diesel fuel and other petroleum products.

SB 1553 – Mosquito Abatement: Permits the Board of Supervisors upon concurrence of the County Health Officer providing such service, to transfer all or any portion of the function of providing vector control service to any mosquito abatement district. We understand that this was recommended to eliminate any questions as to the legality of such an arrangement.

SB 1918 – Pest Abatement Counties: Permits counties to provide the same services as a mosquito abatement district within the unincorporated and incorporated territories of the county, but requires that before exercising such authority within an incorporated territory the consent of the city council must first be obtained and a public hearing be held on such proposal before final adoption.

The California Mosquito Control Association formally opposed this bill, and the only change we were able to effect was the requirement of the Board of Supervisors to have a public hearing.

The regulations pertaining to workers' safety involving insecticides, the Cal OSHA laws, EPA regulations, and the U. S. Fair Labor Standards Act are all laws that will probably present many problems and inconvenience in mosquito control operations. The requirements of the Fair Labor Standards Act will ask Districts to pay time and one-half for any working time over a forty hour week. The prospects of changing this law look very remote.

The continual activity of our State Legislature and the regulations issued by State and Federal Governments compels us to keep a close watch on new proposals which threaten mosquito control operations. We must, in the future, be alert and willing to put some effort into protecting our interests.

BOARD POLICY FOR DISTRICTS

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My qualifications for speaking to you on the establishing of district policies are based on background experience as manager of a mosquito abatement district, and my work in federal, state, and local governmental agencies. This has given me the opportunity to assemble some of the policy determinations and procedural guides that have been developed by various local agencies. I'd like to acknowledge the contributions of Contra Costa, Orange County, and San Joaquin Mosquito Abatement Districts.

There are several categories of policy making. Some are of structure, and there is possible agreement among agencies on these; some are subject to laws and there must be agreement on these. There is considerable similarity in personnel policies, with possible differences in employee benefits. The same might be said of purchasing. But in the category of source reduction, for example, there is wide variation in the application of law which is relatively concise. There are almost as many approaches to this problem as there are control agencies. At an administrative practices seminar, one speaker emphasized that there are "things that must be done, things that you should do, and things that are nice to do". Many of the activities of the District are covered under the Health and Safety Code. I have prepared an outline listing things which under the law must be done, and some refinements under the columns, "should do", and "nice to do". The formation of a district, for example, is covered under Safety Code, Sections 2210, through 2226. Appointment of trustees is covered in Sections 2242 through 2244. Each trustee appointed by the governing body of the city shall be an elector of the city from which he is appointed, and a resident of the portion of the city which is in the district. Each member appointed from a county or a portion of a county shall be an elector of the county, and a resident of the portion of the county which is in the district. Each member appointed at large shall be an elector of the district. In mosquito abatement districts, a representative of each city is appointed by the governing body of the city. At least one representative at large shall be appointed by the board of supervisors, and all Boards shall have at least 5 members. Section 2245 stipulates that there be staggered terms of office so that all of the trustees do not go out of office during any one year. There are a few pest abatement districts in California doing vector control. Under Section 2850 in the Health and Safety Code, the Board of Supervisors shall appoint a board of trustees consisting of not less than five nor more than nine members to act as the governing body of the pest abatement district.

To return to the mosquito abatement district – additional sections in the Health and Safety Code establish the structure of board of trustees and meeting time and place. Section 2247 says that the board shall organize by the election of one of the members as president and one as secretary. In Section 2248, the members of the district board shall serve without compensation, but necessary expenses of each member for travel in connection with meetings or business of the board shall be allowed and paid. A majority of the members shall constitute a quorum for the transaction of business. All of its sessions, whether regular or special, shall be open to the public. Members of boards of trustees are busy people and it's to their advantage to conduct the meetings with as much despatch as possible. In connection with the requirement that the meetings be open to the public, I recently heard Mr. Ken Davis, general manager of Sacramento Municipal Utility District, describe the happenings in his district. Originally, they had a Board meeting which was held during the day where business was conducted by the trustees sitting around the table, requiring probably one hour. Since the district decided to build a nuclear plant which attracted a considerable amount of public interest, the Board must now have evening meetings, the agendum must be published in advance, and they have a meeting in the morning for four hours, and in the evening for four hours. In the morning they decide the matters that are to be considered, and in the evening they air these. The meetings are attended by the press and other media and there is always a vocal public there. Mr. Davis stresses the importance of District employees and directors being active in service clubs and carrying the message of the district to those clubs. Three days ago, in the Sacramento Union, this article appeared:

SMUD RELEASES AGENDA BACKGROUND DATA

"In an apparent move to open up information about its activities to the public and the Board of Directors, the staff of the Sacramento Municipal Utility District has released background material on most of the items on the agenda of Thursday's board meeting.

The material was sent to board members and newspapers. SMUD general manager E. K. Davis said other copies will be available in the SMUD office at 6201 S. Street "for review by the public if anyone inquires." Distribution of the material follows prodding by newspapers and demands from director Fred Anderson, who several times has requested more background on equipment bids and other subjects to study before board meetings."

Mr. Davis felt that there is need to expand public information and public relations programs, and the District produced a movie that explains the operation and the process of rate-setting.

You are required to allow the public to attend the meetings but you are not required to allow disruption of public meetings. Penal Code, Section 403 provides in part that, "Every person who, without authority of law, willfully disturbs or breaks up an assembly or meeting, not unlawful in its character, is guilty of a misdemeanor." The Butte County Counsel discovered that in 1970 the legislature modified the Brown Act by adding Section 54957.9 to the Government Code which provides that in the event a meeting is willfully interrupted by a group of persons, so as to render the orderly conduct of such meeting infeasible, and order cannot be restored by the removal of individuals who are willfully interrupting the meeting, the members of the legislative body conducting the meeting may order the meeting room cleared and continue the session. Only the matters on the agenda may be considered in such a session. Duly accredited representatives of the press or other news media, except those participating in the disturbance, shall be allowed to attend any session held pursuant to this section. Nothing in this section shall prohibit the legislative body from establishing a procedure for readmitting an individual or individuals not responsible for willfully disturbing the orderly conduct of the meeting. In the January 27, 1975 issue of the Sacramento Union, this editorial appeared:

IGNORANCE AN EXCUSE
OPEN MEETING LAW CONTAINS FATAL FLAW

"The Ralph M. Brown Act governing secrecy in public meetings was shown last week to be a paper tiger.

"As interpreted by Sacramento County District Attorney John Price, there can be no prosecution unless it can be shown that public officials holding an illegal secret meeting "knew they were violating the law."

"This, indeed, seems a fatal flaw in the law, which was enacted to protect the people's right to know what public officials are doing.

"Five members of the Sacramento City Council – a majority of that body – met for an unannounced session over dinner in a downtown restaurant to discuss the controversial question of appointing a budget analyst to advise the council.

"Press and public would have been none the wiser if a couple of council members – who happen to oppose the idea of having a budget analyst – had not blown the whistle on their colleagues. (Incidentally, that situation makes one wonder how many other illegal meetings have been held when there was no division of opinion on the council. But, that's not the issue now.)

"What disturbs this newspaper – and should disturb the public, since the Brown Act is for its protection – is the loophole in the law.

"It boggles a poor layman's mind to consider the legal technicality that a district attorney cannot act against council members in a clearly recognized violation without proof the members were aware they were violating the law."

The Health and Safety Code calls for the election of a president and secretary of the Board of Trustees. Under "Should do" it is usual also to elect a vice president. The district should describe the duties of the officers. And under "Nice to do": establish committees, give each committee a charge, establish policy for rotation of officers, and establish a policy for attendance at conferences. The "Nice to do" policies are personal preferences, and these are molded by the circumstances surrounding the individual agency.

In the changing order of our society, employee relations, employee associations, and labor unions have commanded a much larger amount of the attention of the governing bodies of government institutions. Government Code, Section 3502 says that "except as otherwise provided by the legislature, public employees shall have the right to form, join, and participate in the activities of employee organizations of their own choosing, for the purpose of representation on all matters of employer-employee relations. Public employees also shall have the right to refuse to join or participate in the activities of employee organizations, and shall have the right to represent themselves individually in their employment relations with the public agency.

Section 3503: Recognized employee organizations shall have the right to represent their members in their employment relations with public agencies.

Section 3504: The scope of representation shall include all matters relating to employment conditions and employor-employee relations, including but not limited to wages, hours, and other terms and conditions of employment, except however, that the scope of representation shall not include the consideration of the merits, necessity, or organization of any service or activity provided by law or executive order.

Section 3504.5: Except in cases of emergency, the governing body of a public agency and boards and commissions designated by law shall give reasonable written notice to each recognized employee organization affected or any ordinance, rule, resolution, or regulation directly relating to matters within the scope of representation, proposed to be adopted by the governing body or such boards or commissions, and shall give such recognized employee organization the opportunity to meet with the governing body, or such boards or commissions.

Section 3505: The governing body of a public agency or such boards, commissions, administrative officers, or other representatives as may be properly designated by law, or by such governing body, shall meet and confer in good faith regarding wages, hours, and other terms and conditions of employment with representatives of recognized employee organizations.

This greater participation of public employees in labor organizations of various kinds had its beginnings in 1969. President Nixon signed Executive Order 11491 in that year which provided new ground rules on representation and unfair labor acts within the federal government. In California, the Meyers-Milias-Brown Act of January 1969 was the beginning of California's public labor relations history. There have been indications that further legislation dealing with this subject are in prospect for this year. The legislature and the governor have gone on record as favoring collective bargaining for public employees. In order for a district not to be caught unprepared by the intrusion of an employee association or a labor union, it should establish personnel policy that outlines a plan describing working conditions. For example: days per week and hours per day, number of holidays, salaries, vacation and sick leave allowance, medical plan, insurance, and retirement plan. This plan should be a matter of record. A representative of a labor union said that he looked upon governmental organizations as Christians in a lion's den, and that when he wished, he could go

Laws	Should do	Nice to do
Formation: Health & Safety Code Sections 2210-2226 – 2822-2836 Appointment of Trustees: H.&S. Code – 2242-2244 2850 Government Code – 1021 (Trustee shall not be a criminal Staggered terms of office: H.&S. Code 2245 Elect president and secretary: H.&S. Code 2247 Establish time and place of regular meetings: H.&S. 2250 Meetings open to the public: H.S. 2252 Majority constitutes a quorum: H.&S. 2253 Audit: County auditor Government Code 26909 Filing of budget: Gov't. Code 26911 Reimbursement of expenses for attending Board meetings: H. &S. Code 2248, 2851 (permissive) Purchase supplies: H. & S. 2270 (permissive) Gov't. Code 54201-54204 (policy mandatory) Board powers: H. & S. 2270-2292, 2850-2855 (permissive) Nuisance: H. & S. 2271 - 2800.5 Penal Code 370-375. Source reduction: H. & S. 2274-2289; 2857-2868 (permissive, except for notification of land owner and the holding of hearings) Labor relations: Gov't. Code 3502-3511 Labor Code 132a, 224, 2257.3, 2923, 2924	Determine objectives of the District & have them on file. Elect a vice-president Describe the duties of officers. Establish salary schedule. Set travel reimbursement. Budget review. Establish rules of procedure for Board Meetings. Establish amount of reimbursement for attending Board meetings. Have on file a policy statement describing purchasing procedures. Establish a policy with regard to the type of program which the District should pursue, and the end point to which negotiations should go. Develop procedures to implement the course that is chosen.	Establish committees, eg.: budget, finance, policy, property & building, vehicle & equipment, interagency, personnel & salary. Give each committee a charge. Establish a policy for rotation of officers. Display national & state flags. Recite the pledge of allegiance. Have information on the business to be conducted at the meetings, agenda, & reports & recommendations of special committees mailed to Board members in advance of meetings.
Conflict of interest: Gov't. Code, Sections 1090, 1120, 1222, 1360, 1369, 82011, 82048, 87100-87103, 37207, 87300-87312, 91000-91012. Penal Code 70. Safety: Administrative Code Title 8, AB 150 State enforcement agency is the Division of Industrial Safety of the Department of Industrial Relations. Insurance: Gov't. Code 990.4 (permissive - self-insurance or from industry). Reports of and review of the environmental impact of projects: Calif. Environ. Quality Act Division 13, Chapt. 1-6, Sections 21000-21165. Constraints upon water management: River & Harbor Act of 1899, Sect. 10, 30-1151; 33USC 403. National Environmental Policy Act Sect. 404. S. F. Bay Conservation and Development Commission Gov't. Code 66600 et seq. Coastal Zone Conservation Commission Public Resources Code 27000-27428. State lands Commission Public Resources Code 6108-8558. Fish & Game Commission – Code 1600-1602.5 Porter-Cologne Water Quality Control Act 13300-13350	Establish a personnel policy and salary schedule, describing precisely working conditions, grievance procedures, holidays, vacation & sick leave, overtime allowance, if any, medical insurance, life insurance, retirement plan. Be completely knowledgeable about limitations of the law. Know the requirements of the law and correct faulty plant, equipment, vehicles. Get public liability, vehicle, fire and burglary insurance. Establish a policy for handling environmental impact reports & place it on file in the District. Get an exemption from the various restrictive requirements or negotiate long-term permits in advance of needs.	Have a counsel on retainer or have ready access to county counsel of district attorney.

out there and gobble up those Christians! Obviously, in a confrontation between the Board of Trustees and representatives of the Union, the Trustees will be at a disadvantage simply because they are not in the habit of dealing daily with these negotiations whereas labor union leaders are. To preclude putting yourself into a bad position, a statement of policy is very important.

The passage last year of Assembly Bill 2862, adding powers to pest abatement districts, gave essentially the same powers to Boards of Trustees of both mosquito districts and pest abatement districts. Attorneys who have had occasion to become familiar with the law and the granting of the powers to the Board of Trustees have been amazed at the breadth and strength of these powers. Sections 2270 to 2292 of the Health and Safety Code give these powers to mosquito abatement districts; Sections 2855 to 2868 grant them to trustees of pest abatement districts. The district board may take all necessary or proper steps for the extermination of mosquitoes, flies, or other insects; or abate as nuisances all stagnant pools of water and other breeding places for mosquitoes, flies or other insects; purchase such supplies and materials as may be necessary or proper in furtherance of the objects of this chapter; build, construct, repair, and maintain necessary dikes, levees, cuts, canals, or ditches; make contracts to indemnify or compensate any owner of land or other property, for any injury or damage; enter upon without hindrance any lands, within or without the district for the purpose of inspection to ascertain whether breeding places of mosquitoes, flies, or other insects exist upon such lands, or to abate public nuisances in accordance with this article; sell or lease any land; borrow money in any fiscal year and repay it in the same year or in the next ensuing fiscal year; issue warrants payable at the time stated therein to evidence the obligations to repay money borrowed, or any other obligation incurred by the district; provide a civil service system for any or all employees of the district. The Board can 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; and do any and all things necessary or incident to the powers granted by, and to carry out the object specified in this chapter.

Any breeding place for mosquitoes, flies or other insects is a public nuisance. A nuisance may be abated in any action or proceeded by any remedy provided by this chapter or any other law. Whenever a nuisance specified in this chapter exists upon any property, the district board may notify in writing the owner or party in possession or the agent of either, of the existence of the nuisance. The contents of the written notice shall conform to the requirement of Section 2275. Sections 2275 through 2281 described the procedures that shall be followed in connection with notification and hearing granted to the violator. Section 2282: In the event that the nuisance is not abated within the time specified in the notice or at the hearing, the district board may abate the nuisance by destroying the larvae or the pupae and by taking the appropriate measures to prevent the recurrence of further breeding. Section 2283: The cost of abatement of a nuisance shall be repaid to the district by the owner of the property.

Thus, the law gives the Board of Trustees tremendous power for reduction or elimination of sources of vectors. These powers are permissive; they are not required by the law. So it behooves the Board of Trustees to establish a policy for the direction in which they wish to effect the reduction of vector sources. The procedure by which these policies can be carried out vary from district to district and have been worked out in great detail by a number of the districts, depending upon the policy established. In some districts, for example, the policy is to go the full limit of the law, to give notice to violators and provide hearing opportunities, and then if there is not compliance, to inflict the full weight of the law and the full penalty on the violators. At the other extreme are districts whose policy it is not to require the reduction or elimination of sources, but rather to attempt to effect controls through the district's efforts only. It's understandable that there should be a variety of approaches to this problem, because no two districts are exactly the same. However, I have here for you examples of the procedures in force by a number of districts. I have prepared a packet of handouts that includes excerpts from the California Health and Safety Code; a check list of items requiring Board action, and the frequency of occurrence of these items by Jack Kimball, from Mosquito News Volume 26, No. 3 Sept. 1966; a suggested purchasing policy and district fund investment policy established by a CMCA committee; an opinion on the conflicts of interest under proposition 9 by an attorney who had been retained by the Association of California Water Agencies. The issue of possible conflict of interest will be covered in greater detail later this morning. Also included are the conclusions of a paper by William T. Sweigert who is an attorney for Arcade County Water District, in a paper, the title of which is "A New Concept – The Expenditure of Water Agency Funds for the Purpose of Compiling and Disseminating Information Concerning the Benefits and the Concept of the District Form of Local Government."

His conclusion is: "A legislature which too often has harbored proposed legislation which would severely restrict, even totally abolish the independence of the District form of government, has seen fit to edify certain water agencies by recognizing that they do possess rights and properties and engage in activities." This is according to the statutes of 1972 Chapter 330 California Water Code, Sections 22235, 31011, and 35411. To quote "Given a district with the express statutory power to disseminate information to the public concerning these three attributes, isn't it a logical implication, inherent in that grant, that the power exists to expand public funds for the purpose of compiling, analyzing, and preparing such information in proper form, for dissemination to the public, as well as for the costs of the actual dissemination?" In Municipal Corporations, Section 209 "The power of a municipality to expend the funds as for a public purpose, to send delegates to a convention of a municipal league or to pay expenses of public officers in attending other conventions or conferences for the purpose of obtaining information or education of value in the public interest has been upheld. See also, Powell vs. City and County of San Francisco Supera. It is suggested that the time has arrived legally, practically, and philosophically for districts and their electorates to speak out in defense of their local government and assert, in the absence of a constitutional or legislative abolition of all District government, that combined, they possess the sole right and power to determine their survival or demise."

In addition to greater attention to labor considerations, conflicts of interest, and public information, there is more attention being paid now to safety regulations. A federal law was passed, commonly referred to as OSHA (Occupational Safety and Health Act) and this prescribed that certain safety measures would be met in all places of employment, and that if the state did not pass measures that were at least as stringent as the federal law, the federal agencies would come in and enforce the safety requirements. In order to meet the state's responsibility in this area, the legislature passed bills now included in Title 8 of the California Administrative Code, in which the safety conditions to which governmental agencies must comply are spelled out. The Occupational Health Section of the California Department of Health acts as consultants for the safety measures related to health that are to be installed in governmental agencies. Enforcement of these is by the Division of Industrial Safety of the California Department of Industrial Relations. The law became effective in January, 1974 but first attention is being directed at private industry so there is a little breathing spell for governmental agencies. However, it is very important that governmental agencies bring their places of employment up to standard in anticipation that they will come under close scrutiny.

Environmentalists have promoted federal and state laws that require an environmental impact statement or report on actions that might have an effect upon the environment. Anyone who wants to build a project or dig a ditch or do anything that might produce a change of the environment, or anyone who reviews such a project, must adopt by ordinance, resolution, rule, or regulations, objectives, criteria, and procedures for the evaluation of projects and the preparation of environmental impact reports. The objectives, criteria and procedures shall be consistent with the provisions of the law and with guidelines. There has been a profusion of EIR's. Proponents of projects have spent large sums of money for the preparation of EIR's. Business firms of EIR consultants have sprung up to do the preparation of the reports. Now the guidelines are being changed, and new objectives, criteria and procedures must be adopted by April. For the protection of your District, you should have on file a policy statement of the intent of the District for the handling of EIR's.

The progressive reduction or elimination of sources of mosquitoes has always been a challenging enterprise, but recently several governmental agencies have considerably added to the burden. Most of them are concerned with coastal areas, because those areas seem to be more sensitive, e.g.: the S. F. Bay Conservation and Development Commission and Coastal Zone Conservation Commission confine their activities exclusively to the coast, and all of the other agencies are active there. However, all areas of the state are subject to restrictions by some of the agencies.

The laws under which these agencies operate require permits for any alteration of the environment, and acquisition of the permits is extremely time-consuming. Timing is important in mosquito control, and interference with the orderly conduct of urgent District operations is disruptive and counter productive. Politically this is a bad situation, also, in that one public agency is making another public agency less effective, at the expense of the individual who is supporting both agencies.

EVALUATION OF INSECT GROWTH REGULATORS AGAINST CHIRONOMIDS IN EXPERIMENTAL PONDS

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The importance and magnitude of the chironomid midge problem in California in general, and southern California in particular, was described by Mulla and Khasawinah (1969) and Mulla et al. (1971). Most of the conventional chemicals for the control of midges in flood control channels, residential and recreational lakes, sewage oxidation ponds, and water spreading basins, were found to be either ineffective due to resistant buildup in the population, or undesirable due to high costs and high levels of toxicity to nontarget organisms and wildlife in midge breeding sources (Mulla et al. 1974, Pelsue et al. 1974). Several mosquito abatement districts with large midge populations expressed the need for new techniques and materials to control these nuisance insects in urban situations (Pelsue et al. 1974).

Recent evaluation of IGR's for the control of chironomid midges in recreational lakes, field plots, and water spreading basins yielded encouraging results and the future outlook for the use of IGR's as midge control agents looks promising (Mulla et al. 1974). A number of these materials showed high activity against the target midges with little or no effect on nontargets such as microcrustaceans, aquatic predatory insects and mosquito fish (Mulla et al. 1975). These desirable characteristics are important factors in the development of promising IGR's for the control of mosquitoes and nuisance aquatic midges. The following studies, therefore, were undertaken to evaluate the most effective materials, and to determine the best formulations of some IGR's against aquatic midges in experimental ponds.

METHODS AND MATERIALS.—These studies were conducted at the aquatic research facilities at Oasis in the Coachella Valley of southern California and at the University of California in Riverside. These facilities are described by Mulla et al. (1974). Midges breeding in ponds in the Coachella Valley consisted mostly of *Paralauterborniella* and *Tanytarsus* species, while *Tanytarsus* was predominant in Riverside.

Emulsifiable concentrates, wettable powders, granules, microencapsulated formulations of the IGR's were applied at rates indicated in the tables. The required amounts of wettable powders, emulsifiable concentrates and microencapsulated formulations were mixed with 100 ml of water and applied to the water surface with a plastic squeeze bottle. The four granular formulations of TH-6040 (0.5%) were applied by hand from the middle of the pond. Materials evaluated include:

HE-24108: 3-Butyn-2-yl-N-(p-chlorophenyl) carbamate.

Methoprene or Altosid®: isopropyl 11-methoxy-3,7,11-trimethyl-dodeca-2,4-dienoate.

Dimilin® (TH-6040): 1-(4-chlorophenyl)-3-(2,6-difluro-benzoyl)-urea.

R-20458: 1-4(4'-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene.

RO-10-3108: 6,7-Epoxy-3-ethyl-1-(p-ethylphenoxy) -7 -methylnonane (cis/trans mixture).

CGA-13353: 4-(4'-benzylphenoxy)-3-methyl-2-butenoic acid eth. ester.

Water temperature in the Coachella Valley ponds was measured continuously during each experiment by a Tempscribe remote-reading thermograph with its probe placed at the bottom of one of the ponds. Water pH in the ponds ranged from 8.6 - 9.7.

Two or more materials were evaluated at a time, at several different rates, using 2 ponds per application rate and 2 ponds as checks in each test. Methods utilized for larval and adult emergence assessments are described elsewhere (Mulla et al. 1974, 1975).

Larval Assessment.--Benthic chironomid midge larvae were sampled by taking 2 bottom mud samples from each pond with a 16.5 $in.^2$ scraper. The mud sample was washed through a 50-mesh screen sieve, and the residue on the screen was transferred to plastic cups. The midge larvae were floated from the residue by adding saturated solution of $MgSO_4$. The larvae were counted and grouped into Chironominae and Tanypodinae.

Adult Emergence Assessment.--Emergence of Chironomid midges was assessed by placing one emergence cylinder in each pond, fitted with a collection chamber at the top. Every 48 hours, the collection chambers were collected and stored in an ice chest and transported to the laboratory for counting and identification. The emergence cylinders were washed and moved to a new spot each time. Species present in the ponds during each test are shown in the tables along with water temperature, materials, formulations and rates of application. Percent inhibition of emergence of adults was calculated by the formula $[\%EI = 100 - (\frac{C1}{T1} \times \frac{T2}{C2})\, 100]$, as utilized in other studies (Mulla et al. 1971); where C1 = No. of adults in the check plot prior to treatment; C2 = No. of adults in the check plot after treatment; T1 = No. of adults in treated plots prior to treatment; T2 = No. of adults in treated plots after treatment.

RESULTS AND DISCUSSION.—TH-6040 was the most effective IGR tested against chironomid midges in experimental ponds. The 5 percent emulsifiable concentrate and 25 percent wettable powder formulations were equally effective, and completely inhibited the adult chironomid midge emergence for more than 3 days at the rate of 0.1 lb/A actual. At the rate of 0.05 lb/A, the granular formulations of this material, PP-106 and PP-108, inhibited midge emergence completely for over 3 days, while PP-107 and PP-109 were somewhat less effective, but reduced adult emergence drastically (Table 1). These granules were formulated on florex, Celatom and emathlite. The finer size formulations, PP-106 and PP-108, appeared to be more effective than the coarser ones PP-107 and PP-109.

Table 1.–Effect of various formulations of the IGR TH-6040 on the emergence of adult chironomid midges in experimental ponds.[a]

Material and formulation	Rate lb/A	Average number emerging adults/trap/48 hours: Pretreat	Posttreat (days): 2-3	(%EI)	6-7	(%EI)
			Oasis – June 1973			
(5%) EC	0.025	51	7	92	79	2
	0.10	54	0	100	56	1
(25%) WP	0.025	140	45	81	195	1
	0.10	60	0	100	51	1
Check	--	113	193	--	102	--
			Oasis – April 1974			
PP-106 0.5G 20/30 florex	0.05	51	0	100	14	78
PP-107 0.5G 15/30 celatom	0.05	27	3	87	26	25
PP-108 0.5G 30/60 celatom	0.05	43	0	100	27	51
PP-109 0.5G 15/30 emathlite	0.05	50	3	93	17	73
Check	--	29	24	--	37	--

[a]Population mostly *Paralauterborniella* and *Tanytarsus*. Daily mean min. and mean max. water temperature 70-90°F in June 1973, and 72-91°F during April 1974.

Table 2.–Effect of various formualtions of the IGR HE-24108 on the emergence of adult chironomid midges in experimental ponds.

Formulation	Rate lb/A	Average number emerging adults/trap/48 hours: Pre-treat	Posttreat (days): 2-3	(%EI)	4-5	(%EI)	7-8	(%EI)
			Oasis – May 1973[a]					
EC1	0.25	77	90	59	76	10	118	0
	0.50	78	33	78	129	0	112	0
Check	--	92	175	--	101	--	130	--
			Oasis – February-March 1974[b]					
EC1	0.5	70	60	64	3	99	12	94
	1.0	21	23	54	3	100	6	89
50 WP	0.5	21	25	49	4	95	6	89
	1.0	54	57	55	0	100	9	94
Check	--	28	6	--	97	--	74	--

[a]Population consisted mostly of *Paralauterborniella, Tanytarsus.* Other species present in small number included *Tanypus, Procladius*, and *Chironomus*. Daily mean min. and mean max. water temperature was 67-89°F.

[b]Population as above. Daily mean min. and mean max. water temperature was 54-67°F.

At the rates of 0.25 - 0.5 lb/A, adult emergence inhibition was mediocre with HE-24108 (Table 2). However, at the rate of 0.5 - 1.0 lb/A, inhibition of emergence increased gradually and almost complete inhibition was achieved 4 days after treatment with both formulations (50WP and EC 1) producing similar results. At both rates 0.5 and 1.0 lb/A, results were promising during March, but were poor during the month of May, when water temperature reached 89°F.

Neither formulation of Altosid was effective against the chironomid midges in the experimental ponds at the rate of 0.1 lb/A Altosid SR10, Altosid SR10-F produced similar results, and neither material was superior to the other at the rate of 0.1 lb/A (Table 3). Better results may be possible at a higher rate (0.25 - 0.5 lb/A).

At the rates of 0.25 - 0.5 lb/A, various formulations of R-20458 were ineffective and adult emergence inhibition of chironomid midges was negligible (Table 4). Poor results were also obtained with CGA-13353, and both formulations of RO-3108 at the rate of 0.05 and 0.1 lb/A. At a higher rate, 0.5 lb/A, CGA-13353, RO-10-3108 (25%) semigelled, and RO-10-3108 EC4 produced 76, 89 and 82 percent inhibition of chironomid midges respectively. RO-10-3108 semigelled formulation remained active for 6 days, while the other two materials were active for 3-4 days only (Table 5).

TH-6040, as previously reported (Mulla et al. 1975), was highly active against larvae of various species of chironomid midges. At the rate of 0.1 lb/A, TH-6040 (25%) wettable powder formulation eliminated the Chironominae larvae completely and reduced the number of Tanypodinae larvae drastically for more than 8 days (Table 6). No reduction in the number of larvae was apparent in the plots treated with HE-24108 and R-20458 at the rate of 0.25 and 0.5 lb/A. As reported earlier (Mulla et al. 1974), TH-6040 acted as an antimolting agent against mosquitoes, and bulk of the mortality initially occurred in the larval stage, and some in the pupal and adult stages. The large number of dead adult midges found on water surface of treated ponds with this material and HE-24108 after a few days indicate loss of effectiveness. The emergence of adults and their death on the surface is a clue to normal emergence within 2-3 days thereafter, indicating the need for subsequent treatment.

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Table 3.—Effect of various formulations of the IGR Altosid on the emergence of adult chironomid midges in experimental ponds.

Formulation	Rate lb/A	Average number emerging adults/trap/48 hours: Pre-treat	Posttreat (days): 2-3	(%EI)	5-6	(%EI)	7-8	(%EI)
			Oasis – March 1974[a]					
SR10 (white)	0.1	13	8	78	8	66	15	42
SR10-F (black)	0.1	21	13	78	12	69	14	67
Check	--	10	28	--	18	--	20	--
			Oasis – May 1974[b]					
SR10 (white)	0.1	47	7	58	--	--	46	0
SR10-F (black)	0.1	63	9	60	--	--	38	0
Check	--	129	46	--	--	--	23	--

[a]Population mostly *Tanytarsus* and *Cricotopus;* daily mean min. and mean max. water temperature 58-74°F.

[b]Population mostly *Paralauterborniella* and *Tanytarsus;* daily mean min. and mean max. water temperature 70-89°F.

Table 4.—Effect of various formulations of the IGR R-20458 on the emergence of adult chironomid midges in experimental ponds.

Formulation	Rate lb/A	Pre-treat	Average number emerging adults/trap/48 hours — Posttreat (days) 2-3	(%EI)	5-6	(%EI)	7-8	(%EI)
			Oasis – May 1973[a]					
F1	0.25	95	49	0	127	0	230	0
	0.50	161	39	81	64	64	245	0
Check	--	92	175	--	101	--	130	--
			UCR – August – 1973[b]					
EC4	0.5	74	39	27	11	73	5	71
F1 (Thinwall)	0.5	34	70	0	24	0	11	0
F1 (Thickwall)	0.5	76	65	0	6	61	8	55
Check	--	69	50	--	14	--	16	--
			Oasis – November 1974[c]					
EC4	0.5	92	44	40	52	14	23	53
2 S	0.5	34	44	0	22	2	23	0
F1 (Thinwall)	0.5	53	39	8	20	42	20	28
F1 (Thickwall)	0.5	75	80	0	28	43	39	1
Check	--	55	44	--	36	--	29	--

[a] *Paralauterborniella* and *Tanytarsus* were predominant. Daily mean min. and mean max. water temperature 67-89°F.

[b] Mostly *Tanytarsus*; air temp. 65-95°F.

[c] Population as in (a); daily mean min. and mean max. water temp. 54-65°F.

Table 5.—Effect of various IGR's on the emergence of adult chironomid midges in experimental ponds.[a]

Material and formulation	Rate lb/A	Pretreat	Average number emerging adults/trap/48 hours — Posttreat (days) 2-3	(%EI)	5-6	(%EI)
CGA-13353	0.05	23	54	0	120	0
EC 3.3	0.10	104	177	0	269	0
	0.25	101	27	75	87	53
	0.50	31	8	76	56	1
RO-10-3108	0.05	43	79	0	114	0
(25%) semigelled	0.10	16	23	16	43	0
	0.25	105	25	77	66	66
	0.50	18	2	89	8	86
RO-10-3108	0.05	56	50	48	101	6
EC4	0.10	32	182	0	230	0
	0.25	99	16	85	58	68
	0.50	16	3	82	50	0
Check 1[b]	--	58	99	--	106	--
Check 2[c]	--	18	19	--	33	--

[a] Population mostly *Paralauterborniella* and *Tanytarsus*. Daily mean min. and mean max. water temperature 75-93°F.

[b] Test 1 at 0.05 and 0.10 lb/A.

[c] Test 2 at 0.25 and 0.50 lb/A.

Table 6.—Effect of various IGR's on the larval population of chironomid midges in experimental ponds.

Material and formulation	Rate lb/A	Average number midge larvae/16.5-in^2 mud sample pre and post-treat days									
		Chironominae					*Tanypodinae*				
		Pre	2	4	8	12	Pre	2	4	8	12
		Oasis – May 1973									
TH-6040	0.01	51	27	9	19	31	4	10	11	12	22
25 WP	0.025	19	31	0	1	5	7	7	6	7	24
	0.10	48	31	0	0	2	9	5	4	2	9
HE-24108	0.25	17	13	21	40	40	6	4	8	14	25
EC 1	0.50[a]	48	73	29	33	44	8	8	12	25	23
R-20458	0.25	37	70	73	38	45	5	7	9	24	34
EC1 (flow)	0.50[a]	43	48	35	36	34	6	6	9	13	34
Check	--	29	57	31	30	20	5	5	10	15	31
		Oasis – June 1973									
TH-6040	0.025	14	6	2	1	2	18	18	17	23	22
EC (5%)	0.10[a]	5	7	3	2	3	20	27	12	18	16
TH-6040	0.025	27	12	6	5	2	22	20	21	31	29
(25%) WP	0.10[a]	11	14	4	2	2	13	23	13	26	20
Check	--	18	13	13	17	11	27	27	19	23	23

[a]Dead adult midges floating on water surface in large number 4-5 days after treatment.

EVALUATION OF MOSQUITO ADULTICIDES IN IRRIGATED PASTURES USING NONTHERMAL AEROSOLS

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ABSTRACT

Cidial (ethyl mercaptophenylacetate, 0,0-diethyl phosphoro=dithioate) produced good control of adult pasture mosquitoes [*Aedes nigromaculis* (Ludlow)] up to ¾ mile when applied at the rate of 15 oz/min of (14%) active ingredient in locomotive diesel oil with a nonthermal aerosol generator. The synthetic pyrethroids NIA-18739 (5 benzyl-3-furyl (+)-trans-2, 2-dimethyl-3-(2-methyl-l-propenyl) cyclopropane-l-carboxylate) and NIA-26021 (5 benzyl-3-furyl) methyl (+)-cis-2,2-dimethyl-3-(2-methyl-l-propenyl) cyclopropane-l-carboxylate) also were fast acting and highly effective. NIA-26021 (7%) in light medium spray oil controlled adult mosquitoes (¾) mile downwind at the rate of 36 oz/min. NIA-18739 (7%) in the same carrier produced 95% reduction of adult mosquitoes ½ mile downwind at the rate of 22 oz/min.

The need for the development of mosquito adulticiding agents has been documented, and this work has been advanced by Mulla et al. (1972) and Sjogren et al. (1973). In California, adulticides are now being used extensively where insecticide resistance exists in the larval stages to most conventional insecticides (Ramke et al. 1969) or where larval control is difficult to achieve due to the large expanse of mosquito breeding sources (Whitesell 1973).

Results of earlier field evaluation of synthetic pyrethroids for the control of the pasture mosquito *Aedes nigromaculis* (Ludlow) were promising. Resmethrim or SBP-1382 yielded good control of adult mosquitoes when applied by air at the rate of 0.05 lb ai/A in 0.5 gal of ARCO larvicidal oil (Mulla et al. 1972). However, as reported by Sjogren et al. (1973), poor and inconsistent results were obtained with the same material when applied with a 4 nozzle Microgen® (Model L4-75) cold fogger. Better results were obtained at the rate of 28-30 fluid oz/min of 7.5% of SBP-1382 in cotton-seed oil than 105 oz/min of 4.5% of the same material in ARCO larvicidal oil. High rates, as explained by Sjogren et al. (1973) caused droplet fallout, while low rates of discharge produced optimum droplet size, resulting in reduced fallout, yielding good results for greater distances from point of discharge.

The new synthetic pyrethroids NIA-26021 and NIA-18739 and the organophosphorus material phenthoate or Cidial were fast acting and highly effective against larvae and pupae of various mosquito species (Darwazeh and Mulla 1974). The following studies were initiated to evaluate the efficacy of these three materials for the control of adult mosquitoes (*A. nigromaculis*) in irrigated pastures. Studies were also conducted to determine the most effective rate of discharge (ounces/min) from a nonthermal aerosol generator.

METHODS AND MATERIALS.—Emulsifiable concentrate materials were diluted with either light medium spray oil or with locomotive diesel oil and applied with a four nozzle cold fogger (Microgen® Model L4-75), transported on the tail gate of a pickup truck. All materials were applied immediately after sunset (1900-2000), at a time when *A. nigromaculis* activity is at its peak.

The synthetic pyrethroids NIA-26021 [(5-benzyl-3-furyl) methyl (+)-cis-2,2-dimethyl-3-(2-methyl-l-propenyl) cyclopropane-1-car=boxylate)] and NIA-18739 [(5-benzyl-3-furyl) methyl (+)-trans-2,2-dimethyl-3-(2-methyl-l-propenyl) cyclopropane-1-carboxylate)] were evaluated in the Rindy and Pintail duck clubs area, located on Bear Mountain Boulevard, 15 miles south of Bakersfield in Kern County. Test area consisted of duck clubs, irrigated pastures, cotton, corn and alfalfa fields. 2.75 gals of NIA-18739 EC1 were mixed with 2.5 gals of light medium spray oil and applied at the rate of 22 oz/min, with the vehicle traveling at a speed of 4 MPH. One run of 2 miles length from north to south was made. 1-2 MPH westerly breeze prevailed during application, carrying the insecticidal cloud eastward over the mosquito infested fields. NIA-26021 EC1 was mixed in the same ratio in light medium spray oil and applied at the rate of 36 oz/min, making a 1 mile run from north to south, at a speed of 5 MPH. Wind velocity and direction remained unchanged as previously described.

Population assessment was conducted prior to treatment, 12 and 24 hours post-treatment. Four sampling stations (0.25 miles apart) were established at 300, 1760, 3250 and 5280 feet from the line of discharge, and several sites were sampled at each station and the average landing count calculated.

The organophosphorus material Cidial EC4 (ethyl mercaptophenyl=acetate, 0,0-dimethyl phosphorodithioate) was diluted in either locomotive diesel oil or in light medium spray oil. All Cidial tests in locomotive diesel oil were conducted in Rancho Santa Maria (Smith Pasture) in Kern County, while Cidial in light medium spray oil was run in Sections 30-31, south of Bear Mountain Boulevard, west of the Golden State Freeway, along the irrigation canal, 15 miles south of Bakersfield. Cidial percent concentration, rate of application (oz/min) and sampling stations and distance from dispersal point are included in tables 1-3. Percent reduction in the adult population was calculated by utilizing the formula developed by Mulla et al. (1971).

RESULTS AND DISCUSSION.—At the rate of 22 oz/min, NIA-18739 (7%) in light medium spray oil produced 84-94 percent initial reduction of *A. nigromaculis* for a distance of ½ mile from the discharge line. Poor results (62 and 27 percent reduction respectively) were obtained ¾ and 1.0 mile from the discharge point, 24 hours after treatment. The same concentration of NIA-26021 in light medium spray oil, applied at the rate of 36 oz/min, produced complete control for ¾ mile, and 72 percent reduction 1.0 mile

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Table 1.–Field evaluation of synthetic pyrethroids (EC1) in light medium spray oil for the control of adult mosquitoes *(Aedes nigromaculis)*.

			Average number of adults landing				
				Post-treat hours and % reduction			
Adulticide (%)	Rate oz/min	Distance from discharge point (feet)	pretreat	12	(%R)[a]	24	(%R)[a]
NIA-18739 (7)	22	300	44	8	82	7	84
		1760	293	21	93	19	94
		3520	88	20	78	33	62
		5280	41	18	56	30	27
NIA-26021 (7)	36	300	9	1	89	2	78
		1760	10	0	100	1	90
		3520	8	0	100	0	100
		5280	7	1	86	2	72

[a] $(\%R) = 100 - \frac{(T2)}{(T1)}\ 100$, where T1 = no. of adult pre-treat.; T2 = no. of adult post-treat.

from point of application (Table 1). These preliminary results from the evaluation of synthetic pyrethroids as nonthermal aerosol adulticides showed some promise. From the data presented, it seems that 7% concentration of these materials in the carrier is not sufficient for best results. Additional studies on the evaluation of these materials are needed to determine the most suitable carrier as well as the most effective rate (oz/min).

Diluted Cidial (14%) in locomotive diesel oil yielded good control of adult mosquitoes at the rate of 15 oz/min up to ¾ mile. Cidial performance was not improved when concentration percentage and delivery rate were increased (Table 2). These findings substantiate the previously mentioned results reported by Sjogren et al. (1973).

Level of control was not high when Cidial (26%) in light medium spray oil was discharged at the rate of 14 oz/min (Table 3). This poor performance could be attributed to the carrier rather than the toxicant. Results indicate that locomotive diesel oil is a more suitable carrier for the organophosphorus material (Cidial) than the light medium spray oil.

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Table 2.—Field evaluation of Cidial (EC4) in locomotive diesel oil for the control of adult mosquitoes (*Aedes nigromaculis*) in irrigated pastures.

			Average number of adults landing				
				Post-treat hours and % reduction			
Adulticide (%)	Rate oz/min	Distance from discharge point (feet)	Pretreat	12	(%R)[a]	24	(%R)[a]
Cidial (14)	15	300	22	1	88	1	88
		1100	88	4	88	3	91
		1900	60	5	78	3	87
		2600	38	4	73	2	86
		3000	25	3	69	1	90
		3500	25	2	79	0	100
		4000	150	10	83	8	86
		4500	25	7	27	0	100
		5280	20	5	35	20	0
Check	--	--	60	23	--	23	--
Cidial (16)	14	300	63	2	94	4	93
		1320	50	3	89	20	57
		2640	75	1	98	15	79
		3960	50	20	26	50	0
		5280	125	40	32	30	49
Check	--	--	188	101	--	175	--
Cidial (16)	32	300	225	15	94	12	98
		1320	145	30	81	30	91
	2	2600	126	71	44	32	89
Check	--	--	30	32	--	67	--
Cidial (25)	37	300	52	4	95	13	85
		1320	63	27	74	42	60
		2640	57	5	94	33	60
Check	--	--	23	38	--	33	--

[a]$(\%R) = 100 - \frac{C1}{T1} \times \frac{T2}{C2}$ 100; where C1 = no. of adult pre-treat. in check; T1 = no. of adults pre-treat. in treated plots; C2 = no. of adults post-treat. in check plots; and T2 = no. of adults post-treat. in treated plots.

Table 3.—Field evaluation of Cidial (EC4) in light medium spray oil discharged at 14 oz/min for the control of adult mosquitoes (*Aedes nigromaculis*) in irrigated pastures.

			Post-treat hours and % reduction			
Adulticide (%)	Distance from discharge point (feet)	Pretreat	12	(%R)[a]	24	(%R)[a]
Cidial	300	18	4	78	2	89
	600	20	4	80	6	70
	900	35	18	49	12	66
	1320	40	20	50	18	55

[a]For calculation of % reduction see footnote as in Table 1.

CYCLOPS VERNALIS (COPEPODA: CYCLOPOIDA): AN ALTERNATE HOST FOR THE FUNGUS, *COELOMOMYCES PUNCTATUS*

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ABSTRACT

Adult *Cyclops vernalis* copepods, reared in a medium which consistently produced larvae of *Anopheles quadrimaculatus* infected with *Coelomomyces punctatus*, have been found to contain uni- and biflagellate planonts. The development of these planonts within the copepod, in conjunction with other studies, indicates *C. vernalis* is an alternate host for *C. punctatus*.

INTRODUCTION.–Fungi of the genus *Coelomomyces* (Phycomycetes: Blastocladiales) are obligate parasites of mosquitoes and a few other aquatic diptera. Most of the known species have been described from larvae. Sporadic epizootics caused by these fungi have periodically resulted in high mortalities (90-100%) in larval populations of *Anopheles*, *Aedes*, *Culex*, *Culiseta*, and *Psorophora* mosquitoes. This characteristic stimulated several early workers to attempt to study several species of *Coelomomyces* in the laboratory under controlled conditions. Laboratory infections, however, proved extremely difficult to obtain and as a direct result of this, until recently, detailed studies on *Coelomomyces* have been hindered. Two recent reviews (Chapman 1974; Roberts 1974) attest to the deficiency of our knowledge concerning these fungi in such important areas as the life cycle, host range, and ecological parameters which favor the induction of epizootics.

During the 1960's Couch (1968) obtained laboratory infection of *Anopheles quadrimaculatus* with *C. punctatus* and subsequently (Couch 1972) developed a technique for producing thousands of infected larvae. He postulated that the zoospores released from sporangia were the mosquito-infective units. More recently, Federici and Roberts (1973, 1975a,b) obtained laboratory infections with three different *Coelomomyces*-mosquito systems, *C. psorophorae* var. in *Aedes taeniorhynchus*, *C. psorophorae* var. in *Culiseta inornata*, and *C. punctatus* in *A. quadrimaculatus*, and based on experimental studies with these systems they suggested the zoospore was not the infective unit, and hence, that unknown alternate stages existed in more complex life cycles of these fungi. In another recent and significant study Whisler et al. (1974), working with *C. psorophorae* var. in *C. inornata*, found that the copepod *Cyclops vernalis* is an obligate alternate host for *C. psorophorae*. They postulated that the zoospores released from sporangia infect copepods and a stage subsequently develops in the copepod which produces mosquito-infective biflagellate spores.

The purpose of this paper is to present some recent results which indicate that this same copepod, *C. vernalis*, is also an alternate host for *C. punctatus*, and to interpret some results of a previous study (Federici and Roberts 1975a) in light of this finding.

MATERIALS AND METHODS.–Sixty adult *C. vernalis* copepods, which had been reared in a medium that consistently produced *C. punctatus* infected larvae of *A. quadrimaculatus*, were obtained from the Boyce Thompson Institute for Plant Research, Yonkers, N. Y. This medium had been prepared as described previously (Federici and Roberts 1975b). When they arrived the copepods were placed in 300 ml of distilled water in a 500 ml beaker to which ten drops of an egg yolk diet (1 hard boiled egg yolk/500 ml of distilled water) were added. The copepods were examined daily and individuals which differed in appearance from the majority of the normally gray and slightly translucent copepods were examined at 400X with a phase microscope. Copepods which were confirmed to have a mycelium developing in the hemocoel were reared separately in small petri dishes (Falcon no. 3002) until they died. Shortly after death they were crushed under a coverslip on a glass slide and examined again with a phase microscope.

RESULTS.–Three days after the copepods had been placed in the freshly prepared medium five were observed to be slightly more opaque than the others. Three of these were gray while the other two were yellow. Using phase microscopy a small pleomorphic mycelium was observed in the hemocoel of the gray copepods. By day five this mycelium had branched and grown substantially, thereby producing many extensive lobate hyphae which occupied a significant portion of the hemocoel. Hyphae were most easily observed in the posterior portion of the abdomen where they grew between the gut and epidermis. By the morning of day six the copepods were dead. In all three the mycelium had cleaved into thousands of flagellated planonts[1] which were observed throughout the hemocoel. These planonts, the majority of which were uniflagellate, the others being biflagellate, were liberated by crushing the copepod under a coverslip on a microscope slide (Figure 1-3).

The hemocoel of the yellow copepods contained an extensive lobate mycelium within which most of the yellow pigmentation was located. These copepods also died six days after being placed in the freshly prepared medium, but the mycelium had not cleaved nor apparently undergone any further development.

In the course of these observations it was noted that although none of the females carried egg sacs on arrival, most developed them within three days after being placed in the freshly prepared medium.

DISCUSSION.–The results of this study including the demonstration of a mycelium and observations on its development in the hemocoel of the copepod *C. vernalis*, and the cleavage of this mycelium into flagellated planonts, in conjunction with the results of Whisler et al. (1974) and Federici and Roberts (1975b), provide strong evidence that this copepod is an alternate host for *C. punctatus*. Final proof of this is dependent upon positive identification of the mos-

[1]The term planont is used here because the precise function and sexual nature of these motile cells has not yet been determined.

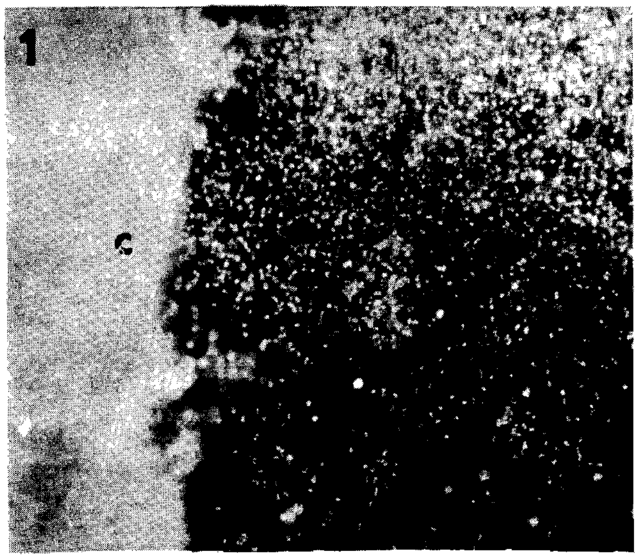
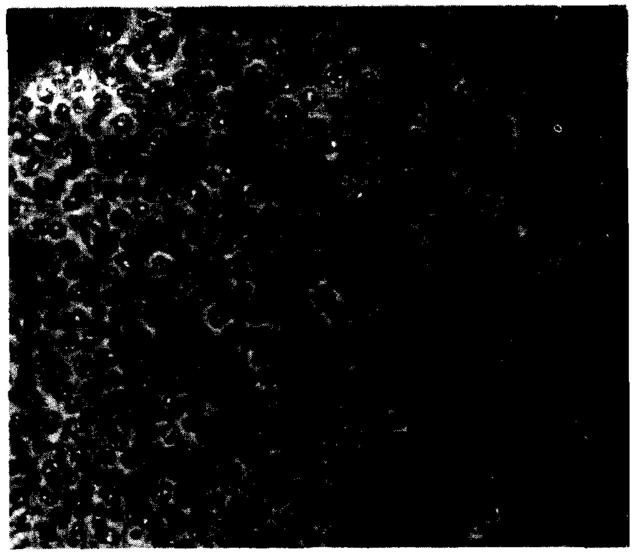


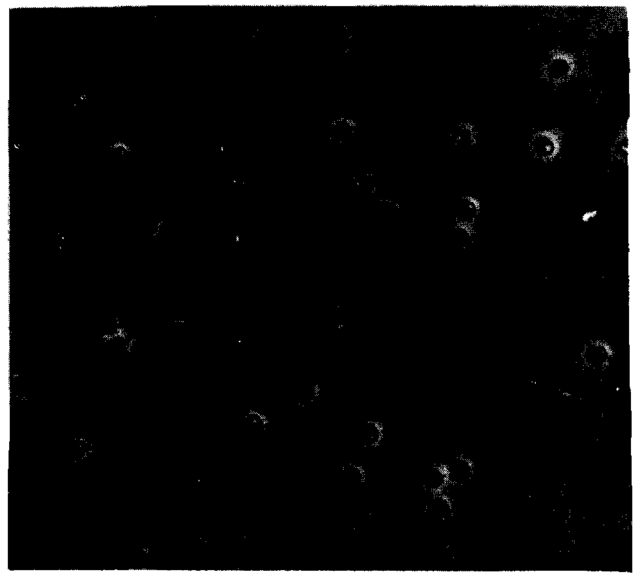
Figure 1.–Portion of a crushed copepod (c) and thousands of liberated flagellated planonts (330X).

quito-infective and copepod-infective units during the infection process, and demonstration of the subsequent induction of a typical *C. punctatus* infection in each host.

Whisler et al. (1974) proposed that the uniflagellate spores liberated from sporangia infected the copepod, and the biflagellate spores liberated from the copepod infected mosquito larvae. Although a similar process may take place in the *A. quadrimaculatus-C. punctatus-C. vernalis* system several phenomena remain to be resolved. When sporangia from a single larva are induced to dehisce by placing them in four ml of deionized water in a small petri dish, many biflagellate, and in some cases, multiflagellate zoospores are observed, in addition to the predominantly uniflagellate zoospores, 24 hours after the initiation of dehiscence. The relative number of bi- and multiflagellate to uniflagellate spores increases between 24 and 48 hours (Federici, unpublished observations). It is not clear whether these multiflagellate zoospores result from fusion of uniflagellates, or whether they are the product of abnormal cleavage during zoospore formation. Furthermore, the infective properties of these multiflagellates, if any, have not been determined. In addition to the above, although uni- and biflagellate spores occur in the copepod, the fusion of the former to form the latter has not been observed. These alternative considerations are diagramatically summarized in Figure 4.

Although the precise life cycle of *C. punctatus* is unknown, the identification of a copepod as an alternate host provides an explanation for (1) the absence of mosquito-infective units prior to seven days after the introduction of zoospores into an appropriate medium, and (2) the "dilution phenomenon", where a burst of infection resulted six days after dilution of media twenty-one days old or older (Federici and Roberts 1975b). In the former case the hypothesized alternative stages do in fact exist and develop over a period of approximately seven days within the copepod hemocoel. The "dilution phenomenon" appears to be related to the nutritional and physiological state of the copepod. The majority of adult female copepods in cultures at least twenty-one days old frequently do not bear egg





Figures 2-3.–Flagellated planonts at higher magnification (1300X). These spores appear more spherical than normal because they have hypertrophied in the distilled water under the coverslip.

sacs, especially under conditions where they are crowded and/or their nutrition is poor. Yet when the medium is diluted with an equal volume of water, and food is added, most females develop egg sacs within one to three days. In such media, infection first results six days after dilution. This result can be obtained by diluting media anytime subsequent to twenty-one days after preparation of the media, up to a period of at least three months. As noted in the present study, the female copepods developed egg sacs three days after being placed in a freshly prepared medium and on day six, three dead copepods resulted which contained uni- and biflagellate planonts. Apparently the factors which inhibit or retard the formation of egg sacs, whether they be lack of proper nutrition, high population density, or a variety of other complex variables, also indirectly retard or prevent the development of the fungus mycelium. Yet, once the proper conditions for egg sac development are restored the mycelium develops normally. This is not to say that the fungus develops only in females, but that a correlation ex-

Possible Life Cycles For *Coelomomyces punctatus*

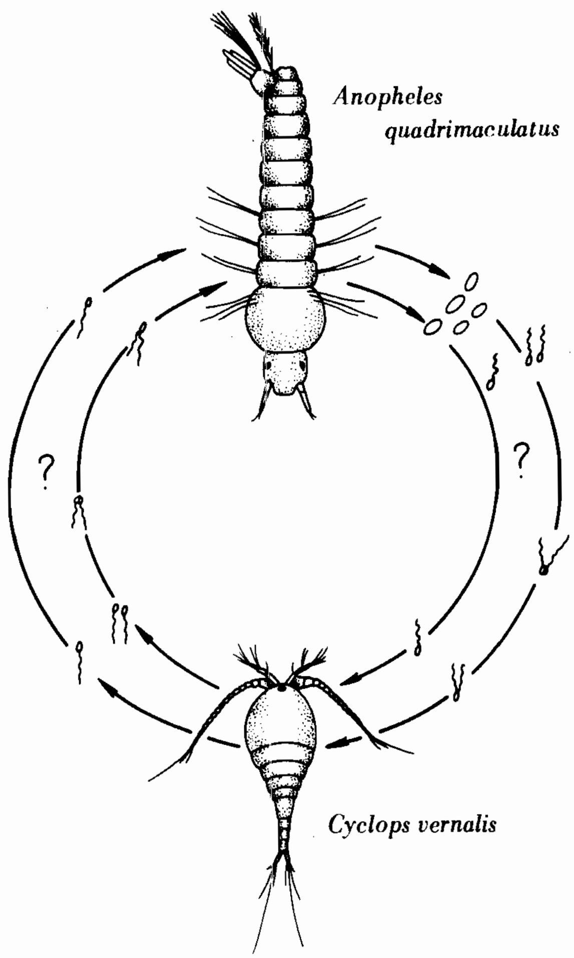


Figure 4.–Possible life cycles for *Coelomomyces punctatus*. Uniflagellate "zoospores" (haploid?) are liberated from sporangia and eventually result in copepod infection. A mycelium develops within the copepod during a period of approximately six days and subsequently cleaves forming flagellated planonts. These planonts fuse and eventually result in infection of mosquito larvae. In this case the mycelium which develops in the mosquito would be a sporophyte while that which develops in the copepod would be a gametophyte, the planonts in the latter case being gametes. Another, less likely, alternative is that the stage which develops in the mosquito is the gametophyte and that in the copepod a sporophyte.

ists between the conditions conducive to egg sac development and the production of infective units six days after medium dilution. This ability of the copepod to apparently harbor a "latent" infection for long periods is a potentially important phenomenon.

It is becoming increasingly apparent that the induction of *Coelomomyces* epizootics in nature is a result of complex interactions between populations of the fungus and its copepod and mosquito hosts. Our ability to develop possible control strategies which employ these fungi will be directly dependent on the level of understanding we obtain of these complex interactions.

ACKNOWLEDGMENTS.–The author expresses his appreciation to Drs. D. W. Roberts and J. L. Fowler, and Miss G. Smedley and P. Pong of the Boyce Thompson Institute for Plant Research, Yonkers, N. Y., for supplying the copepods used in this study.

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OPERATIONAL USE OF THE INSECT GROWTH REGULATOR ALTOSID® IN KERN MOSQUITO ABATEMENT DISTRICT, 1974

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Prior to the 1974 mosquito season the chemical larvicidal program at Kern Mosquito Abatement District (MAD) consisted primarily of petroleum oil treatment by airplane and ground rig. Besides becoming an increasingly expensive and scarce resource, oils have never given completely satisfactory control results. The Kern District, as were many others, was greatly in need of a safe and effective alternative.

Schaefer and Wilder (1972, 1973) demonstrated the practicability of utilizing the insect growth regulator Altosid®, (isopropyl 11-methoxy-3,7,11,-trimethyl-2,4-dodecadienoate), for controlling the floodwater species, *Aedes nigromaculis* (Ludlow) and *Aedes melanimon* Dyar. When Zoecon Corporation was granted an Experimental Sales Permit for Altosid the district management[1] promptly planned their first operational program with the chemical for the 1974 season.

METHODS.—The 10% microencapsulated formulation, Altosid SR-10, was applied in an aqueous dilution, at the rate of 4 oz (=.025 lb AI)/acre by both airplane and ground rig. The Call-Air airplane was calibrated to deliver 0.5 gal of Altosid-water mixture per acre. Power driven jeep-mounted ground sprayers delivered 10 gal of mixture per acre.

Nearly all larvae treated were *Aedes nigromaculis*, but a few isolated *Ae. melanimon* sites were also sprayed. Requests for air applications were written up by field personnel when the larvae were 2nd or 3rd instar. The pilot would then fly the sites the following morning when these larvae were 3rd or 4th instar respectively. Ground personnel usually treated sites harboring 3rd or 4th instar larvae but treated 2nd instar mosquitoes on at least several occasions.

Periodically pupae were collected from treated areas to measure Altosid effectiveness. The pupae were brought to the laboratory where they were held in emergence vessles containing tap water. Fourth instar larvae collected as soon as 4 hrs after treatment were occasionally assessed for emergence. Percent control was calculated from pupal mortality and adult emergence.

RESULTS AND DISCUSSION.—Table 1 shows acreage treated by the aircraft. Air application accounted for almost all Altosid used, and irrigated pastures received the majority of the treatments.

Eighty percent of the total acres of breeding sites received multiple treatments of Altosid. Table 2 gives a breakdown of the application frequencies on pasture and alfalfa, which were the only sites treated more than once. Each cumulative figure represents different breeding locations, ranging in size from 10 to 60 acres, treated the indicated number of times. Pastured lands treated 13, 17, and 20 times were each from the same respective area. These areas were large single pastures, and treatments overlapped only part of the time. However, the treatments always eliminated the mosquito population in the entire pasture and for all practical purposes these areas can be considered single breeding sites.

Only 140 acres were treated from the ground. Pastures comprised nearly all of this acreage.

Table 1.—Altosid SR-10 applied by air by Kern MAD, 1974.

Area	Gallons applied	Total acres treated
Pasture	163.0	5180
Alfalfa	28.6	941
Desert	1.9	60
Duck Clubs	1.2	40
Corn	0.3	10
Barley	0.6	20
Cotton	1.2	38
Beets	0.9	30
Total	197.7	6319

Table 2.--Frequency of treatment record of Altosid SR-10 applied by air by Kern MAD, 1974.

Pasture		Alfalfa	
No. times treated	Cumulative acres	No. times treated	Cumulative acres
1	675	1	432
2	605	2	389
3	260	3	120
5	447		
6	730	Total:	941
7	130		
8	120		
13	684		
17	702		
20	827		
Total:	5180		

[1]Manager Arthur F. Geib (deceased 1974), Entomologist Robert D. Sjogren, and Superintendent Richard H. DeWitt.

The overall impression of Altosid's effectiveness against *Aedes nigromaculis* was favorable. When larvae received the proper dosage during their critical period of vulnerability, control was 100%. Not all applications were monitored but in those where some degree of control failure was noted the reasons could be traced to the normal variables inherent in any control operation, i. e., heavy vegetation, skipped swaths, and wind. High water temperatures and ultra violet radiation may also have reduced effectiveness in some instances (see Schaefer and Wilder 1972). However, this effect is more subtle and difficult to assess. Timing is a new and critical variable to be considered in the Altosid program since populations must be treated before they pupate. Good control was achieved when larvae were treated as 3rd and 4th instar. Ground crews reported achieving control when larvae were treated as 2nd instar but, since no samples were obtained from these areas, firm conclusions cannot be presented.

An attempt was made to establish baseline Altosid susceptibility figures for *A. nigromaculis* prior to the initial field applications. Due to a host of factors, this could not be accomplished. Consequently, no precise method to assess possible onset of Altosid resistance was developed. If continued treatment reveals an increased frequency of control failure at a given application rate, we will be able to conclude resistance if we can confidently discount other variables. However, reliable quantitative data will be lacking. We will especially watch closely those areas pressured heavily during the first season (see Table 2) for signs of treatment failure.

At the rate of 4 oz Altosid (=.025 lb AI)/acre our chemical cost was $2.00/acre applied by air. This compares to a cost of $1.20 – $1.80/acre for larvicide oil applied at 2-3 gal/acre. Although oil costs are lower, the level of control was rarely higher than 80%, and reflights were sometimes necessary. The most dramatic cost savings with Altosid occurs from ground application. Altosid can be applied with good coverage at 4 oz (=.025 lb AI) in 10 gal of water/acre. We find it difficult to produce effective control with oil using sprayers calibrated for less than 10 gal oil/acre. Actual output varies from 5-10 gal depending upon vegetation density. Oil cost ranges from $3.00 – $6.00/acre.

There are several distinct advantages to using Altosid. Its safety to humans is a great asset, as is its mild impact on nontarget organisms. Its non phytotoxic nature eliminates dangers associated with drifting. The especially acute corrosion problems associated with oils are also avoided with Altosid. We will be better able to calculate a monetary savings in this regard when more ground rigs are converted to exclusive Altosid spraying.

The greatest drawback with Altosid resides in its confined use against only *Aedes* mosquitoes. This presents no problems for the airplane since the pilot knows his target beforehand and can arrange his chemical schedule accordingly. However, the ground crews must carry Altosid for *Aedes* spp. and oil for *Culex* spp. This is not an insurmountable problem but operators do frequently find themselves encountering more of one or the other genera than anticipated.

Altosid's ineffectiveness against pupae is a disadvantage. Under an Altosid regimen we must employ adulticiding measures in the inevitable event that a breeding source is detected too late. This problem was largely avoided in our oil program since these chemicals are effective against pupae.

Instability at elevated water temperatures and high ultra violet light intensity may contribute to control failures during the hottest months of the season. Against *A. nigromaculis*, which has a rapid life cycle and a relatively homogeneous instar age, the short effective life of Altosid (approximately 24 hrs) is sufficient to inhibit growth. However, factors reducing the life of the chemical by a few hours greatly threaten its potential effectiveness.

CONCLUSIONS.–We achieved satisfactory control of *Aedes* mosquitoes in our operational season using Altosid, and will expand this use in 1975. More emphasis will be placed on ground application from power and hand units. This will call for more careful inspection and better planning by the ground crews. Improved inspection will also result in less acres being treated with oil by air since fewer sites will escape early detection.

The most productive improvement in the Altosid program would be the development of a stable slow-release granular formulation. If we could treat known breeding areas on a pre-flood basis we could add considerable flexibility to our operation.

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